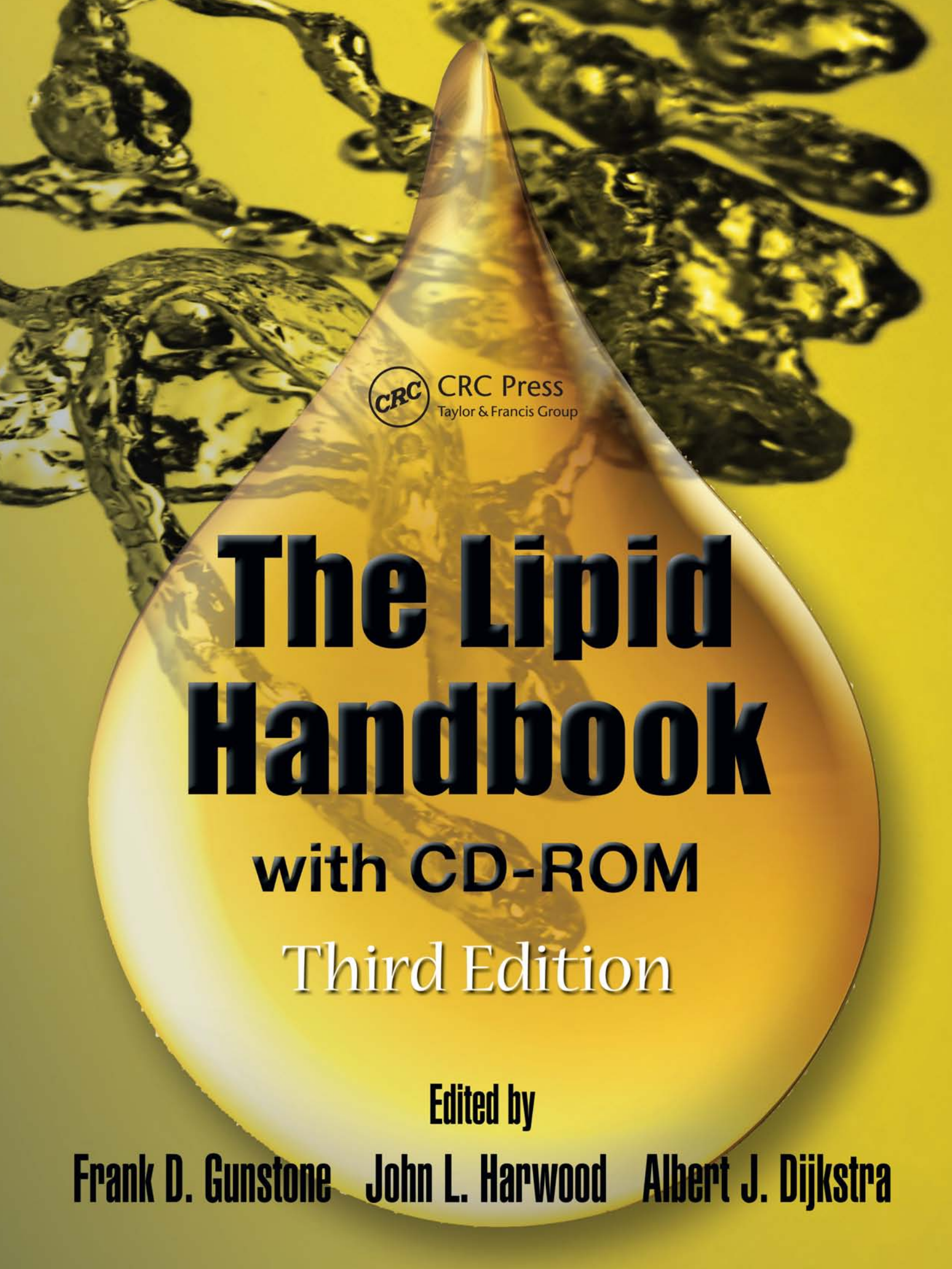
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The Lipid Handbook
with CD-ROM
Third Edition
Edited by
Frank D. Gunstone
John L. Harwood
Albert J. Dijkstra

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PREFACE

The Lipid Handbook was first published in 1984, with a second edition in 1994. We now present the third edition of this successful book, with Albert Dijkstra replacing Fred Padley as a member of the editorial team. The decision to revise this book was made late in 2004 and most of the writing was completed during 2005. We planned the book to take account of the many changes in lipid science and technology that have occurred in the past 10 years, but we sought to maintain the approach and organisation of material used in the earlier editions. Compared to the second edition, some chapters have been combined — "Fatty acid structure" with "Lipid structure" (Chapter 1), "Separation and isolation" with "Analytical methods" (Chapter 6), along with the two chapters on "Physical properties" (Chapter 7). Other chapters have been divided — The former chapter on "Processing" now appears as separate chapters devoted to "Production and refining of oils and fats" (Chapter 3) and to "Modification processes and food uses" (Chapter 4). One new chapter —"Nonfood uses" (Chapter 9) has been introduced. All chapters have been rewritten (often by a new author) and we have sought to present information on the basis of thinking and practice in the present day. One interesting change is that the processing sections refer to patents now easily accessible through espacenet.com or uspto.gov.

In addition, the Dictionary section has been extended on the basis of the latest Taylor & Francis Group databases. This contains a wealth of information covering chemical structures, physical properties, and references to hundreds of lipid and lipid-related molecules, only some of which can be detailed in the text. We are grateful to Taylor & Francis for allowing us to include this information and we thank Fiona Macdonald for assistance in selecting and organising it.

In order to make our task manageable in the time scale agreed between the publishers and the editors and to present authoritative coverage of our topics, we have secured the assistance of several contributors from Europe, Hong Kong and the United States. Only one contributor (P. J. Quinn) and two of the editors (F. D. Gunstone and J. L. Harwood) were involved with the previous edition and almost the entire text now has different authors. This brings fresh minds to the volume.

By bringing a wide range of information into a single volume, we hope that the book will be useful to all who work in the lipid field as scientists or technologists, in industrial or academic laboratories, as newcomers, or as those who already know their way around the field. Lipid science is of increasing interest for metabolic, nutritional, and environmental reasons and we offer this revised and updated volume as a contribution to that growth. For 20 years the book has provided assistance to a generation of those working with lipids and we offer *LH-3* (our acronym for this work) to the next generation.

The third edition is also available on a CD-ROM (included with the book). This will provide a compact form of the so-called "Handbook" and will be easily searchable, thereby providing easy access to material hidden in tables and figures and in the extensive list of references, which now come with full titles.

F. D. Gunstone
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EDITORS

Frank D. Gunstone, Ph.D., is professor emeritus of the University of St. Andrews (Scotland) and holds an honorary appointment at the Scottish Crop Research Institute (Invergowrie, Dundee, Scotland). He received his Ph.D. from the University of Liverpool (England) in 1946 for studies with the late Professor T. P. Hilditch, and subsequently, there followed an academic career in two Scottish Universities: Glasgow (1946 to 1954) and St. Andrews (1954 to 1989). He continues to be professionally active and has spent over 60 years studying fatty acids and lipids with many publications to his credit. Since his retirement in 1989, Dr. Gunstone has written or edited several books. He has given many invited lectures and has received distinguished awards in the United States (1973, 1999, 2005, and 2006), Britain (1962 and 1963), France (1990), Germany (1998), and Malaysia (2004). For many years he has been the editor of *Lipid Technology*, an activity that gives him continued contact with lipid scientists of many differing interests.

John L. Harwood, Ph.D., is head of the School of Biosciences at Cardiff University (Wales, United Kingdom). He received his Ph.D. from the University of Birmingham in 1969, with studies on the metabolism of inositol lipids with Professor J. N. Hawthorne and, subsequently, learned about plant fatty acid synthesis at the University of California with Professor P. K. Stumpf. Following a tenure at the University of Leeds, he moved to Cardiff where he was promoted via reader to professor in 1984. He is currently editor of four journals, including executive editor of *Progress in Lipid Research*. Dr. Harwood has published nearly 500 scientific papers and communications, plus authoring three books (including *Lipid Biochemistry*) and editing 14 others. He has given many plenary and named lectures, received his D.Sc. in 1979 and is in receipt of personal prizes. He also has awards for his publications and those of his students. He is an honorary visiting scientist at the Malaysian Palm Oil Board (Kuala Lumpur), Centre d'Etudes Nucléaires (Grenoble), and the Hungarian Academy of Sciences (Szeged).

Albert J. Dijkstra, Ph.D., specialised in gas kinetics with Professor A. F. Trotman-Dickenson at University College of Wales, Aberystwyth, before defending his Ph.D. thesis at Leyden University in 1965. He joined ICI, first at the Petrochemical & Polymer Laboratory in Runcorn, Cheshire, then at the ICI Holland Rozenburg Works, The Netherlands, and finally at the ICI Europa headquarters in Everberg, Belgium. He became involved in edible oils and fats in 1978 when he joined the Vandemoortele Group in Izegem, Belgium, as its R&D director. Dr. Dijkstra is the inventor in a dozen patents and has published numerous articles on edible oil processing. He was the first non-American to receive the American Oil Chemists' Society (AOCS) Chang Award (1997) and the first to receive the EuroFedLipid Technology Award (2002). Although officially retired, he continues to be active in the field of edible oils and fats as author and scientific consultant.

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1

FATTY ACID AND LIPID STRUCTURE

C. M. Scrimgeour and J. L. Harwood

1.1 Fatty acid structure

1.1.1 Introduction and nomenclature of fatty acids

Fatty acids are aliphatic, usually straight chain, monocarboxylic acids. The broadest definition includes all chain lengths, but most natural fatty acids have even chain lengths between C_4 and C_{22}, with C_{18} the most common. Natural fatty acid structures reflect their common biosynthesis — the chain is built in two-carbon units and *cis* double bonds are inserted at specific positions relative to the carboxyl carbon. Over 1000 fatty acids are known with different chain lengths, positions, configurations and types of unsaturation, and a range of additional substituents along the aliphatic chain. However, only around 20 fatty acids occur widely in nature; of these, palmitic, oleic, and linoleic acids make up ~80% of commodity oils and fats. Figure 1.1 shows the basic structure of fatty acids and a number of the functional groups found in fatty acids. A list of many of the known structures, sources, and trivial names is available online (Adlof and Gunstone, 2003).

Table 1.1 illustrates the naming of some commonly encountered fatty acids (additional examples are found in the following sections). Fatty acids are named systematically as carboxylic acid derivatives, numbering the chain from the carboxyl carbon (IUPAC-IUB, 1976). Systematic names for the series of saturated acids from C_1 to C_{32} are given in Table 1.2. The -anoic ending of the saturated acid is changed to -enoic, -adienoic, -atrienoic, -atetraenoic, -apentaenoic, and -ahexaenoic to indicate the presence of one to six double bonds, respectively. Carbon–carbon double bond configuration is shown systematically by *Z* or *E*, which is assigned following priority rules for the substituents. However, the terms *cis* and *trans* (abbreviated *c* and *t)* are widely used to describe double bond geometry, as with only two types of substituents there is no ambiguity that requires the systematic *Z*/*E* convention (Figure 1.1). However, a recent proposal for systematic naming for use in lipidomic and bioinformatic databases requires the use of *Z* or *E* (Fahy et al., 2005a, 2005b).

Systematic names for fatty acids are cumbersome in general use and both shorthand alternatives and trivial names are widely used. Trivial names seldom convey any structural information, often reflecting a common or early source of the acid. The shorthand names use two numbers separated by a colon for the chain length and number of double bonds, respectively. Octadecenoic acid with 18 carbons and 1 double bond is, therefore, 18:1. The position of double bonds is indicated in a number of ways — explicitly, defining the position and configuration or locating double bonds relative to the methyl or carboxyl ends of the chain. In the biomedical literature, it is common to number the chain from the methyl end rather than the systematic numbering from the carboxyl end, to emphasise the biosynthetic relationship of different double bond patterns. Numbering from the methyl end is written n-x or ωx, where x is the double bond carbon nearest the methyl end. If there is more than one double bond, a *cis* configuration, methylene-interrupted pattern is implied. Although the n-x notation is recommended, both n-x and ωx are widely used in the current biomedical literature and wider nutritional contexts. The Δ notation is used to make it explicit that the numbering is from the carboxyl end. Other substituents may also be included in the shorthand notation; for example 12-OH 18:1 9c for ricinoleic acid (12-hydroxy-9-*cis*-octadecenoic acid). The order and style used for shorthand names varies widely in the literature.

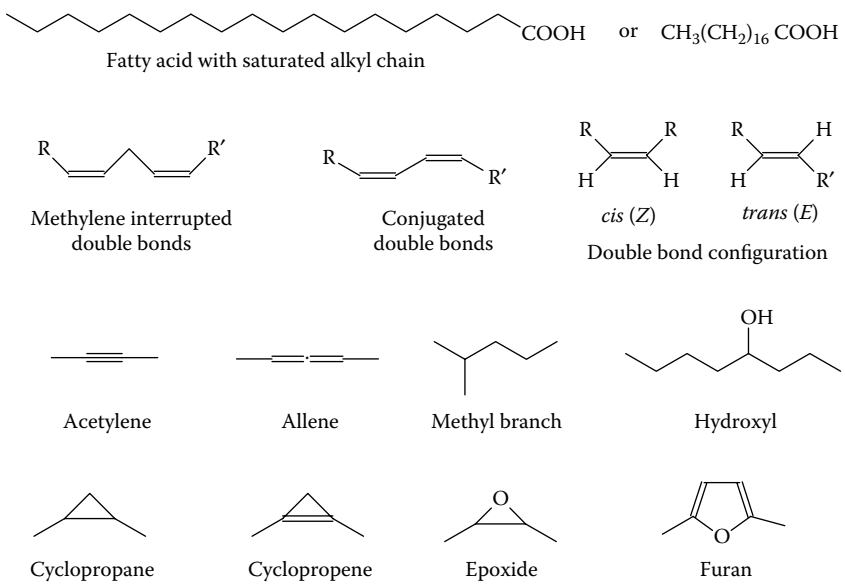


FIGURE 1.1 Fatty acid structure and some functional groups found in fatty acids.

TABLE 1.1 Structure, systematic, trivial, and shorthand names of some common fatty acids

Structure	Systematic Name	Trivial Name/ Abbreviation	Shorthand Name	n- or ω
$CH_3(CH_2)_{10}COOH$	Dodecanoic	lauric	12:0	
$CH_3(CH_2)_{12}COOH$	Tetradecanoic	myristic	14:0	
$CH_3(CH_2)_{14}COOH$	Hexadecanoic	palmitic	16:0	
$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$	*Z*-9-hexadecenoic	palmitoleic	16:1 9*c*	7
$CH_3(CH_2)_{16}COOH$	Octadecanoic	stearic	18:0	
$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$	*Z*-9-octadecenoic	oleic	18:1 9*c*	9
$CH_3(CH_2)_5CH{=}CH(CH_2)_9COOH$	*Z*-11-octadecenoic	*cis*-vaccenic	18:1 11*c*	7
$CH_3(CH_2)_5CH{=}CH(CH_2)_9COOH$	*E*-11-octadecenoic	vaccenic	18:1 11*t*	7
$CH_3(CH_2)_3(CH_2CH{=}CH)_2(CH_2)_7COOH$	*Z,Z*- 9,12-octadecadienoic	linoleic (LA)	18:2 9*c*,12*c*	6
$CH_3(CH_2CH{=}CH)_3(CH_2)_7COOH$	*Z,Z,Z*- 9,12,15-octadecatrienoic	α-linolenic (ALA)	18:3 9*c*,12*c*,15*c*	3
$CH_3(CH_2)_3(CH_2CH{=}CH)_3(CH_2)_4COOH$	*Z,Z,Z*- 6, 9,12-octadecatrienoic	γ-linolenic (GLA)	18:3 6*c*,9*c*,12*c*	6
$CH_3(CH_2)_{18}COOH$	eicosanoic[a]	arachidic	20:0	
$CH_3(CH_2)_3(CH_2CH{=}CH)_4(CH_2)_3COOH$	*Z,Z,Z,Z*- 5,8,11,14-eicosatetraenoic[a]	arachidonic (ARA)	20:4 5*c*,8*c*,11*c*,14*c*	6
$CH_3(CH_2CH{=}CH)_5(CH_2)_3COOH$	*Z,Z,Z,Z,Z*- 5,8,11,14,17-eicosapentaenoic[a]	EPA	20:5 5*c*,8*c*,11*c*,14*c*,17*c*	3
$CH_3(CH_2)_{20}COOH$	docosanoic	behenic	22:0	
$CH_3(CH_2)_7CH{=}CH(CH_2)_{11}COOH$	*Z*-13-docosenoic	erucic	22:1 13*c*	9
$CH_3(CH_2CH{=}CH)_6(CH_2)_2COOH$	*Z,Z,Z,Z,Z,Z*- 4,7,10,13,16,19-docosahexaenoic	DHA	22:6 4*c*,7*c*, 10*c*,13*c*,16*c*,19*c*	3
$CH_3(CH_2)_{22}COOH$	tetracosanoic	lignoceric	24:0	
$CH_3(CH_2)_7CH{=}CH(CH_2)_{13}COOH$	*Z*-15-tetracosenoic	nervonic	24:1 15*c*	9

[a] Icosa- replaced eicosa- in systematic nomenclature in 1975, but the latter is still widely used in the current literature.

The following sections describe classes of naturally occurring fatty acids, emphasising acids that are nutritionally and biologically important, are components of commodity oils and fats, or are oleochemical precursors. The structures of many fatty acids are contained in the dictionary section of this book. Up to date information on fatty acid occurrence in seed oils can be found online (Aitzetmuller et al., 2003) and this is the source of much of the data in Section 1.1.2. Further information on fatty acid structure is available online at http://www.lipidlibrary.co.uk/ and http://www.cyberlipid.org/. The structures of naturally occurring fatty acids are most easily rationalised by considering their biosynthesis; a few basic processes build and extend the chain and insert double bonds, producing the common families of fatty acids. We do not consider the details of these biochemical processes here (see Section 10.1), but the reader should be aware of the result of the various enzyme processes that build and modify fatty acids. Saturated fatty acids are built from two carbon units, initially derived from acetate, added to the carboxyl end of the molecule, usually until there are 18 carbons in the chain. Double bonds are introduced by desaturase enzymes at specific positions relative to the carboxyl group. Elongases further

TABLE 1.2 Systematic, trivial, and shorthand names and melting points of saturated fatty acids

Systematic Name	Trivial Name	Shorthand Name	Melting Point[a] (°C)
methanoic	formic	1:0	8.4
ethanoic	acetic	2:0	16.6
propanoic	propionic	3:0	–20.8
butanoic	butyric	4:0	–5.3
pentanoic	valeric	5:0	–34.5
hexanoic	caproic	6:0	–3.2
heptanoic	enanthic	7:0	–7.5
octanoic	caprylic	8:0	16.5
nonanoic	pelargonic	9:0	12.5
decanoic	capric	10:0	31.6
undecanoic		11:0	29.3
dodecanoic	lauric	12:0	44.8
tridecanoic		13:0	41.8
tetradecanoic	myristic	14:0	54.4
pentadecanoic		15:0	52.5
hexadecanoic	palmitic	16:0	62.9
heptadecanoic	margaric	17:0	61.3
octadecanoic	stearic	18:0	70.1
nonadecanoic		19:0	69.4
eicosanoic	arachidic	20:0	76.1
heneicosanoic		21:0	75.2
docosanoic	behenic	22:0	80.0
tricosanoic		23:0	79.6
tetracosanoic	lignoceric	24:0	84.2
pentacosanoic		25:0	83.5
hexacosanoic	cerotic	26:0	87.8
heptacosanoic	carboceric	27:0	87.6
octacosanoic	montanic	28:0	90.9
nonacosanoic		29:0	90.4
triacontanoic	melissic	30:0	93.6
hentriacontanoic		31:0	93.2
dotriacontanoic	lacceric	32:0	96.0

[a] Data from *The Lipid Handbook,* 2nd Edition (1994), Chapman & Hall, London. With permission.

extend the chain in two carbon units from the carboxyl end. These processes produce most of the fatty acids of commercial importance in commodity oils and fats, and which are considered to be of most value in food and nutrition.

A great diversity of fatty acid structures is produced by variations on the basic process. The start, particularly, of the chain elongation process may be derived from acids other than acetate, resulting in odd or branched chains. Enzymes closely related to the desaturases may introduce functional groups other than double bonds, but usually with similar positional patterns. The result is a great variety of fatty acid structures, often restricted to a few related plant genera in which the altered enzymes have evolved. Additional structural variety is introduced by subsequent modification of fatty acids, e.g., oxidation at or near the carboxyl or methyl end. The Euphorbiacae and Compositae (Asteracae) are particularly adept at producing many and varied fatty acid structures. Fatty acids may be modified further, producing other groups of natural products, such as polyacetylenes, ecosanoids, and oxylipins. The following sections illustrate these various structures, but are not exhaustive.

References

Adlof, R.O. and Gunstone, F.D. (2003) Common (non-systematic) names for fatty acids. http://www.aocs.org/member/division/analytic/fanames.asp

Aitzetmuller, K. et al. (2003) A new database for seed oil fatty acids — the database SOFA, *Eur. J. Lipid Sci. Technol.,* **105**, 92–103. http://www.bagkf.de/sofa/

Fahy, E. et al. (2005a) A comprehensive classification system for lipids. *J. Lipid Res.,* **46,** 839–861.

Fahy, E. et al. (2005b) A comprehensive classification system for lipids. *Eur. J. Lipid Sci. Technol.,* **107**, 337–364.

IUPAC-IUB (1976) Nomenclature of Lipids, World Wide Web version, prepared by G.P. Moss. http://www.chem.qmul.ac.uk/iupac/lipid/

1.1.2 Fatty acids

1.1.2.1 Saturated acids

Saturated fatty acids form a homologous series of monocarboxylic acids ($C_nH_{2n+1}COOH$). Table 1.2 lists the saturated acids from C_1 to C_{32} with their systematic and trivial names and melting points. Naturally occurring saturated acids are mainly of even chain length between C_4

and C_{24}. Fats rich in saturated acids are high melting and are characteristic of many tropical species. Odd chain acids are usually minor or trace components of plant and animal lipids, but some are more abundant in bacterial lipids.

Short chain acids, particularly butyric (4:0), are found mainly in ruminant milk fats. Medium chain fatty acids (8:0, 10:0, 12:0, and 14:0) occur together in coconut and palm kernel oils, both tropical commodity oils. In both of these oils, lauric acid (12:0) predominates (45 to 55%), with 14:0 next most abundant. A number of Lauracae and Myristacae species contain in excess of 80% of 12:0 or 14:0, respectively. *Cuphea*, a temperate genus, has species rich in individual medium chain acids, e.g., *C. pulcherrima* >90% 8:0, *C. koehneana* >90% 10:0, and *C. calophylla* ~85% 12:0. These include some of the highest levels of single fatty acids in seed oils.

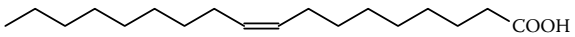
Palmitic acid (16:0) is the most abundant and widespread natural saturated acid, present in plants, animals, and microorganisms. Levels of 20 to 30% are common in animal lipids, 10 to 40% in seed oils. Palm oil is a rich commodity oil source and contains over 40% of palmitic acid. Stearic acid (18:0) is also ubiquitous, usually at low levels, but is abundant in cocoa butter (~34%) and some animal fats, e.g., lard (5 to 24%) and beef tallow (6 to 40%). A few tropical plant species contain 50 to 60+% of 18:0, e.g., *Shorea, Garcinia, Allanblackia*, and *Palaquium*. Arachidic acid (20:0) is 20 to 30% of the seed oils of some tropical Sapindaceae species, but is usually a minor component of plant and animal lipids. Groundnut oil is the only commodity oil with significant amounts (~1%).

Saturated acids are often most easily obtained by hydrogenation of more readily available unsaturated acids, e.g., docosanoic acid (22:0) could be obtained by hydrogenation of erucic acid (22:1). Chain shortening and chain extension reactions give access to odd or even chain lengths not readily found in natural sources. Saturated acids with 10 or more carbons are solids, and melting points increase with chain length (see Table 1.2). Melting points alternate between odd and even chain length, with odd chain lengths having a lower melting point than the preceding even chain acid. Polymorphism occurs, where one or more lower melting, metastable forms exist.

1.1.2.2 Monoenoic acids

Straight-chain, *cis*-monoenoic acids with an even number of carbons are common constituents of many lipids and commodity oils. *Trans*- monoenes are rare components of natural oils and fats (see Section 1.2.6). The *cis* (*Z*) double bond is usually inserted by a Δ9-desaturase enzyme into preformed saturated acids; this may be followed by two-carbon chain extension at the carboxyl end. Starting with 16:0, this results in n-7 monoenes, while desaturation of 18:0 leads to the n-9 family. Monoenes may also result from desaturation at the Δ4 or Δ5 positions since oils with unsaturation at these positions occur in a few plant genera.

The most common monoene is oleic acid (18:1 9*c*). Oleic acid (**1**) is found in most plant and animal lipids and is the major fatty acid in olive oil (70 to 75%) and several nut oils, e.g., macadamia, pistachio, pecan, almond, and hazelnut (filbert) contain 50 to over 70%. High oleic varieties of sunflower and safflower contain 75 to 80% oleic acid.



Oleic acid

(1)

Cis-vaccenic acid (18:1 11*c*, n-7) is common in bacterial lipids and a minor component of plant and animal lipids, co-occurring with the more abundant oleic acid. *Cis*-vaccenic is relatively abundant in sea buckthorn pulp, which is also rich in its n-7 biosynthetic precursor 16:1 9*c*. Petroselinic acid (18:1 6*c*) makes up over 50% of seed oil fatty acids of Umbelliferae species, such as carrot, parsley, and coriander, and is also found in the Araliaceae, Garryaceae, and Geraniaceae species. The biosynthesis of petroselinic acid involves a Δ4 desaturase acting on palmitic acid (16:0) followed by two carbon chain elongation (Cahoon et al., 1994).

Palmitoleic acid (16:1 9*c*, n-7) is a ubiquitous minor component in animal lipids; somewhat more abundant in fish oils. A few plant oils are richer sources, e.g., nuts such as macadamia (20 to 30%) and the pulp of sea buckthorn (25 to 40%). C_{20} monoenes (11*c* and 13*c*) are present in brassica seed oils and the 9c and 11c isomers are found in fish oils. 20:1 5c is >60% of meadowfoam (*Limnanthes alba*) seed oil fatty acids. Erucic acid (22:1 13*c*, n-9) is up to 50% of Cruciferae oils, e.g. rape, mustard, crambe and over 70% in some *Tropaeolum* species. Nervonic acid (24:1 15*c*, n-9) occurs at 15 to 20% in *Lunaria annua* seed oil, along with higher levels of erucic acid.

Some monoenes are used as or have potential use as oleochemicals. Erucic acid, as the amide, is used as an antislip agent for polythene film. 20:1 5*c* from meadowfoam oil can be used to prepare estolide lubricants and other novel materials. ω-Olefins, such as 10-undecenoic acid available from pyrolysis of castor oil, are useful oleochemical intermediates.

Cis-monoenes with 18 or less carbons are liquids at room temperature or low-melting solids; higher homologues are low-melting solids. *Trans*-monoenes are higher melting, closer to the corresponding saturated acids. Double bond position also influences the melting point; both *cis*- and *trans*-C_{18} monoenes are higher melting when the double bond is at even positions than at odd positions; a pattern most distinct for double bonds between C_4 and C_{14}. The solid acids may exist as a number of polymorphs, with different melting points, resulting from subtly different packing in the crystal (Table 1.3).

1.1.2.3 Methylene-interrupted polyunsaturated acids

Most unsaturated fatty acids with two or more double bonds show a characteristic methylene-interrupted pattern

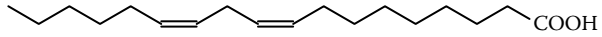
TABLE 1.3 Trivial names and melting points of some monoene fatty acids

Fatty Acid	Trivial Name	Melting Point[a] (°C)
16:1 9*c* (n-7)	palmitoleic	0.5
16:1 9*t* (n-7)	palmitelaidic	32
18:1 9*c* (n-9)	oleic	16.2, (13.3)
18:1 9*t* (n-9)	elaidic	45.5
18:1 6*c*	petroselinic	31, (29)
18:1 11*c* (n-7)	*cis*-vaccenic	15.5
18:1 11*t* (n-7)	vaccenic	44.1
20:1 5*c* (n-16)		27
20:1 11*c* (n-9)	gondoic	25
22:1 13*c* (n-9)	erucic	33.5 (–52, –7, 2, 14)
24:1 15*c* (n-9)	nervonic	45, 41

[a] Data from *The Lipid Handbook,* 2nd Edition (1994), Chapman & Hall, London. With permission. Also references in Section 1.1.3. Polymorph melting points in parentheses.

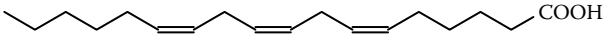
of unsaturation, with one CH_2 between *cis* double bonds. This pattern results from the operation of a few specific desaturases and chain-elongation enzymes. Plants generally insert double bonds at the Δ9, Δ12, and Δ15 positions in C_{18} fatty acids, giving n-9, n-6, and n-3 compounds, respectively. Animals can also insert double bonds at the Δ9 position, but not at Δ12 or Δ15; instead, further double bonds are introduced between the carboxyl group and the Δ9 position by Δ5 and Δ6 desaturase enzymes and the chain can then be extended in two carbon units at the carboxyl end of the molecule. The resulting n-6 and n-3 polyenes are shown in Figure 1.2. The step leading to DHA appears to be the result of a Δ4 desaturase, but is usually the net result of two elongations, a Δ6 desaturase and subsequent two-carbon chain shortening. Leonard et al. (2004) have reviewed the biosynthesis of long chain polyenes. Along with a few saturates (mainly 16:0 and 18:0, but also 10:0 to 14:0) and oleic acid, the n-6 and n-3 polyenes make up the fatty acids found in most plants, animals, and commodity oils and fats.

Linoleic acid (18:2 n-6, **2**) is present in most plant oils and is abundant (>50%) in corn, sunflower, and soybean oils, and exceeds 70% in safflower oil. γ-linolenic acid (18:3 n-6, **3**) is usually a minor component of animal lipids, but is relatively abundant in some plant oils, e.g., evening primrose (~10%), borage (~20%), blackcurrant (~15%), and echium (~25%). Other n-6 acids, dihomo-γ-linolenic acid (20:3 n-6) and arachidonic acid (20:4 n-6) are present in animal tissues, but do not usually accumulate at significant levels in storage fats. These two C_{20} acids are the precursors of the PG_1 and PG_2 prostaglandin families, respectively. Some fungi, e.g., *Mortierella* species produce up to 50% arachidonic acid in storage lipids and are a commercial source of this acid (Ratledge, 2004).



Linoleic acid

(2)



γ-linolenic acid

(3)

α-linolenic acid (18:3 n-3, **4**) is ubiquitous in plant leaf lipids and is present in several commodity seed oils: 8 to 10% in soybean and canola, >50% in linseed oil, and 65 to 75% of perilla oil. The seed oils of many *Labiatae* species are >50% α-linolenic acid. In plant leaves, chloroplast lipids contain up to 50% α-linolenic acid accompanied, in some species, by its C_{16} homologue, 16:3 7*c*, 10*c*,13*c* (Mongrand et al., 1998). Stearidonic acid (18:4 n-3, **5**) is a minor component of animal lipids and fish oils and is found in some seed oils, e.g., blackcurrant (up to 5%) and echium (~7%). The n-3 long-chain, polyunsaturated fatty

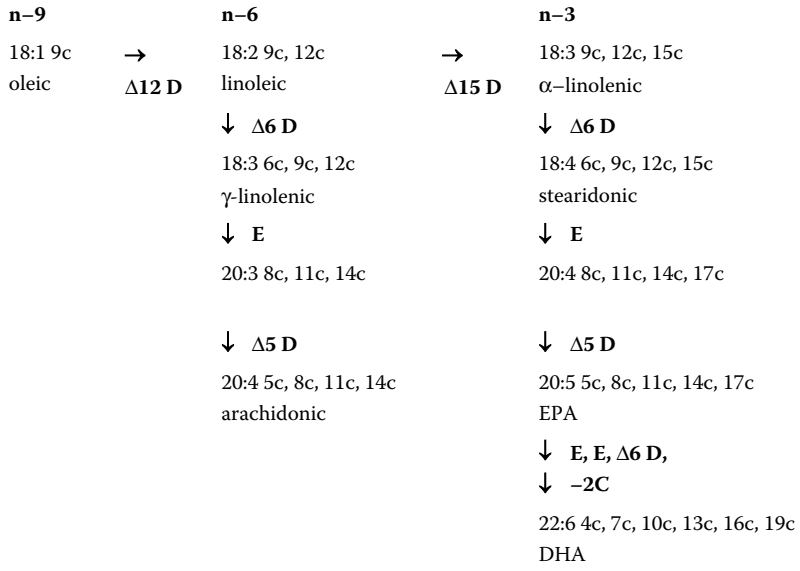
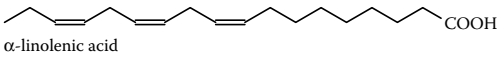


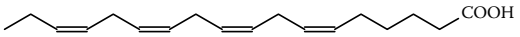
FIGURE 1.2 Biosynthesis of n-6 and n-3 polyenes (D = desaturase, E = elongase, -2C = two-carbon chain shortening).

acids (LC-PUFA) 20:5 (EPA, **6**) and 22:6 (DHA, **7**) are important nutritionally and are mainly obtained from oily fish and fish oils where they are present at levels from 5 to 20%. EPA is the precursor of the PG_3 prostaglandin series. Attempts are being made to produce EPA and DHA in plant lipids by the incorporation of appropriate enzymes because of the desire to have new sources of these important acids. Two types of microorganisms, a dinoflagellate *Crypthecodinium cohnii* and marine protist *Schizochytrium* species, are commercial single-cell oil sources of DHA (Ratledge, 2004).



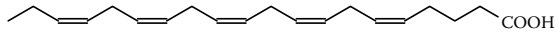
α-linolenic acid

(4)



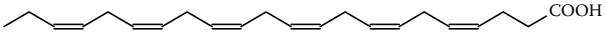
Stearidonic acid

(5)



EPA

(6)



DHA

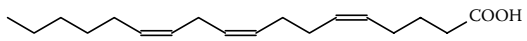
(7)

While the n-3 and n-6 polyenes are the most widely occurring and of prime biological and nutritional interest, a large number of other methylene-interrupted polyenes are known, produced by the same desaturation and elongation steps, but starting with fatty acids of different chain length and initial unsaturation. For example, animals deprived of linoleic or linolenic acids can use oleic acid as substrate for the Δ6 desaturase and subsequent steps, resulting in an n-9 polyene series. The accumulation of 20:3 n-9 (Mead's acid) in animals is considered to be a symptom of essential fatty acid (i.e., linoleic acid) deficiency.

The presence of two or more *cis* double bonds results in a large lowering of the melting point compared to saturates of the same chain length and these polyenes are all liquid at room temperature. Linoleic acid melts at −5°C.

1.1.2.4 *Bis*- and polymethylene-interrupted acids

Fatty acids with *bis*- or polymethylene-interrupted double bonds, or a mixture of methylene and polymethylene separated unsaturation, occur in some plant species and marine organisms. Often these have a double bond inserted at the Δ5 position in addition to one or more double bonds in more usual positions. *Bis*-methylene-interrupted acids with a Δ5*c* double bond are common in gymnosperms (conifers), a typical example being pinolenic acid (18:3 5*c*,9*c*,12*c*) (**8**), occurring at levels of 25 to 30% in a number of pine and larch species (Wolff et al. 2001). Among angiosperms, *Limnanthes alba* (meadowfoam) seed oil contains the polymethylene-interrupted 22:2 5*c*,13*c* (~20%) and other Δ5 acids. *Bis*-methylene-interrupted acids with a Δ5*t* double bond occur in *Thalictrum* species (see Section 1.2.6).



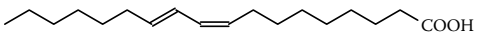
Pinolenic acid

(8)

Sponges and some other marine invertebrates contain a wide range of fatty acids with 5*c*,9*c* double bonds, with chain lengths (both odd and even) ranging from C_{16} to C_{34}, known as demospongic acids. Additional double bonds are usually n-7 or n-9 and methyl branching may also be present (Dembitsky et al., 2003).

1.1.2.5 Conjugated acids

Fatty acids with two or more conjugated double bonds are found in some plants and animals. Ruminant fats contain small amounts (~1%) of "conjugated linoleic acid" (CLA), resulting from bio-hydrogenation of linoleic and α-linolenic acids in the rumen, which gives mainly the 18:2 9*c*,11*t* isomer (rumenic acid, **9**). The only reported long chain, conjugated diene from a plant is 18:2 10*t*,12*t* (~10%), which occurs in *Chilopsis linearis* along with the more abundant conjugated triene 18:3 9*t*,11*t*,13*c*. Estolides in stillingia oil (*Sapium sebiferum*) and *Sebastiana* species contain 10:2 2*t*,4*c* linked to a short chain allenic hydroxy acid (Spitzer et al., 1997; Figure 1.3). Conjugated dienes (and higher polyenes) are prepared chemically from methylene-interrupted fatty acids by alkaline isomerisation. Under controlled conditions, linoleic acid produces a mixture containing only the 9*c*11*t* and 10*t*12*c* CLA isomers (Sæbø, 2001). These isomers have potential uses in modifying body composition and as anticancer agents.



Rumenic acid

(9)

Conjugated trienes and tetraenes are found in several plant species. They are produced biologically from methylene-interrupted polyenes by a conjugase enzyme similar to Δ12 desaturase, which shifts an existing double bond into conjugation with a new double bond (Dyer et al., 2002). Table 1.4 gives the structure, common name, source, and melting point of the known conjugated trienes and tetraenes from plants. Conjugated trienes and tetraenes containing *cis* double bonds readily isomerise to the all *trans* form on heating or on exposure to light. Tung oil, containing >60% α-eleostearic acid (**10**), oxidises and

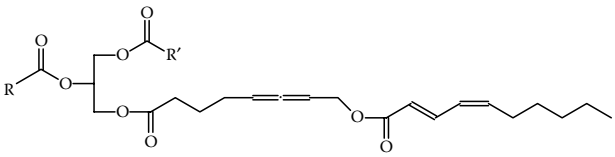
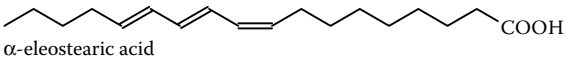


FIGURE 1.3 Estolide from stilingia oil. R, R' 16:0, 18:0, 18:1, 18:2, 18:3.

polymerises readily and is used as a drying agent in paints and varnishes. Along with CLA, there has been recent interest in the biological and nutritional properties of conjugated polyenes.



α-eleostearic acid

(10)

1.1.2.6 *Trans* acids

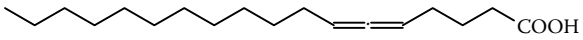
Monoenes and methylene-interrupted polyenes are predominantly *cis*. A few *trans* monoenes and dienes with typical double bond positions are known, e.g., 18:1 9*t* in *Butyrospermum parkii* (12.5%) and *Dolichos lablab* (15%), co-occurring with 18:1 9*c*, and 18:2 9*t*12*t*, (~15%) in *Chilopsis linearis*, associated with conjugated acids. *Thalictrum* (and some other Ranunculaceae species) contain several acids with a Δ5*t* bond, 16:1 5*t* (~2%), 18:1 5*t* (~20%), 18:2 5*t*,9*c* (~6%), and 18:3 *5t*,9*c*,12*c* (~45%). A similar pattern with Δ3*t* unsaturation is seen in some *Aster* species. 16:1 3*t* occurs widely in leaves associated with chloroplast lipids. Vaccenic acid, 18:1 11*t,* is the most abundant *trans* monoene in ruminant lipids, which contain a complex mixture of both *cis* and *trans* positional isomers resulting from biohydrogenation of linoleic and linolenic acids. Conjugated acids usually contain one or more *trans* double bonds (see Section 1.2.5).

Trans isomers, mainly monoenes, are produced during catalytic partial hydrogenation, and can be present in substantial amounts in hardened fats, generally as a mixture of positional isomers. Heat treatment during deodorisation of commodity oils may result in low levels of *trans* isomers, particularly of polyenes. The undesirable nutritional properties of *trans* acids have led to alternative ways of producing hardened fats, such as interesterification or blending with fully saturated fats, and to the use of milder deodorisation procedures.

1.1.2.7 Acetylenic and allenic acids

Fatty acids with acetylenic and allenic unsaturation are rare. The two types of unsaturation are isomeric and can be interconverted. In the allenic function, the double bonds are rigidly held at right angles and introduce a twist in the molecule, resulting in optical activity when they are asymmetrically substituted.

The estolide oil in stillingia oil contains the allenic hydroxy acid 8-hydroxy-5,6-octadienoic acid (Spitzer et al., 1997; Figure 1.3). The (*R,E*) form of 2,4,5-tetradecatrienoic acid is an insect sex pheromone. Fatty acids with a 5,6 allene are found in the seed oils of a few Labiatae species: laballenic acid (18:2 5,6; **11**) is up to 25% of *Phlomis tuberosa* and some *Leucas* species; lamenallenic acid (18:3 5,6,16*t*) is up to 10% in *Lamium purpureum*.



Laballenic acid

(11)

Fatty acids containing an acetylenic group are tariric acid (18:1 6*a*, **12**), up to 85% of some *Picramnia* species and crepenynic acid (18:2 9*c*,12*a*, **13**) 50 to 75% of some

TABLE 1.4 Common name, source, and melting point of some conjugated fatty acids

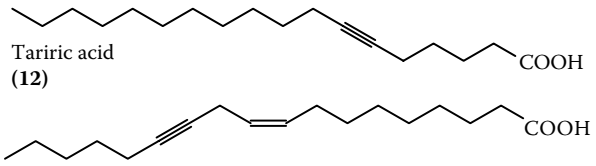
Fatty Acid	Common Name	Source	Melting Point[a] (°C)
10:2 2t,4c		*Sapium sebiferum* (stillingia oil) (~5 to 10%)	
18:2 8*t*,10*t*	CLA		56
18:2 9*t*,11*t*	CLA		54
18:2 9*c*,11*c*	CLA		43
18:2 9*c*,11*t*	CLA	ruminant fats	20
18:2 10*t*,12*t*	CLA	*Chilopsis linearis* (~10%)	56
18:2 10*t*,12*c*	CLA		23
18:2 10*c*,12*c*	CLA		39
18:3 8*t*,10*t*,12*t*	β-calendic	*Calendula officinalis* (tr)	78
18:3 8*t*,10*t*,12*c*	calendic	*Calendula officinalis* (60%)	40
18:3 8*c*,10*t*,12*c*	jacaric	*Jacaranda mimosifolia* (36%)	44
18:3 9*t*,11*t*,13*t*	β-eleostearic	*Aleurites fordii* (11%)	72
18:3 9*c*,11*t*,13*t*[b]	α-eleostearic	*Aleurites fordii* (Tung oil), *Parinarium* spp., *Momordica* sp. (>60%)	49
18:3 9*t*,11*t*,13*c*	catalpic	*Catalpa* spp. (~40%)	32
18:3 9*c*,11*c*,13*t*	–	–	62
18:3 9*c*,11*t*,13*c*	punicic	*Punica granatum* (~70%), *Momordica balsamina* (~60%)	45
18:4 9*c*,11*t*,13*t*,15*c*[c]	α-parinaric	*Parinarium laurinum* (>50%), *Impatiens* spp. (>20%)	86
18:4 9*t*,11*t*,13*t*,15*t*	β-parinaric	–	96

[a] Data from *The Lipid Handbook,* 2nd Edition (1994), Chapman & Hall, London. With permission.

[b] Occurs also as the 18-hydroxy (kamlolenic acid, *Mallotus philippinensis* (70%)) and 4-oxo (licanic acid, *Licania rigida* (80%)) derivatives.

[c] Occurs also as the 4-oxo derivative (*Chrysobalanus icaco* (18%)).

Crepis species. In *C. alpina,* the acetylenic bond is introduced by a Δ12-desaturase-like enzyme (Lee et al., 1998). Crepenynic acid is the starting point for the biosynthesis of a large number of fatty acid-derived acetylenic and polyacetylenic secondary natural products (e.g., matricaria ester). Stearolic acid (18:1 9a), the acetylenic analogue of oleic acid (from which it is easily prepared), is not often found in nature, other than as a minor component. However, it is more abundant in some *Pyrularia* species, *P. edulis* containing over 50%.



Tariric acid
(12)

Crepenynic acid
(13)

1.1.2.8 Branched chain acids

Straight chain fatty acids are the norm, but a wide variety of branched chain structures are known, mainly from bacterial and some animal sources. These acids are usually saturated or monoenes and the alkyl branch is a methyl group. Acids with a methyl group on the n-2 or n-3 carbon (*iso* and *anteiso*, respectively; Figure 1.4) are common in bacteria; their occurrence and distribution being strong taxonomic indicators. The biosynthesis of these acids involves the normal two-carbon chain extension, but instead of starting with a two-carbon acetate-derived unit, they start with 2-methyl propionic acid (from valine) or 2-methyl butanoic acid (from leucine), respectively. The resulting *iso* and *anteiso* acids, thus, have an even and odd total number of carbons, but α-oxidation may subsequently shorten the chain resulting in both odd and even carbon *iso* and *anteiso* acids. The shorthand nomenclature for these acids can be confusing, as the total number of carbons is shown, while the systematic name uses the number of carbons in the longest alkyl chain. For example, 15-methyl hexadecanoic acid is *iso*-17:0.

Iso and *anteiso* acids found in animal fats, particularly ruminant fats, are mostly derived from bacteria in the diet or digestive system. However, some specific acids are of animal origin: 18-methyleicosanoic acid is the major thioester-bound fatty acid on the surface of wool and mammalian hair fibres, producing a continuous hydrophobic layer (Jones and Rivett, 1997). *Iso* and *anteiso* acids are rarely found in plant oils, apart from 14-methylhexadecanoic acid, which is found as a taxonomically useful minor component (~1%) in the Pinacae family. These acids

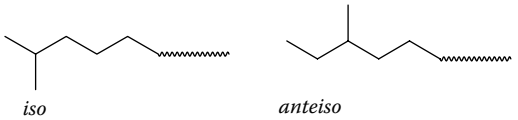
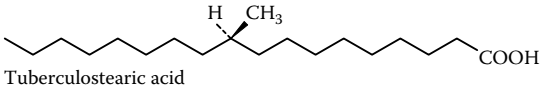


FIGURE 1.4 *Iso* and *anteiso* branched-chain structures.

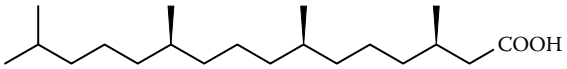
are, however, abundant in the surface waxes of plant leaves.

Fatty acids with a mid-chain methyl branch are characteristic of some bacteria. For example, 10-*R*-methyloctadecanoic acid (tuberculostearic acid) (**14**) is the major normal chain length fatty acid in *Mycobacterium tuberculosis,* the causative agent of tuberculosis, and is found in a number of other actinomycetes. The biosynthesis involves methylation of oleic acid, the methyl carbon being derived from the C-1 pool. C_{16} to C_{24} mid-chain methyl branched acids are also found in *Mycobacterium* species.

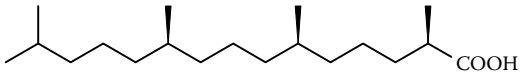


Tuberculostearic acid
(14)

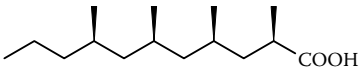
Polymethyl fatty acids include those of isoprenoid origin, derived from partial metabolism of the phytyl chain from dietary chlorophyll. Phytanic (**15**) and pristanic acids (**16**) are the most common examples and are minor components of fish oils. A different pattern is seen in fatty acids from bird uropygial glands where the methyl groups are found on alternate, usually even, carbons, with two to four methyl groups present, e.g., (**17**) found in the preen gland wax of the graylag goose. Dimycocerosate esters, found in mycobacteria, contain a range of polyketide-derived polymethyl fatty acids. These also have the methyls on alternate even carbons (Onwueme et al., 2005).



Phytanic acid
(15)



Pristanic acid
(16)



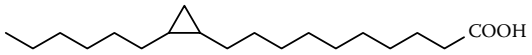
(17)

1.1.2.9 Cyclic fatty acids

Cyclic fatty acids, with a carbon ring along or at the end of the alkyl chain, occur naturally in some bacteria and plants. In addition, a variety of carbocyclic structures are formed from methylene-interrupted polyenes during heating, for example, during deep frying. The sources, synthesis, and biological properties of cyclic fatty acids have been reviewed by Sebedio and Grandgirard (1989).

Fatty acids with a mid-chain cyclopropane group are found mainly in bacteria, with *cis*-9,10-methylenehexadecanoic (9,10 cpa 17:0); *cis*-9,10-methyleneoctadecanoic acid (9,10 cpa 19:0; dihydrosterculic acid); and *cis*-10,11-methyleneoctadecanoic acid (10,11 cpa 19:0; lactobacillic acid, **18**) most common. They are found in diverse bacterial species, both aerobic and anaerobic, and in both Gram-negative

and Gram-positive species. Depending on culture conditions, they may be up to 35% of the membrane lipids.



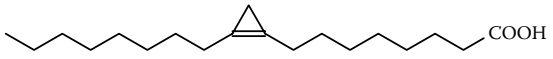
Lactobacillic acid

(18)

Biosynthesis of the cyclopropane ring involves addition of a methylene group, derived from S-adenosylmethione (the "C_1 pool"), to an existing double bond, for example, lactobacillic acid is derived from *cis*-vaccenic acid, the most abundant monoene in many bacteria. The cyclopropane acids that have been found in protozoa, slime moulds, and invertebrates are most likely derived from bacteria in their diet. The distribution and biosynthesis of cyclopropane acids in bacteria has been reviewed by Grogan and Cronan (1997).

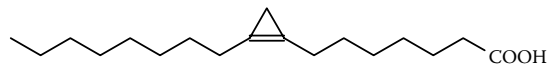
Cyclopropane acids are often found at low levels (~1%) in plant oils containing cyclopropene acids (see below). *Litchi chinensis*, however, contains ~40% dihydrosterculic acid (9,10 cpa 19:0) along with small amounts of shorter chain homologues.

Cyclopropene acids are found in plant oils of the Malvalaceae, Sterculiaceae, Bombacaea, Tiliaceae, and Sapicidacaea families. These are mainly sterculic acid (9,10-methyleneoctadec-9-enoic acid; 9,10 cpe 19:1; **19**) and malvalic acid (8,9-methyleneheptadec-8-enoic acid; 8,9 cpe 18:0; **20**). Sterculic acid is usually the more abundant (>50% in *Sterculia foetida* oil) accompanied by smaller amounts of malvalic acid. 2-hydroxysterculic acid may also occur in these oils, probably an intermediate in the biosynthesis of malvalic acid by α-oxidation of sterculic acid. 9,10-methyleneoctadec-9-en-17-ynoate (sterculynic acid) occurs in *Sterculia alata* (~8%). The biosynthesis of the cyclopropene ring is not fully understood, but is thought to proceed from oleic acid to the cyclopropane, produced by the same mechanism as in bacteria, followed by further desaturation. Long chain cyclopropane and cyclopropylidene fatty acids have been found in sponges, for example, **(21)** from the *Amphimedon* species (Nemoto et al., 1997). Their biosynthesis is unknown.



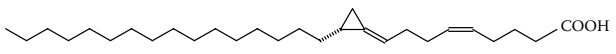
Sterculic acid

(19)



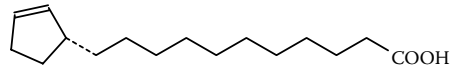
Malvalic acid

(20)



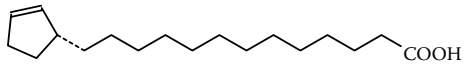
(21)

Fatty acids with terminal rings are thought to be produced by incorporating a cyclic acid rather than acetate at the start of the chain, although the biosynthetic origin of the cyclic acid has not always been unequivocally established. Up to 80% of the seed oils of *Hydnocarpus* species and other genera of the Flacourtiaceae are terminal cyclopentenyl acids of various chain lengths. The most abundant is usually the C_{16} hydnocarpic acid (**22**), but in *Oncoba* and *Caloncoba* species the C_{18} chaulmoogric acid (**23**) predominates (~70%). Gorlic acid (**24**), C_{18} with a Δ6 double bond, is usually 10 to 20% of these oils. Related homologues from C_6 to C_{20} are often found at low levels. *Arum maculatum* seed oil contains ~20% of 13-phenyltridecanoic acid (**25**)



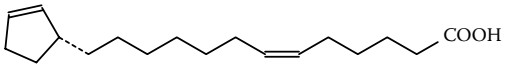
Hydnocarpic acid

(22)



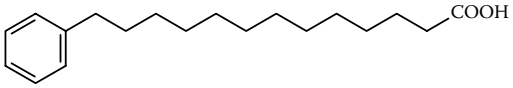
Chaulmoorgic acid

(23)



Gorlic acid

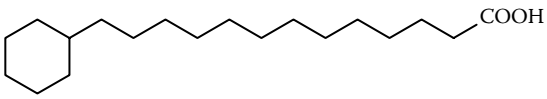
(24)



13-phenyltridecanoic acid

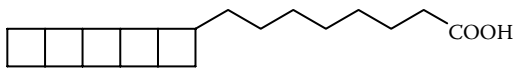
(25)

Bacteria isolated from the extreme environment of hot springs produce fatty acids with a terminal cyclohexyl group. In strains of the acidophilic and thermophilic *Bacillus acidocardarius*, 11-cyclohexylundecanoic acid and 13-cyclohexyltridecanoic acid (**26**) account for 70 to over 90% of the fatty acids in the bacteria (Oshima and Ariga, 1975). One of the most unusual fatty acid structures reported to date is a terminal concatenated cyclobutane or ladderane, containing up to five *cis*-fused four membered rings (e.g. **27**). These occur as glycerol and methyl esters in the unusually dense membranes of anammox bacteria (Damste et al., 2002).



13-cyclohexyltridecanoic acid

(26)



(27)

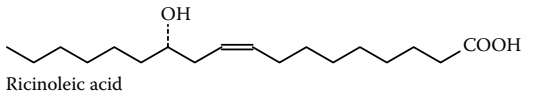
1.1.2.10 Fatty acids with oxygen-containing functional groups

Most fatty acids contain only double bonds, but a number of fatty acids and their metabolites have oxygen-containing functional groups, most commonly a hydroxyl or epoxide.

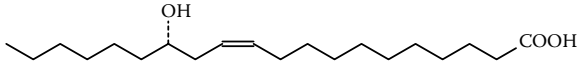
Some of these are introduced by enzyme-mediated oxidation of methylene-interrupted fatty acids, e.g., by lipoxygenase or the initial stages of fatty acid catabolism, the latter giving hydroxyl groups near the carboxyl or methyl end of the chain. Autoxidation, occurring in the absence of enzymes also gives oxygen-containing products (hydroxy, keto, epoxy, etc.) with less positional specificity.

In a few plant oils, hydroxy and epoxy groups are introduced in mid-chain positions by enzymes with the same positional specificity as desaturases. Castor oil, rich in ricinoleic acid (12-OH 18:1 9c), is the only commodity oil containing a fatty acid with a functional group other than double bonds. Oils containing vernolic acid (an epoxy acid) have been investigated as oleochemical precursors.

Ricinoleic acid (*R*-12-hydroxy-9-*cis*-octadecenoic acid; 12-OH 18:1 9*c*; **28**) is 80 to 90% of castor oil (from *Ricinus communis*). It occurs at similar levels in *Hiptage* species and is found in a number of other species. In *Azima tetracantha*, *Argyreia cuneata*, and *Anogeissus latifolia,* it occurs at levels of 10 to 25% along with lower amounts of the cyclopropene, which contain malvalic and sterculic acids (see Section 1.2.9). The sclerotia of the ergot fungus (*Claviceps purpurea*) contain up to 50% ricinoleic acid (see below). Isoricinoleic acid (*R*-9-hydroxy-12-*cis*-octadecenoic acid; 9-OH 18:1 12*c*) is over 70% of the *Wrightia* species. Lesquerolic acid (*R*-14-hydroxy-11-*cis*-eicosenoic acid; **29**), the C_{20} homologue of ricinoleic acid, occurs in *Lesquerella* species (50 to 70%). It is produced from ricinoleic acid by an elongase specific for hydroxy acids (Moon et al., 2001). Related acids found in *Lesquerella* species include densipolic acid (12-OH 18:2 9*c*, 15*c*) and auricolic acid (14-OH 20:2 11*c*, 17*c*). Hydroxy (and keto) acids are also found with conjugated double bonds (see Table 1.4). These include kamlolenic acid (18-OH 18:3 9*c*, 11*t*, 13*t*) in *Mallotus philippinensis* (70%) and coriolic acid (13-OH 18:2 9*t*,11*c*) in *Coriaria* species (~70%).



Ricinoleic acid
(28)



Lesquerolic acid
(29)

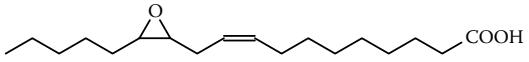
A hydroxyl group along the acyl chain can be esterified to other fatty acids, forming an estolide. In castor oil, ricinoleic acid is present only in simple triacylglycerols, but in the ergot fungus *Claviceps purpurea*, ricinoleic acid is extensively esterified with both nonhydroxy acids and other molecules of ricinoleic acid in polyestolide groups (Batrakov and Tolkachev, 1997). Seed oils of *Lesquerella* and related species rich in lesquerolic acid contain estolides (Hayes et al., 1995).

Cutin, a cross-linked polyester constituent of plant cuticle, contains a number of C_{16} and C_{18} mono, di, and trihydroxy fatty acids. The C_{16} acids, derived from palmitic acid contain a terminal hydroxyl group and a mid-chain hydroxyl between C_7 and C_{10}. The predominant C_{18} acids, derived from oleic acid, are 18-hydroxyoleic, 9,10,18-trihydroxystearic and 9,10-epoxy-18-hydroxystearic acids. The primary hydroxyls are mainly ester linked, while the mid-chain hydroxyls are only partially esterified. Polyhydroxy acids are not usually found in seed oils; however, 9,10,18-trihydroxy-12-*cis*-octadecenoic acid occurs as ~14% of *Chamaepeuce afra* oil.

2-hydroxy or α-hydroxy acids occur in sphingolipids, skin lipids, wool wax, bacterial cell wall lipids, and in a few seed oils. In some *Thymus* species 2-hydroxylinolenic occurs up to ~13%, along with linolenic acid and its C_{17} homologue (17:3 8*c*,11*c*,14*c*). The hydroxy acid is probably an intermediate in the biosynthesis of the C_{17} acid (see also hydroxysterculic acid, Section 1.2.9). *Salvia nilotica* oil contains α-hydroxy oleic, linoleic, and linolenic acids along with traces of C_{17} acids.

3-hydroxy or β-hydroxy fatty acids are found in bacterial lipids, both medium to normal chain-length saturates and in mycolic acids. Mycolic acids are very long chain compounds, typically C_{60} to C_{90}, branched at C_2, with unsaturation or cyclopropane groups along the long chain in addition to the 3-hydroxy group.

Vernolic acid (12-epoxy-9-*cis*-octadecenoic acid, **30**) is the most widespread epoxy acid in plant oils occurring in a number of Compositae, Malvaceae and Euphorbiaceae species. It makes up 60 to 80% of *Vernonia* oils and is over 90% of *Bernardia pulchella* oil. (+)-vernolic acid with the 12*S*,13*R* configuration is the most usual form, but the other optical isomer (–)-vernolic acid, has been isolated from some seed oils of the Malvaceae. In *Crepis palaestina* and *Vernonia galamensis,* the epoxide group is introduced by a Δ12-desaturase-like enzyme (Lee et al., 1998). However, in *Euphorbia lagascae,* the epoxygenase is a cytochrome P450 acting on linoleic acid (Cahoon et al., 2002).



Vernolic acid
(30)

Other epoxy acids include coronoric acid (9,10-epoxy-12-*cis*-octadecenoic acid), which occurs in a number of mainly Compositae species and is ~15% of *Chrysanthemum coronarium* oil. It is also found in sunflower and other oils after prolonged storage of the seeds. 9,10-epoxyoctadecanoic acid is found at low levels in *Tragopogon porrifolius* oil, and alchornoic acid (14,15-epoxy-11-*cis*-eicosanoic acid), the C_{20} homologue of vernolic acid, occurs in *Alchornea cordifolia* (~50%).

A number of oxygen-containing fatty acid derivatives are produced from methylene-interrupted fatty acids following

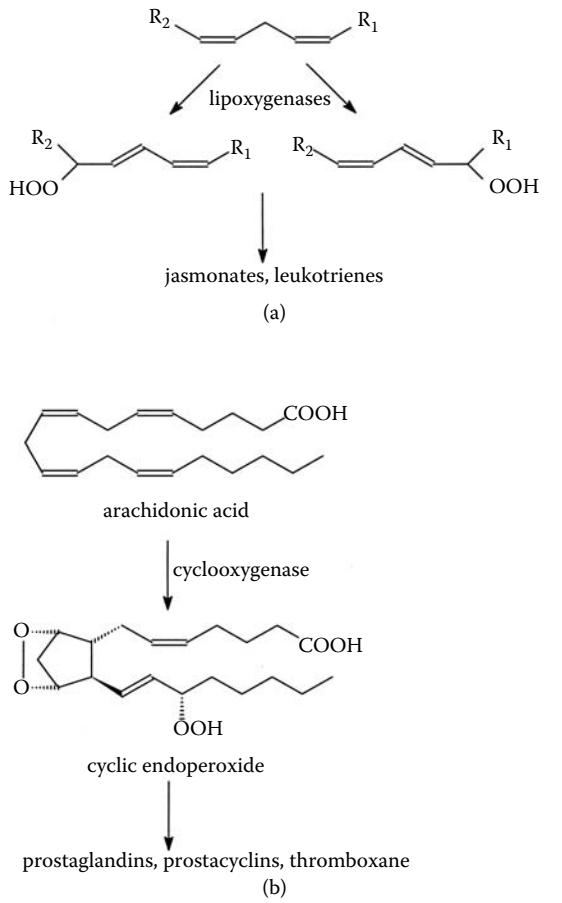


FIGURE 1.5 Formation of hydroperoxides and cyclic endoperoxides catalysed by (a) lipoxygenase and (b) cyclooxygenase enzymes.

the formation of a hydroperoxide or cyclic endoperoxide catalysed by lipoxygenase and cyclooxygenase enzymes, respectively (Figure 1.5). Subsequent cyclisation and modification leads to physiologically active products, such as eicosanoids (in mammals) and jasmonates and divinyl ether fatty acids (in plants), and also to furanoid fatty acids. Although the enzyme-catalysed, oxygen addition is stereo and regiospecific, the range of starting acids and subsequent modifications results in many different products; only a few representative structures are shown here.

Eicosanoids are biologically active C_{20} fatty acid metabolites acting as short-lived hormones or mediators, and include prostaglandins, thromboxanes, and leukotrienes. The PG_1, PG_2, and PG_3 families of prostaglandins are derived from dihomo-g-linolenic acid (20:3 n-6), arachidonic acid (20:4 n-6), and eicosapentaenoic acid (EPA, 20:5 n-3), respectively, via their cyclic endoperoxides (Figure 1.6). Christie (2005) has recently reviewed ecosanoid structure and function. Among other functions, prostaglandins are involved in the inflammatory response, platelet aggregation, vasodilation, and smooth muscle function.

Jasmonates are produced in plants following lipoxygenase catalysed conversion of 16:3 n-3, 18:2 n-6, and

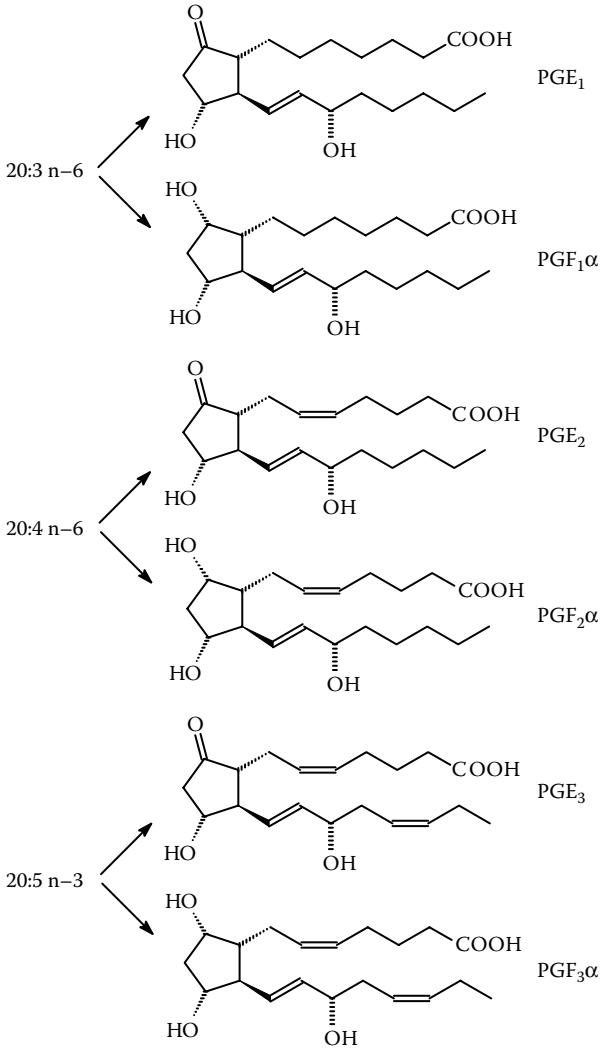
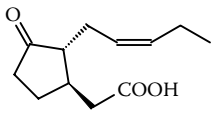


FIGURE 1.6 The PG_1, PG_2, and PG_3 families of prostaglandins.

18:3 n-3 to conjugated hydroperoxides, which are then converted to a range of metabolites. The most widely studied is (–)- jasmonic acid (**31**), which is derived from 13-hydroperoxylinolenic acid. The cyclised product is chain shortened to C_{12} by β-oxidation. Jasmonates have hormone properties, regulating plant growth and development and are involved in leaf senescence and in defence against pathogens and in wound signalling (Farmer et al., 2003).

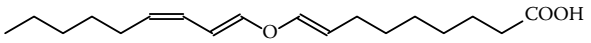


Jasmonic acid

(31)

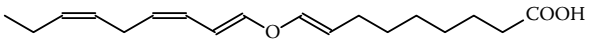
Divinyl ether synthase in plant leaves and roots converts hydroperoxides generated by lipoxygenase to divinyl ethers. In the potato, the 9-hydroperoxides of linoleic and linolenic acids lead to colneleic (**32**) and colnelenic acids

(**33**), respectively. Structurally similar compounds are derived from the 13-hydroperoxides in *Ranunculus* leaves, e.g., etherolenic acid (**34**) (Hamberg, 1998). These compounds are thought to be plant defence compounds protecting against pathogen attacks.



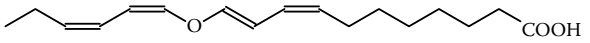
Colneleic acid

(32)



Colnelenic acid

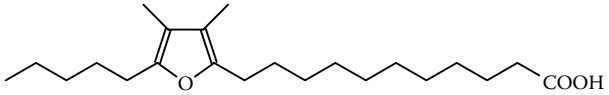
(33)



Etherolenic acid

(34)

Hydroperoxides derived from linoleic and linolenic acids by reaction with lipoxygenase are also the origin of a family of furanoid fatty acids. The most abundant member (F_6, **35**) is a C_{20} furanoid acid with two methyl groups on the furan ring. The ring methyls are derived from the C_1 pool. Furanoid fatty acids were initially isolated from fish and fish oils, but are now thought to originate in photosynthetic organisms eaten by fish, and are ubiquitous at trace levels in most plant-derived oils and fats (Gorst-Allman et al., 1988). No direct physiological role has been found for these compounds. Spiteller (2005) has reviewed the occurrence, biosynthesis, and reactions of furan fatty acids and suggested that they contribute to the cardioprotective effects of a fish-rich diet.



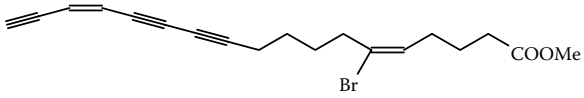
Furanoid acid - F_6

(35)

1.1.2.11 Other fatty acid structures

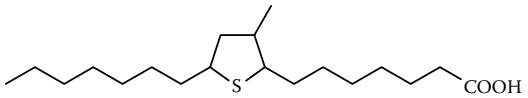
Fatty acids with other functional groups occur rarely; a few examples of naturally occurring acids containing halogens, sulfur, and nitro groups are listed below.

Natural halogen-containing fatty acids have been reviewed recently by Dembitsky and Srebnik (2002). These are often of marine origin, reflecting the availability of chlorine and bromine in seawater, or are produced in environments where there are anthropogenic sources of chlorine, e.g., during bleaching or in chlorinated organic compounds. Many of the chlorine-containing compounds result from addition to a double bond. A mixture of six isomeric chlorohydrins of palmitic and stearic acid occurs in the jelly fish *Auritia aurita* (9-chloro-10-hydroxy- and 10-chloro-9-hydroxy-hexadecanoic acid, 9-chloro-10-hydroxy-, 10-chloro-9-hydroxy-, 11-chloro-12-hydroxy-, and 12-chloro-11-hydroxy-octadecanoic acids). 9,10-dichloro-octadecanoic acid is found in the European eel *Anguilla anguilla*. A number of bromine-containing polyacetylenic methyl esters are found in some lichen species, e.g., (**36**) from the *Parmelia* species. A number of toxic ω-fluoro fatty acids have been isolated from the South African plant *Dichapetalum toxicarium*. The origin of the fluorine is fluoroacetic acid, which can accumulate in the leaves of *Dichapetalum* species. The most abundant is 18-fluoro-oleic acid.

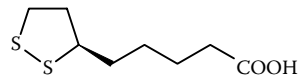


(36)

Sulfur-containing fatty acids have been reported at trace levels (<0.01%) in unprocessed canola oil (Wijesundera and Ackman, 1988). Tentative structures are 9,12-; 8,11-; and 7,10-epithiostearic acids, with a ring methyl, e.g., (**37**). α-lipoic acid or thioctic acid (1,2-dithiolane-3-pentanoic acid, **38**), found in microorganisms and the liver, is derived from octanoic acid and sulfur from a protein-bound iron-sulfur cluster (Booker 2004). It is a powerful antioxidant, which may protect against heart disease and reduce diabetic complications. It is an antidote for *Amanita* mushroom poisoning.



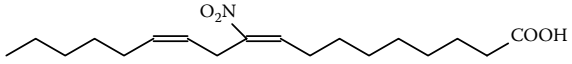
(37)



Lipoic acid

(38)

Two isomeric nitrolinoleic acids (**39** and **40**) have been isolated from plasma lipoproteins and red cell membranes. The nitro groups are probably derived from nitric oxide and these compounds may be signal transducers for the vascular effects of nitric oxide (Lim, 2002).



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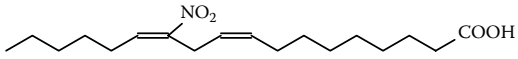


(40)

References

Unless otherwise referenced, composition and species distribution data are taken from the SOFA database: http://www.bagkf.de/sofa/

Batrakov, S.G. and Tolkachev, O.N. (1997) The structures of triacylglycerols from sclerotia of the rye ergot *Claviceps purpurea* (Fries) Tul. *Chem. Phys. Lipids*, **86**, 1–12.

Booker, S.J. (2004) Unraveling the pathway of lipoic acid biosynthesis. *Chem. Biol.*, **11**, 10–12.

Cahoon, E.B. et al. (2002) Transgenic production of epoxy fatty acids by expression of a cytochrome P450 enzyme

from *Euphorbia lagascae* seed. *Plant Physiol.,* **128**, 615–624.
Cahoon, E.B. et al. (1994) Petroselinic acid biosynthesis and production in transgenic plants. *Prog. Lipid Res.*, **33**, 155–163.
Christie, W.W. (2005) The lipid library: http://www.lipidlibrary.co.uk/
Damste, J.S.S. et al. (2002) Linearly concatenated cyclobutane lipids form a dense bacterial membrane. *Nature*, **419**, 708–712.
Dembitsky, V.M. et al. (2003) Lipid compounds of freshwater sponges: family Spongillidae, class Demospongiae. *Chem. Phys. Lipids*, **123**, 117–155.
Dembitsky, V.M. and Srebnik, M. (2002) Natural halogenated fatty acids: their analogues and derivatives. *Prog. Lipid Res.*, **41**, 315–367.
Dyer, J.M. et al. (2002) Molecular analysis of a bifunctional fatty acid conjugase/desaturase from tung. Implications for the evolution of plant fatty acid diversity. *Plant Physiol.*, **130**, 2027–2038.
Farmer, E.E. et al. (2003) Jasmonates and related oxylipins in plant responses to pathogenesis and herbivory. *Curr. Op. Plant Biol.,* **6**, 372–378.
Gorst-Allman, C.P. et al. (1988) Investigations of the origin of the furan fatty acids (F-acids). *Lipids*, **23**, 1032–1036.
Grogan, D.W. and Cronan, J.E. (1997) Cyclopropane ring formation in membrane lipids of bacteria. *Microbiol. Molec. Biol. Rev.*, **61**, 429–441.
Hamberg, M. (1998) A pathway for biosynthesis of divinyl ether fatty acids in green leaves. *Lipids*, **33**, 1061–1071.
Hayes, D.G. et al. (1995) The triglyceride composition, structure, and presence of estolides in the oils of *Lesquerella* and related species. *J. Amer. Oil Chem. Soc.*, **72**, 559–569.
Jones, L.N. and Rivett, D.E. (1997) The role of 18-methyleicosanoic acid in the structure and formation of mammalian hair fibres. *Micron*, **28**, 469–485.
Lee, M. et al. (1998) Identification of non-heme diiron proteins that catalyse triple bond and epoxy group formation. *Science*, **280**, 915–918.
Leonard, A.E. et al. (2004) Elongation of long-chain fatty acids. *Prog. Lipid Res.*, **43**, 36–54.
Lim, D.G. et al. (2002) Nitrolinoleate, a nitric oxide-derived mediator of cell function: synthesis, characterisation, and vasomotor activity. *Proc. Nat. Acad. Sci.*, **99**, 15941–15946.
Mongrand, S. et al. (1998) The $C_{16:3}/C_{18:3}$ fatty acid balance in photosynthetic tissues from 468 plant species. *Phytochemistry*, **49**, 1049–1064.
Moon, H. et al. (2001) A condensing enzyme from the seeds of *Lesquerella fenderi* that specifically elongates hydroxy fatty acids. *Plant Physiol.*, **127**, 1635–1643.
Nemoto, T. et al. (1997) Amphimic acids and related long-chain fatty acids as DNA topoisomerase I inhibitor from an Australian sponge, *Amphimedon* sp.: isolation, structure, synthesis, and biological evaluation. *Tetrahedron*, **53**, 16699–16710.
Onwueme, K.C. et al. (2005) The dimycocerosate ester polyketide virulence factors of mycobacteria. *Prog. Lipid Res.*, **44**, 259–302.
Oshima, M. and Agriga, T. (1975) ω-Cyclohexyl fatty acids in acidophilic thermophilic bacteria. *J. Biol. Chem.,* **250**, 6963–6968.
Ratledge, C. (2004) Single cell oils — a coming of age. *Lipid Technol.*, **16**, 34–39.
Sæbø, A. (2001) Commercial production of conjugated linoleic acid. *Lipid Tech. Newsl.,* **7**, 9–13.
Sebedio, J.L. and Grandgirard, A. (1989) Cyclic fatty acids: natural sources, formation during heat treatment, synthesis and biological properties. *Prog. Lipid Res.*, **28**, 303–336.
Spiteller, G. (2005) Furan fatty acids: occurrence, synthesis, and reactions. Are furan fatty acids responsible for the cardioprotective effects of a fish diet? *Lipids*, **40**, 755–771.
Spitzer, V. et al. (1997) Structure analysis of an allene-containing estolide tetraester triglyceride in the seed oil of *Sebastiana commersoniana* (Euphorbiaceae). *Lipids*, **32**, 549–557.
Wijesundera, R.C. and Ackman, R.G. (1988) Evidence for the probable presence of sulfur-containing fatty acids as minor constituents in canola oil. *J. Amer. Oil Chem. Soc.*, **65**, 959–963.
Wolff, R.L. et al. (2001) Fatty acid composition of Pinaceae as taxonomic markers. *Lipids,* **36**, 439–451.

1.1.3 Fatty acid conformation

Fatty acids have a common basic structure: a long alkyl chain and a carboxylic acid group at one end. The introduction of functional groups along the chain, usually double bonds, produces a range of closely related structures differing in chemical reactivity and shape. This section concerns the possible shapes that these molecules can adopt. Fatty acids are flexible molecules; there is potential rotation about the C-C bonds in the alkyl chain, and different conformations or tertiary structures are possible.

Details of the molecular shape of fatty acids have been obtained in two ways: x-ray crystallography and molecular mechanics calculations. For x-ray studies, the molecules

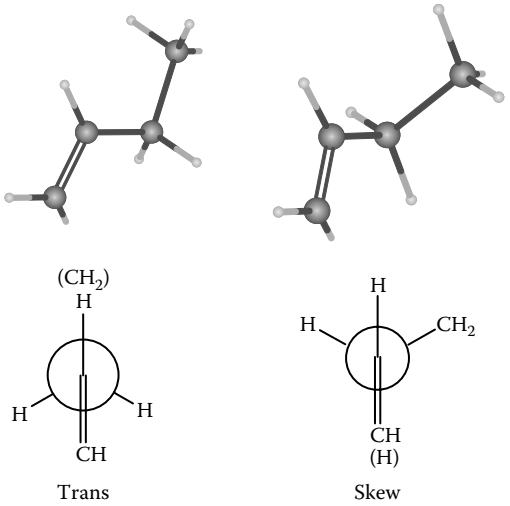


FIGURE 1.7 Conformations adjacent to double bonds. The shorthand notation follows Rich (1993). Torsion angles (τ) about C-C bonds: τ ~180° = trans = *t*; τ ~ ±120° = skew, skew′ = s, s′. Configuration of C=C bonds: τ = 0° = *cis* = C; τ = 180° = *trans* = T.

are necessarily constrained in a highly ordered structure, similar to that found in solid fats and lipid bilayers. Often several polymorphic solid phases occur, differing subtly in packing and molecular conformation. Molecular mechanics, on the other hand, considers the molecule in complete isolation, considering interactions between atoms within the molecule and minimising the energy of interactions between them. The molecular mechanics approach shows us which shapes are probable in the absence of other interactions. In fatty acids, there are often several conformations differing only a little in energy and the molecule's environment is likely to be important in determining the most probable shape. If several conformers are of similar energy, it is likely that the molecule will populate several to some extent, unless rigidly constrained in a crystal-like structure. Figure 1.7 defines the terms used here to describe the conformation adjacent to the double bond.

In a saturated fatty acid, the alkyl chain usually adopts a structure with each methylene anti to the next, resulting in a straight zigzag chain. Functional groups distort this straight chain to a greater or lesser extent and one measure of this distortion is the distance between the carboxyl carbon and the terminal carbon. A bend shortens this distance relative to the fully extended saturated acid — the shorter this distance, the more bent the molecule. We can also define the "width" of the molecule as the distance from the midpoint of this line to carbons in the middle of the chain (Figure 1.8). This parameter is particularly useful when the bend in the molecule is near the middle of the chain.

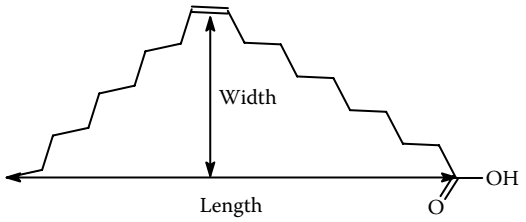


FIGURE 1.8 Length and width parameters of bent-chain fatty acids.

The available data for a number of fatty acids, both from x-ray crystallography and molecular mechanics calculations, are summarised in Table 1.5. X-ray crystallography is limited to compounds for which satisfactory crystals can be obtained. For low-melting fatty acid phases, this requires skilled work and low temperature data collection; in consequence, few polyenes have been studied.

TABLE 1.5 Conformational parameters of some fatty acids

Fatty Acid	Method[a], Crystal Form	Length[b] C1 to CH_3 (Å)	Width[b] (Å)	Torsion Angles (°)	Conformation[c]	References
				in chain		
18:0	calc	21.4		all 180		
18:0	mm	21.4		all ~180		Mizushina et al., 2004
18:0	x, C form	21.54		all ~180		Malta et al., 1971
18:0	x, E form	21.65		all ~180		Kaneko et al., 1990
18:0	x, poly type E	21.64		all ~180		Kaneko et al., 1994b
18:0	x, poly type B	19.14		C1,C2,C3,C4 = 70.3 allylic, C=C, allylic		Kaneko et al., 1994a
18:1 9*c*	calc	19.42	5.7	180, 0, 180	tCt	
18:1 9*c*	mm	12.80	8.50			Mizushina et al., 2004
18:1 9*c*	x, low melt	17.78	5.9	132.5, ~0, –127.4	sCs′	Abrahamsson and Ryderstedt-Nahringbauer, 1962
18:1 9*c*	x, beta	19.70	4.4	173.9, –0.7, 172.9	tCt	Kaneko et al., 1997
18:1 9*t*	calc	21.18		180, 180, 180	tTt	
18:1 9*t*	x	21.39		–118.8, –179.9, 118.6	s'Ts	Low et al., 2005
18:1 6*c*	x, low melt	19.59		157.1, 0.1, –160.2	sCs′	Kaneko et al., 1992a
18:1 6*c*	x, high melt	18.27		90.9, 0.8, 130.2	sCs	Kaneko et al., 1992b
		18.79		136.5, 1, 119.3		
18:2 9*c*,12*c*	x	19.15	2.4	–119.4, –2.3, 122.7 (9,10)	sCs′	Ernst and Sheldrick, 1979
				123.5, –3.3, –120.9 (12,13)	s'Cs	
18:2 9*c*,12*c*	mm	4.45	10.6			Mizushina et al., 2004
18:3 9*c*,12*c*,15*c*	mm	5.54	10.30			Mizushina et al., 2004
22:1 13*c*	x, alpha	22.49	7.3	–106, 1, –175	s'Cs′	Kaneko et al., 1996
22:1 13*c*	x, alpha 1	20.73	8.1	97, 1, 102	sCs	Kaneko et al., 1996

[a] calc = calculated using normal bond lengths and angles; mm = molecular mechanics; x = x-ray crystallography.
[b] x-ray data, calculated from coordinates in cited reference; mm values from Mizushina et al., 2004.
[c] see Figure 1.1.7.

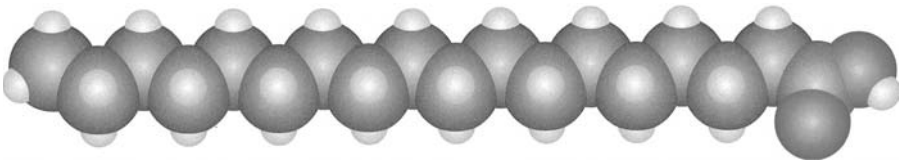


FIGURE 1.9 Space-filling diagram of the fully extended conformation of stearic acid. The molecule occupies an approximate cylinder 24.5 by 2.5 Å.

Using normal C-C bond lengths (1.54 Å), C-C-C bond angles (109.5º), and C-C-C-C torsion angles of 180º, the calculated C1 to C18 distance in stearic acid is 21.4 Å. Molecular mechanics calculations give the same value, and in two crystal forms the length is similar (21.6 Å) and the measured torsion angles are close to 180º (Figure 1.9).

However, the low melting β-form does not have a completely straight chain; rotation about the C2-C3 bond gives a skew conformation, with a C1-C2-C3-C4 torsion angle of 70º, the other torsion angles still being close to 180º. The result is a bend at C3 and a noticeable shortening of the C1-C18 distance to 19.1 Å. The straight all *trans* conformation has the lowest energy, but rotation about a C-C bond does not result in a great increase in energy (3 to 4 kJ/mol), so partially skew conformations may be favoured in certain environments.

A *cis*-double bond introduces a pronounced bend in the alkyl chain. Using standard bond lengths and angles and confining the molecule to a plane, the C1-C18 distance in oleic acid is 19.4 Å, considerably shorter than in stearic acid (21.4 Å). The width (C=C to midpoint of C1-C18) is 5.7 Å. In this conformation, the C7-C8-C9-C10 and C9-C10-C11-C12 torsion angles are 180º, i.e., a trans conformation (see Figure 1.7). The crystal structure of the β-form is close to this structure with a trans, *cis,* trans conformation about the double bond (see Figure 1.10) and a C1-C18 distance of 19.7 Å and width 4.4 Å. In the low melting form, the double bond carbons are twisted to one side of the plane of the rest of the molecule in a skew, *cis*, skew′ conformation (see Figure 1.10). This results in a sharper bend in the molecule, with a C1-C18 distance of 17.9 Å and width 5.9 Å. Molecular mechanics calculations give a yet more bent molecule (length 12.8 Å, width 8.5 Å) achieved through twisting bonds in the alkyl chain to smaller torsion angles.

X-ray structures are available for other monoenes. The low melting form of petroselinic acid also has a skew, *cis*, skew′ conformation. The high melting form of petroselinic and the α and α^1 forms of erucic acid have skew, *cis*, skew conformations, where the double bond carbons are twisted to opposite sides of the plane containing the alkyl chains (Figure 1.10). The trans, *cis*, trans conformation appears unique to oleic acid.

Trans unsaturation, in contrast, results in little bending of the alkyl chain. Using standard bond lengths and angles and confining the molecule to a plane, the C1-C18 distance in elaidic acid (18:1 9*t*) is only 0.2 Å shorter than stearic acid. The x-ray structure shows a skew′, *trans*, skew conformation, resulting from the double bond being twisted across the line of the alkyl chain, but with no significant shortening of the C1-C18 distance compared with stearic acid.

Obtaining adequate crystals of low melting polyenes is difficult and the only full x-ray structure available is of linoleic acid. The conformations about the 9,10 and 10,12 bonds are skew, *cis*, skew′ and skew′, *cis*, skew, respectively, giving an extended "angle iron" conformation (see Figure 1.11). The C1-C18 distance is 19.2 Å, comparable to the C_{18} monoenes. Analogous extended structures for linolenic and arachidonic are compatible with the available crystal

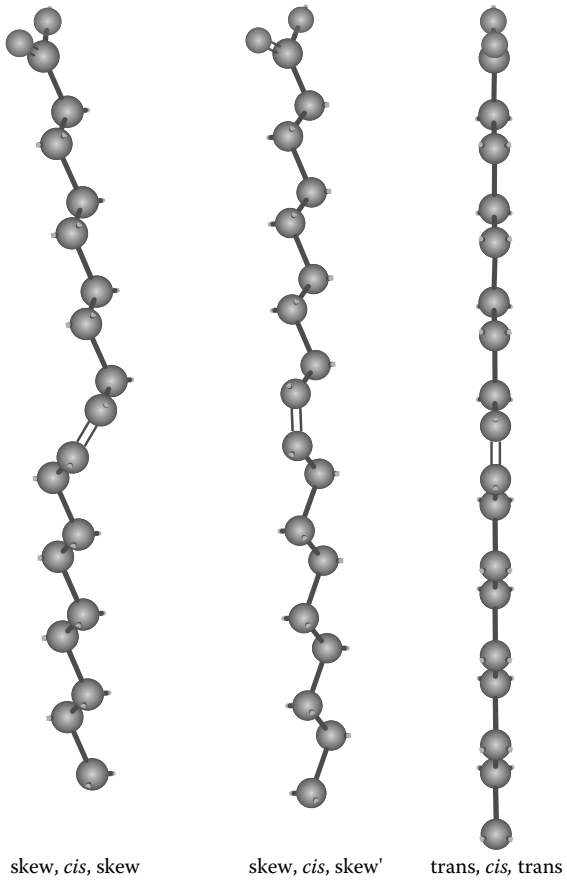


FIGURE 1.10 Possible conformations about the double bond. These are drawn for oleic acid, viewed from above the double bond with the molecule bent down into the plane of the paper.

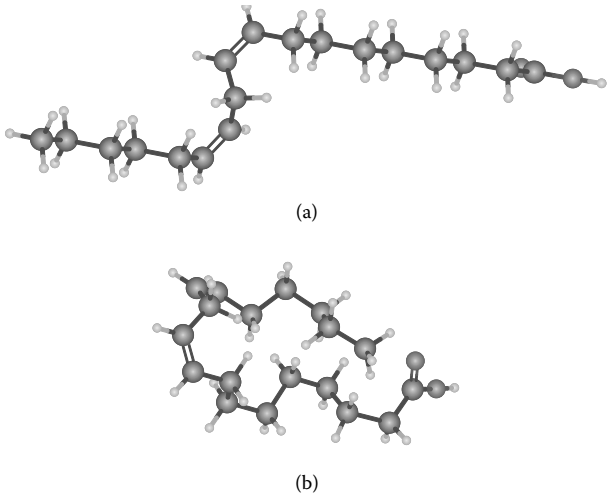


FIGURE 1.11 Conformations of linoleic acid: (a) angle-iron, (b) hairpin. Drawn from torsion angles given in Rich (1993).

data. Molecular mechanics calculations give very different results for these molecules. The alternating of skew, *cis*, skew′, skew′, *cis*, skew pattern is no longer seen and most alkyl carbon torsion angles are skew rather than anti, except for those near the methyl end. These conformations result in sharply bent hairpin molecules (Figure 1.11).

Conjugated acids do not have conformational mobility between double bonds and, therefore, have a more restricted range of conformations than methylene-interrupted molecules. This may be responsible for the specific biochemical effects of some conjugated acids where a particular molecular shape can interact with an enzyme while others cannot.

The combination of x-ray crystallography and molecular mechanics gives an idea of the shapes that fatty acids can adopt. The results from x-ray studies can be applied directly to situations where fatty acids or their derivatives are in ordered structures, solid phases, monolayers, or bilayers. In these environments, they adopt extended forms where there is some flexibility in shape through twisting adjacent to the double bond. Molecular mechanics calculations on isolated molecules reveal a number of low energy conformations, where more extensive twisting gives sharply bent hairpin structures. The accessibility of these conformations may be important in the behaviour of individual molecules interacting with receptor sites and enzymes.

References

Abrahamsson, S. and Ryderstedt-Nahringbauer, I. (1962) The crystal structure of the low melting form of oleic acid. *Acta Cryst.,* **15,** 1261–1268.

Ernst, J. and Sheldrick, W.S. (1979) Die Strukturen der essentiellen ungesättigten Fettsäuren, Kristallstruktur der Linolsäure sowie hinweise auf die Kristallstrukturen der α-Linolensäure und der Arachidonsäure. *Z. Naturforsch B.,* **34,** 706–711.

Kaneko, F., et al. (1990) Structure of stearic acid *E* form. *Acta Cryst.,* C**46,** 1490–1492.

Kaneko, F. et al. (1992a), Structure of the low-melting phase of petroselinic acid. *Acta Cryst.,* C**48,** 1054–1057.

Kaneko, F., et al. (1992b), Structure of the high-melting phase of petroselinic acid. *Acta Cryst.,* C**48,** 1057–1060.

Kaneko, F. et al. (1994a) Double-layered polytypic structure of the B form of octadecanoic acid, $C_{18}H_{36}O_2$. *Acta Cryst.,* C**50,** 245–247.

Kaneko, F. et al. (1994b) Double-layered polytypic structure of the E form of octadecanoic acid, $C_{18}H_{36}O_2$. *Acta Cryst.,* C**50,** 247–250.

Kaneko, F. et al. (1996) Mechanism of the $\gamma \rightarrow \alpha$ and $r^1 \rightarrow \alpha^1$ reversible solid-state phase transitions of erucic acid. *J. Phys. Chem.,* **100**, 9138–9148.

Kaneko, F. et al. (1997) Structure and crystallization behavior of the β phase of oleic acid. *J. Phys. Chem. B,* **101**, 1803–1809.

Low, J.N. et al. (2005) Elaidic acid (*trans*-9-octadecanoic acid), *Acta Cryst.,* E**61**, o3730–o3732.

Malta, V. et al. (1971) Crystal structure of the *C* form of stearic acid. *J. Chem. Soc.* (*B*),548–553.

Mizushina, Y. et al. (2004) Inhibitory action of conjugated C_{18}-fatty acids on DNA polymerases and DNA topoisomerases. *Lipids,* **39**, 977–983.

Rich, M.R. (1993) Conformational analysis of arachidonic and related fatty acids using molecular dynamics simulations. *Biochem. Biophys. Acta,* **1178**, 87–96.

1.2 Lipid structure

1.2.1 Acylglycerols (glycerides)

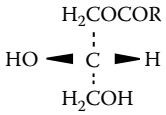
Acylglycerols are esters of glycerol (propane-1,2,3-triol) and fatty acids. Partial glycerides are important intermediates in metabolism, and triacylglycerols are the major constituents of most natural fats and oils.

In order to designate the stereochemistry of glycerol-containing components, the carbon atoms of glycerol are numbered stereospecifically. When the glycerol molecule is drawn in a Fischer projection with the secondary hydroxyl group to the left of the central prochiral carbon atom, then the carbons are numbered 1, 2, and 3 from top to bottom. Molecules that are stereospecifically numbered in this fashion have the prefix "*sn*" immediately preceding the term "glycerol" in the name of the compound to distinguish them from compounds that are numbered in a conventional fashion. The prefix "*rac*" in front of the full name shows that the compound is an equal mixture of both antipodes, whereas "*x*" is used if the configuration is unknown or unspecified.

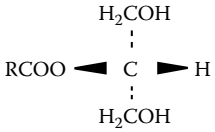
Any glycerolipid will be chiral when the substituents at the *sn*-1 and *sn*-3 positions are different. Mirror-image molecules or enantiomers possess opposite but equal optical rotations. However, if both substituents are long-chain acyl groups, then the optical rotation will be extremely small (Myher, 1978).

1.2.1.1 Monoacylglycerols (monoglycerides)

These are fatty acid monoesters of glycerol and exist in two isomeric forms (**1**) and (**2**).



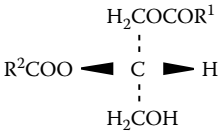
(**1**) 1-Monoacyl-*sn*-glycerol (α isomer)



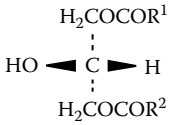
(**2**) 2-Monoacyl-*sn*-glycerol (β isomer)

1.2.1.2 Diacylglycerols (diglycerides)

These are fatty acid diesters of glycerol and occur in two isomeric forms (**3**) and (**4**).



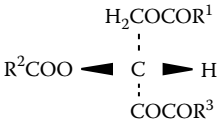
(**3**) 1,2-Diacyl-*sn*-glycerol (α,β isomer)



(**4**) 1,3-Diacyl-sn-glycerol (α,α′- isomer)

1.2.1.3 Triacylglycerols (triglycerides)

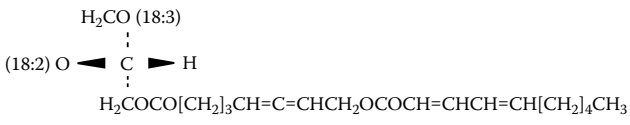
These are fatty acid triesters of glycerol. The fatty acids may be all different, two may be different, or all may be alike (see (**5**)).



(**5**) Triacyl-*sn*-glycerol

1.2.1.4 Glycerides containing four or more acyl groups

When triacylglycerols contain a hydroxy fatty acid, the hydroxyl group then can be esterified with further fatty acids. These are sometimes described as estolides. Examples of these glycerides have been found in plants (Hitchcock, 1975) with the occurrence of tetra-, penta- and hexa-acid glycerides. The tetra-acid triacylglycerol (**6**) from *Sapium sebiferum* oil (Sprecher et al., 1965) is an example.



(**6**)

References

Hitchcock, C. (1975) The structure and distribution of plant acyl lipids. In *Recent Advances in the Chemistry and Biochemistry of Plant Lipids,* Eds., T. Galliard and E.I. Mercer, Academic Press, New York, pp.1–19.

Myher, J.J. (1978) Separation and determination of the structure of acylglycerols and their ether analogues. In *Handbook of Lipid Research,* vol. 1, Ed., A. Kuksis, Plenum, New York, pp. 123–196.

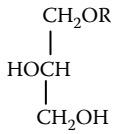
Sprecher, H.W. et al. (1965) Structure of an optically active allene-containing tetra-ester triglyceride isolated from the seed oil of *Sapium sebiferum*. *Biochemistry,* **4**, 1856–1863.

1.2.2 Glycerol ethers

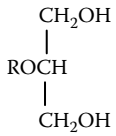
Glycerol ethers can be divided into two general types: alkyl ethers and alk-1-enyl ethers (or plasmalogens).

1.2.2.1 Alkyl ethers

1. Monoalkyl ethers (**7**) and (**8**)



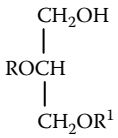
(**7**) *sn*-1 or α isomer



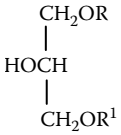
(**8**) *sn*-2 or β isomer

The α-isomer is the more common and is usually found to have the D or *sn*-1 configuration. Examples include batyl alcohol (*sn*-1-*O*-octadecylglycerol) and chimyl alcohol (*sn*-1-*O*-hexadecyl alcohol). *sn*-3-*O*-tetramethylhexadecylglycerol is a reported example of an α-isomer with the L configuration. β-Mono-phytanylglycerol (2-*O*-tetramethylhexadecylglycerol) has been reported and is an example of a β–monoalkyl ether.

2. Dialkyl ethers (**9**) and (**10**)



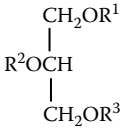
(**9**) *sn*-2,3 or α, β isomer



(**10**) *sn*-1,3 or α, α′ isomer

Although diethers have been synthesised in the 1,2 and 1,3 forms, the only naturally occurring diether is *sn*-2,3-di-*O*-tetramethylhexadecylglycerol (diphytanylglycerol).

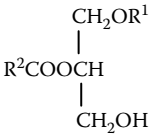
3. Trialkyl ethers



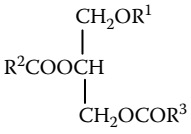
(**11**)

These compounds have not yet been reported in nature, but have been synthesised with various combinations of saturated or unsaturated chains. They have been proposed as nonfattening dietary lipids (Mangold and Paltauf, 1983).

4. Acylated alkyl ethers (**12**) and (**13**)



(**12**) *sn*-1-Alkyl-2-acylglycerol



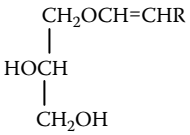
(**13**) *sn*-1-Alkyl-2,3-diacylglycerol

Both the monoacyl and diacyl derivatives are found in Nature.

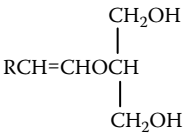
1.2.2.2 Alk-l-enyl ethers

As with the alkyl ethers of glycerol, the monoalk-1-enyl ethers predominate in Nature with small amounts of the dialk-1-enyl ethers, but no trialk-1-enyl ethers so far detected.

1. Monoalk-1-enyl ethers (**14**) and (**15**)



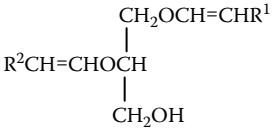
(**14**) *sn*-1 or α isomer



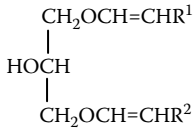
15) *sn*-2 or α isomer

Only the *sn*-1 isomer occurs naturally. Chains of 16 and 18 carbons are usual.

2. Dialk-1-enyl ethers (**16**) and (**17**)

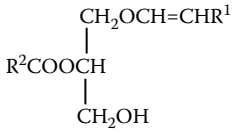


(**16**) *sn*-1,2 or α, β isomer

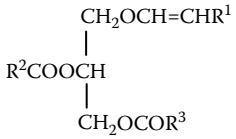


(**17**) *sn*-1,3 or α, α′ isomer

3. Acylated alk-1-enyl ethers (neutral plasmalogens) (**18**) and (**19**)



(**18**) *sn*-1-Alk-1′-enyl-2-acylglycerol



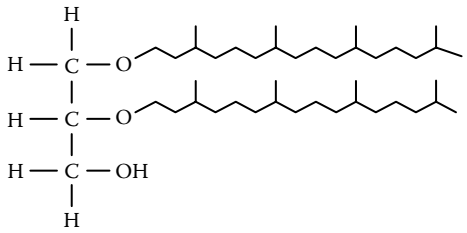
(**19**) *sn*-1-Alk-1′-enyl-2-3-diacylglycerol

Both the monoacyl and diacyl derivatives have been reported.

1.2.2.3 Ether lipids of Archaebacteria

Archaebacteria vary from eubacteria in that their membrane lipids consist almost entirely of C_{20}-C_{20} diacylglycerol diether derivatives: *sn*-2,3-diphytanylglycerol diether (**20**) and its dimer, dibiphytanyldiglycerol tetraether, in which the two C_{20}-C_{20} diether moieties are linked head to head.

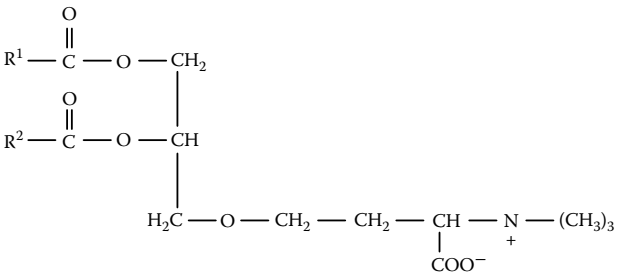
Variants of this structure, including terpenyl-, glyco- and sulfoglyco- derivatives, have been reported and identified. These lipids are found in all three classes of Archaebacteria — the extreme halophiles, the methanogens, and the thermoacidophiles (see Kates, 1990, 1993).



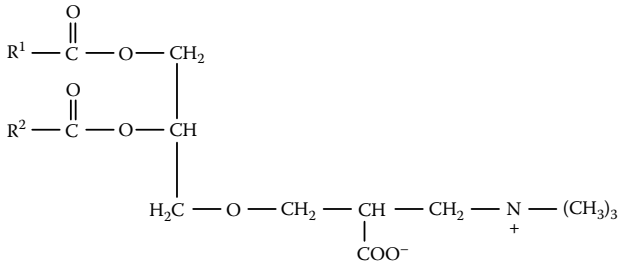
(**20**) 2,3-Di-*O*-phytanyl-*sn*-glycerol diether

1.2.2.4 Betaine lipids

Two betaine lipids, diacylglycerol-*O*-*(N,N,N*-trimethyl*)* homoserine (DGTS (**21**)) and diacylglycerol-*O*-(hydroxymethyl)-(*N,N,N*-trimethyl)-β-alanine (DGTA (**22**)), have been reported from a variety of lower plants and algae, often as major components (see Dembitsky, 1996). A new betaine lipid, diacylglycerol-*O*-carboxyl-(hydroxymethyl)-choline (DGCC) has been recently reported in *Pavlova* (chromophyta) (Dembitsky, 1996).



(**21**) DGTS



(**22**) DGTA

References

Dembitsky, V.M. (1996) Betaine ether-linked glycerolipids: chemistry and biology. *Prog. Lipid Res.* **35**, 1–51.

Kates, M. (1972) Ether-linked lipids in extremely halophilic bacteria. In: *Ether Lipids: Chemistry and Biology*, F. Snyder, Ed., New York: Academic Press, pp. 351–398.

Kates, M. (1990) Glyco-, phosphoglyco- and sulfoglycoglycerolipids of bacteria. In: *Handbook of Lipid Research,* vol. 6 M. Kates, Ed., Plenum: New York, pp. 1– 122.

Kates, M. (1993) Membrane lipids of Archaea. In: *The Biochemistry of Archaea (Archeabacteria)* M. Kates, D.J. Kushner, and A.T. Matheson, Eds., Amsterdam: Elsevier, pp. 261–295.

Mangold, H. K. and Paltauf, F. Eds. (1983) *Ether Lipids: Biochemical and Biomedical Aspects.* London: Academic Press.

Synder, F. (1969) Biochemistry of lipids containing ether bonds. *Prog. Chem. Fats Other Lipids,* **10**, 287– 335.

1.2.3 Phospholipids

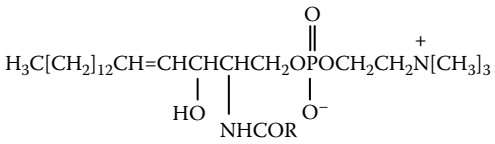
Phospholipids are divided into two main classes depending on whether they contain a glycerol or a sphingosyl backbone.

1.2.3.1 Glycerophospholipids

The compounds are named after and contain structures that are based on phosphatidic acid (3-*sn*-phosphatidic acid). The X moiety attached to the phosphate includes nitrogenous bases (amino alcohols) or polyols. In Table 1.6 the major phosphoglycerides are listed. Some of the less common compounds are indicated in the remarks within the table.

1.2.3.2 Sphingophospholipids

Lipids that contain sphingosine (*trans-D-erythro*-1,3-dihydroxy-2-amino-4-octadecene) or a related amino alcohol are known as sphingolipids. The most common phospholipid in this class is the phosphorylcholine ester of an *N*-acylsphingosine (or ceramide) that is more commonly called sphingomyelin (**23**).



(**23**) *N*-Acyl-*trans*-4-sphingenine-1-phosphorylcholine (sphingomyelin containing sphingosine as the sphingosyl moiety)

Although sphingomyelin is a major lipid of certain membranes in animal (particularly nervous) tissues, it is of minor importance in plants and probably absent from bacteria. Even in animals, the nonphosphorus sphingolipids (see Section 1.2.4) are more widely distributed.

Glycolipids based on phytosphingosine that contain both inositol and phosphate were identified first by Carter and coworkers (Carter et al., 1969). Their general structure is shown in Figure 1.12. Both phytosphingosine- and dehydrosphingosine-based variants have been reported. However, all contain inositol linked via a phosphodiester linkage to the ceramide and via a glycosidic bond to a chain of sugar residues of variable composition (Hetherington and Drobak, 1992). The components were previously called "phytoglycolipids," but Laine and Hsieh (1987) suggested that the term "glycophosphosphingolipid" be used to distinguish them from other plant glycosphingolipids.

A number of glycophosphosphingolipids have been reported (Laine and Hsieh, 1987; Stults et al., 1989). Two structures are shown in (**24**) and (**25**). (See Hetherington and Drobak (1992) for more details.) The analysis of plant glycophosphospingolipids has been thoroughly discussed by Laine and Hsieh (1987). In addition, various phosphoglycolipids have been identified in Archaebacteria (Kates, 1990).

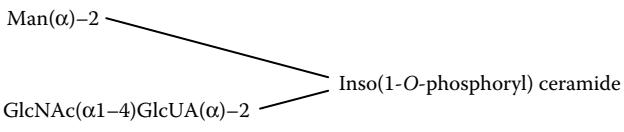
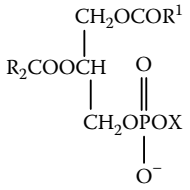
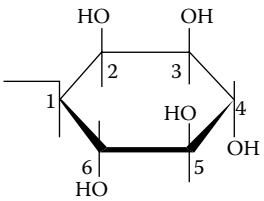


TABLE 1.6 Structure and distribution of important phosphoglycerides of general formula



Substituent (X)	Phospholipid	Remarks
H	Phosphatidic acid (PtdOH)	Negatively charged lipid. Important metabolic intermediate, only occurring in trace amounts.
$-CH_2CH(\overset{+}{N}H_3)COO^-$	Phosphatidylserine (PtdSer)	Negatively charged lipid. Serine is the L isomer. Widespread but minor lipid in eukaryotes. *N*-Acylated derivatives have been found.
$-CH_2CH_2\overset{+}{N}H_3$	Phosphatidylethanolamine (PtdEtn)	Widespread and major lipid. The partly methylated derivatives (phosphatidyl-*N*-monomethyl-ethanolamine, phosphatidyl-*N*-dimethyl-ethanolamine) are found in small amounts in many organisms. They are metabolic intermediates in the conversion of phosphatidylethanolamine to phosphatidylcholine (see Section 10.2.1). *N*-Acylated derivatives of phosphatidylethanolamine (and lyso-phosphatidylethanolamine) are found in small amounts in many tissues. In some tissues they may be significant components (Schmid et al., 1990). Following phospholipase D action, *N*-acylethanolamines are released and are part of the endocannabinoid signalling system (Chapman, 2004).
$-CH_2CH_2\overset{+}{N}(CH_3)_3$	Phosphatidylcholine (PtdCho)	Has a net neutral charge. The major animal phospholipid and the main component of nonchloroplast membranes of plants. Found in small quantities in some bacteria.
$-CH_2CH(OH)CH_2OH$	Phosphatidylglycerol (PtdGro)	Negatively charged lipid. Head group glycerol has *sn*-1 configuration. The major phospholipid in photosynthetic tissues and many bacteria. Some bacteria contain *O*-aminoacyl groups (lysine, ornithine, arginine, or alanine) attached to position 3 of the base glycerol. Bisphosphatidic acid, the fully acylated analogue of PtdGro, has been found in some plant tissues
	Phosphatidylinositol (PtdIns)	Negatively charged lipid. Inositol is the *myo* isomer. Widespread and usually minor lipid. Further phosphorylations can take place at different positions of the inositol and give rise to phosphatidylinositol-4-phosphate, phosphatidylinositol-3,4-*bis*phosphate, phosphatidylinositol-4,5-*bis*phosphate and phosphatidylinositol-3,4,5-*tris*phosphate. These have been found in small amounts in many eukaryotes and are important for signalling (Payrastre, 2004).
Phosphatidylglycerol	Diphosphatidylglycerol (DPG) (cardiolipin)	Negatively charged lipid. Common in bacteria. Localized in the inner mitochondrial membrane of eukaryotes.

Note: R^1 and R^2 are long-chain alkyl groups.

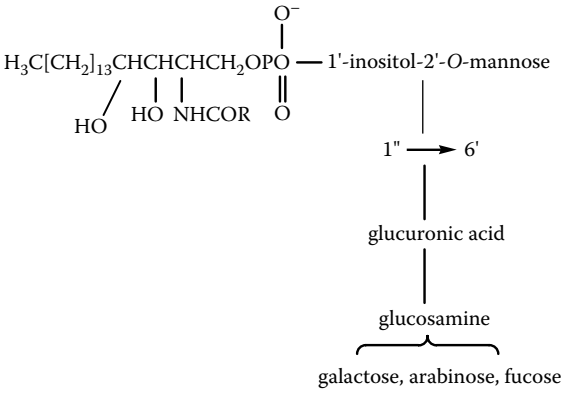


FIGURE 1.12 Phytoglycolipid.

(**24**) Found in corn.

GlcNAc(α1–4)GlcUA(α1–2)Inso(1-*O*-phosphoryl)ceramide

(**25**) Found in tobacco.
GlcUA = glucuronic acid

1.2.3.3 Variants on the diacyl structure

Variants on the diacyl structure are summarized in Table 1.7.

1.2.3.3.1 Monoacyl derivatives

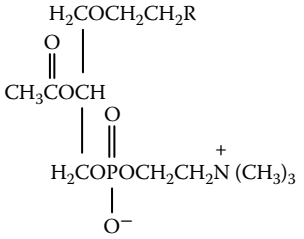
Lyso-derivatives (monoacylglycerophospholipids) are found in small amounts in most tissues, but their presence in large amounts is usually indicative of lipid degradation either before or during extraction. An exception is the presence of lysophosphatidylcholine and lysophosphatidylethanolamine as the major phospholipids of cereal grains. Lysophospholipids, and especially lysophosphatidiate, are important signalling molecules (Pyne, 2004) (see Section 10.6).

1.2.3.3.2 Plasmalogens

These are the monoacyl monoalk-1-enyl ether forms of phospholipids. Choline and ethanolamine plasmalogens are the most common forms, although serine plasmalogen has also been found. The percentage of the plasmalogen form of a given phospholipid may be quite high (e.g., in the mammalian brain) and is usually underestimated because most thin-layer chromatographic systems fail to resolve the plasmalogen from the diacyl form of a given lipid class. They are present in most animal tissues, but are rare in plants.

Plasmanic acid and plasmenic acid represent the alkyl and alk-1-enyl analogues of phosphatidic acid, respectively. Thus, the choline plasmalogen (1-alkyl-2-acyl-*sn*-glycero-3-phosphocholine) is termed plasmanylcholine, while a 1-alk-1-enyl derivative would be plasmenylcholine (Synder, 1996). There are a number of reports of phospholipids in bovine heart and spermatozoa that have *O*-alkyl groups at both *sn*-1 and *sn*-2 positions. Halophilic bacteria contain large amounts of dialkylglycerolipids, but of the opposite stereochemical configuration (*sn*-2, 3 or D series; see Synder, 1996).

Platelet-activating factor (1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) (**26**) is a biologically active phospholipid of great current interest (e.g., see Ishii and Shimizu, 2000).



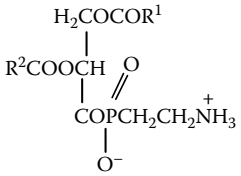
(**26**) 1-Alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (platelet-activating factor, PAF)

1.2.3.3.3 Ether derivatives

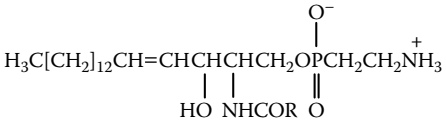
Monoacyl monoether and diether forms have been detected in very small amounts in various tissues (Mangold and Paltauf, 1983).

1.2.3.4 Phosphonolipids

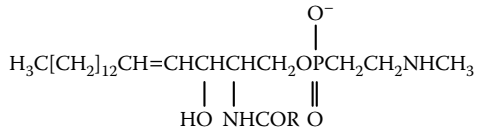
Phosphono analogues of various phospholipids have been reported. These include phosphono analogues of sphingosylphosphatides, which have been found in various invertebrates, and of phosphatidylethanolamine (Figure 1.13). Phosphonolipids are major constituents in three phyla and are synthesized by phytoplankton, the base of the food chains of the ocean (Kittredge and Roberts, 1969). The following phosphonic acids have been found in nature: 2-amino ethylphosphonic acid, 2-methylaminoethylphosphonic acid, 2-dimethylaminoethylphosphonic acid, 2-trimethylaminoethylphosphonic acid, and 2-amino-3-phosphonopropionic acid. The first of



Phosphono form of phosphatidylethanolamine



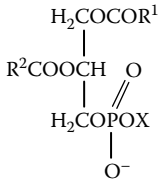
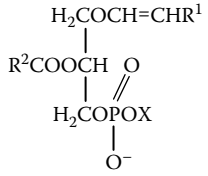
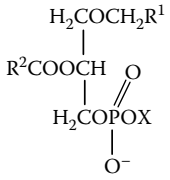
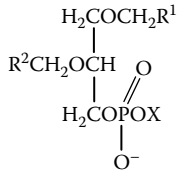
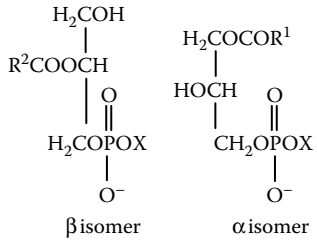
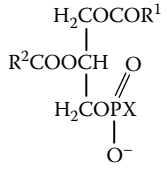
Ceramide aminoethyl phosphonate (ceramide ciliatine)



Ceramide *N*-methylaminoethyl phosphonate

FIGURE 1.13 Phosphonolipids.

TABLE 1.7 Variations in phosphoglyceride structures

Structure[a]	Structure Form	Common Phosphoglyceride[a]
	Diacyl ester form	All
	Monoacyl monoalk-1-enyl ether form (plasmalogen)	PtdSer, PtdEtn, PtdCho
	Monoacyl monoether form	PtdEtn, PtdCho
	Diether form	PtdGro, PtdGroP
	Monoacyl (lyso) form	PtdSer, PtdEtn, PtdCho, PtdOH
	Phosphono form	PtdEtn

[a] For abbreviations, see Table 1.6. R^1 and R^2 represent saturated or unsaturated alkyl chains. X represents a nitrogenous base or polyol residue. PtdGroP is phosphatidylglycerol phosphate.

these phosphonic acids is the main component of the phosphonolipids. Phosphonolipids have been reviewed by Hori and Nozawa (1982).

References

Ansell, G.B., Dawson, R.M.C., and Hawthorne, J.N. Eds., (1973) *Form and Function of Phospholipids,* Amsterdam: Elsevier Scientific.

Carter, H.E., Strobach, D.R., and Hawthorne, J.N. (1969) Biochemistry of the sphingolipids. XVIII. Complete structure of tetrasaccharide phytoglycolipid. *Biochemistry,* **8**, 383– 388.

Chapman, K.D. (2004) Occurrence, metabolism and prospective functions of N-acylethanolamines in plants. *Prog. Lipid Res.* **43**, 302–327.

Hetherington, A.M. and Drobak, B.K. (1992) Inositol-containing lipids in higher plants. *Prog. Lipid Res.* **31**, 53– 63.

Hori, T. and Nozawa, Y. (1982) Phospholipids. In *Phospholipids,* J.N. Hawthorne and G.B. Ansell, Eds., Amsterdam: Elsevier Biomedical Press, pp. 95–128.

Ishii, S. and Shimizu, T. (2000) Platelet-activating factor (PAF) receptor and genetically engineered PAF receptor mutant mice. *Prog. Lipid Res.* **39**, 41–82.

Kanfer, J.N. and Hakomori, S.-I. (1983) *Sphingolipid Biochemistry,* New York: Plenum.

Kates, M. (1972) *Techniques in Lipidology,* 2nd ed. Amsterdam: Elsevier.

Kates, M. (1990) Glyco-, phosphoglyco- and sulphoglycoglycerolipids of bacteria. In *Handbook of Lipid Research,* M. Kates, Ed., vol. 6, New York: Plenum Press, pp. 1–122.

Kittredge, J.S. and Roberts, E. (1969) Carbon-phosphorus bond in Nature. *Science,* **164,** 37–42.

Laine, R.A. and Hsieh, T.C. -Y. (1987) Inositol-containing sphingolipids. *Methods Enzymol.,* **138**, 186–195.

Mangold, H.K. and Paltauf, F. Eds., (1983) *Ether Lipids: Biochemical and Biomedical Aspects,* London: Academic Press.

Payrastre, B. (2004) Phosphoinositides. In *Bioactive Lipids,* A. Nicolaou, A. and G. Kokotos, Eds., Bridgwater, U.K. The Oily Press, pp. 63–84.

Pyne, S. (2004) Lysolipids: sphingosine 1-phosphate and lysophosphatidic acid. In *Bioactive Lipids,* A. Nicolaou and G. Kokotos, Eds., Bridgwater, U.K.: The Oily Press, pp. 85–106.

Ratledge, C. and Wilkinson, S. G. Eds., (1988) *Microbial Lipids,* vol. 1, London: Academic Press.

Schmid, H.H.O., Schmid, P.C., and Watarajan, V. (1990) N-Acylated glycerophospholipids and their derivatives. *Prog. Lipid Res.,* **29,** 1– 43.

Stults, C.L.M., Sweeley, C.C., and Macher, B.A. (1989) Glycosphingolipids: structure, biological source and properties. *Methods Enzymol.*, **179**, 167– 214.

Synder, F. (1996) Ether-linked lipids and their bioactive species: occurrence, chemistry, metabolism, regulation and function. In *Biochemistry of Lipids, Lipoproteins and Membranes,* D.E. Vance and J.E. Vance, Eds., Amsterdam: Elsevier, pp. 183–210.

1.2.4 Glycosphingolipids

A large number of different glycosphingolipids are known, which differ in the nature and number of their glycosyl residues. A general reference is that of Kanfer and Hakomori (1983). Insect glycolipids are dealt with specifically by Wiegandt (1992) and a useful introductory article is that by Merrill and Sweeley (1996).

1.2.4.1 Ceramides and glycosylceramides

Attachment of a fatty acid to the amino group of sphingosine or other related amino alcohol gives rise to a ceramide. The most commonly found sphingosyl alcohols are shown in Figure 1.14. (see Merrill and Sweeley, 1996).

Attachment of glucose or of galactose by *O*-ester linkage to the primary alcohol of the sphingosyl moiety yields a ceramide hexoside (or cerebroside) (see (**27**)): X = H for simple ceramides, X = a monosaccharide (usually galactose) for cerebrosides in animals and often glucose in plants. The linkage is 1-*O*-β. X = 2 to 6 sugar units for ceramide polyhexosides where the first sugar residue is glucose. The sphingosine base can vary. It is usually phytosphingosine in plant sphingolipids. The fatty acids in the R group are frequently 2-hydroxy compounds for the galactosyl-ceramides. In animals, the galactocerebroside is the most common. Confusingly, futher attachment of hexosides to glucocerebroside yields the neutral ceramides (see (**27**)). These are usually written by a shorthand nomenclature, e.g., Glc-Gal-Gal-Glc-Cer would be glucosyl(1→4)galactosyl(1→4) galactosyl(1→4)glucosyl(1→1)ceramide.

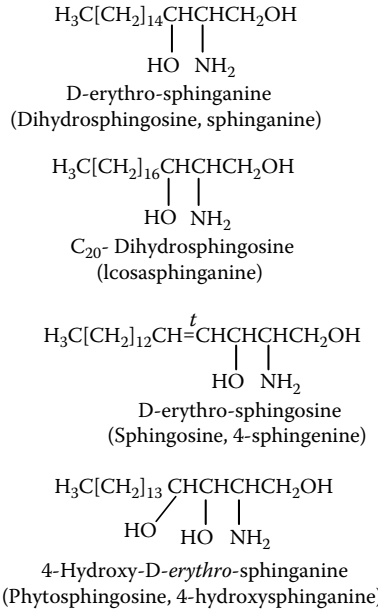
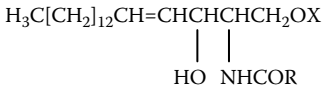


FIGURE 1.14 Some amino alcohols found in sphingolipids.



(**27**) Cerebrosides and neutral ceramides.

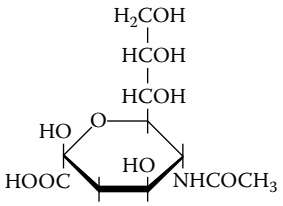
The nomenclature of simple glycosphingolipids as recommended by the IUPAC-IUB Commission on Biochemical Nomenclature is shown in Table 1.8. Further classification can be based on shared partial oligosaccharide sequences as shown in Table 1.9 and as discussed by Merrill and Sweeley (1996).

Some of the neutral ceramides have immunochemical properties, such as the so-called "Forsmann antigen" (CerGlc(4→1)Gal(4→1)Gal(3→1)-α-*N*-acetylgalactosamine). In general, each mammalian organ has a characteristic pattern of neutral ceramides, with kidney, lung, spleen, and blood containing quite large amounts.

In microorganisms, glucose or galactose are the usual carbohydrate residues, although glucuronic acid has been reported in the ceramides of *Pseudomonas paucimobilis*. Inositol and mannose residues may also be attached to sphingosine bases in fungi (Ratledge and Wilkinson, 1988). However, sphingolipids are not quantitatively important in most microorganisms. A number of acylated sphingosines have been reported, where the hydroxyl as well as the amino group of the base can be reacted. For example, both triacetyl and tetraacetyl derivatives have been described in *Hansenula ciferri* (Brennan et al., 1974). Some organisms, including mammals, have small amounts of ceramide phosphorylethanolamine, ceramide phosphate, and phosphoglycosphingolipids (Merrill and Sweeley, 1996).

1.2.4.2 Gangliosides

These are glycosphingolipids that contain a ceramide linked to a glucosylgalactosylsialic acid (β-D-*N*-acetylneuraminic acid) moiety. Other monosaccharide and sialic acid residues may also be present. The structure of sialic acid is shown in (**28**).



(**28**) Sialic acid

Gangliosides have been reported with structures shown in Table 1.10.

TABLE 1.8 Nomenclature for the classification of glycosphingolipids

Root Name	Abbreviation	Partial Structure[a] IV	III	II	I
Ganglio	Gg	Galβ1-3GalNAcβ1-4Galβ1-4Glc-Cer			
Lacto	Lc	Galβ1-3GlcNAcβ1-3Galβ1-4Glc-Cer			
Neolacto	nLc	Galβ1-4GlcNAcβ1-3Galβ1-4Glc-Cer			
Globo	Gb	GalNAcβ1-3Galα1-4Galβ1-4Glc-Cer			
Isoglobo	iGb	GalNAcβ1-3Galα1-3Galβ1-4Glc-Cer			
Mollu	Mu	GalNAcβ1-2Manα1-3Manβ1-4Glc-Cer			
Arthro	At	GlcNAcβ1-4GlcNAcβ1-3Manβ1-4Glc-Cer			

[a] Roman numerals define sugar positions in the root structure.
From Sweeley, C.C. (1991).

TABLE 1.9 Names and abbreviations of simple glycosphingolipids

Structure	Trivial Name of Oligosaccharide[a]	Symbol[b]
Gal (α1-4)Gal(β1-4)GlcCer	Globotriaose	$GbOse_3$
GalNAc(β1-3)Gal(α1-4)Gal(β1-4)GlcCer	Globotetraose	$GbOse_4$
Gal(α1-3)Gal(β1-4)GlcCer	Isoglobotriaose	$iGbOse_3$
GalNAc(β1-3)Gal(α1-3)Gal(β1-4)GlcCer	Isoglobotetraose	$iGbOse_4$
Gal(β1-4)Gal(β1-4)GlcCer	Mucotiaose	$McOse_3$
Gal(β1-3)Gal(β1-4)Gal(β1-4)GlcCer	Mucotetroase	$McOse_4$
GlcNAc(β1-3)Gal(β1-4)GlcCer	Lactotriaose	$LcOse_3$
Gal(β1-3)GlcNAc(β1-3)Gal(β1-4)GlcCer	Lactotetraose	$LcOse_4$
Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)GlcCer	Neolactotetraose	$nLcOse_4$
GalNAc(β1-4)Gal(β1-4)GlcCer	Gangliotriaose	$GgOse_3$
Gal(β1-3)GalNAc(β1-4)Gal(β1-4)GlcCer	Gangliotetraose	$GgOse_4$
Gal(α1-4)GalCer	Galabiose	$GaOse_2$
Gal(1-4)Gal(α1-4)GalCer	Galactriaose	$GaOse_3$
GalNAc(1-3)Gal(1-4)Gal(α1-4)GalCer	*N*-Acetylgalactosaminylgalatriaose	$GalNAc1\text{-}3GaOse_3$

[a] Name of glycolipid is formed by converting ending "-ose" to "-osyl," followed by "ceramide," without space, e.g., globotriaosylceramide.
[b] Should be followed by Cer for the glycolipid, without space, e.g., $McOse_3Cer$, Mc_4Cer.
Abbreviations: GlcNAc, *N*-acetylglucosamine; Gal, galactose; Glc, glucose; Cer, ceramide.
Taken from the IUPAC-IUB Commission on Biochemical Nomenclature (1977).

TABLE 1.10 Abbreviated representation of gangliosides

Lipid[a]		Designation According to Wiegandt[b]	Svennerholm[c]
1	I^3NeuAc-GalCer	G_{Gal}1NeuAc	–
2	II^3NeuAc-LacCer	G_{Lac}1NeuAc	G_{M3}
3	II^3NeuGc-LacCer	G_{Lac}1NeuNG1	–
4	$II^3(NeuAc)_2$LacCer	G_{Lac}2NeuAc	G_{D3}
5	II^3NeuAc/NeuGc-LacCer	G_{Lac}2NeuAc/NeuNG1	–
6	II^3NeuGc-LacCer	G_{Lac}2NeuNG1	–
7	II^3NeuAc-$GgOse_3$Cer	G_{Gtri}1NeuAc	G_{M2}
8	II^3NeuAc-$GgOse_4$Cer	G_{Gtet}1NeuAc	G_{M1}
9	IV^3NeuAc-n$LcOse_4$Cer	G_{Lntet}1aNeuAc	$G_{M1\text{-}GlcNAc}$
10	IV^6NeuAc-n$LcOse_4$Cer	G_{Lntet}1bNeuAc	–
11	IV^2Fuc, II^3NeuAc-$GgOse_4$Cer	G_{Gfpt}1NeuAc	–
12	IV^3NeuAc-n$LcOse_4$Cer	-	–
13	$II^3(NeuAc)_2$-$GgOse_4$Cer	G_{Gtet}2bNeuAc	G_{D1b}
14	IV^3NeuAc, II^3NeuAc-$GgOse_4$Cer	G_{Gtet}2aNeuAc	G_{D1a}
15	$II^3(NeuAc)_3$-$GgOse_4$Cer	G_{Gtet}3bNeuAc	–
16	IV^3NeuAc,$II^3(NeuAc)_2$-$gOse_4$Cer	G_{Gtet}3aNeuAc	G_{T1}
17	IV^3NeuAc,$II^3(NeuAc)_3GgOse_4$Cer	G_{Gtet}4bNeuAc	–
18	$IV^3(NeuAc)_2$,$II^3(NeuAc)_3GgOse_4$Cer	G_{Gtet}5NeuAc	–
19	IV^3NeuAc, II^3NeuAc-$GgOse_5$Cer	G_{Gpt}2aNeuAc	–

[a] To indicate linkage points and anomeric form: Fuc should be written(←1αFuc); NeuAc should be written (←2αNeuAc); $(NeuAc)_2$ should be written $(\leftarrow 2\alpha NeuAc8)_2$; etc. If these features are assumed or defined, the short form used in this column is more convenient for use in texts and tables.

[b] The subscripts to G (for ganglioside), from lipid 7 onwards have the meanings: Gtri = gangliotriose, Gtet = gangliotetraose, Lntet = lactoisotetraose, Gpt = gangliopentaose, Gfpt = gangliofucopentaose (from Wiegandt, 1973).

[c] G = ganglioside, M = monosialo, D = disialo, T = trisialo. Arabic numerals indicate sequence of migration in thin-layer chromatograms (from Svennerholm, 1963).

Notes on composition and occurrence (see Gurr et al., 2002): Gangliosides appear to be confined to the animal kingdom. In man, cattle, and horses, the main ganglioside outside the brain is G_{Lact}1NeuAc; N-glycolyneuraminic acid is the chief sialic acid in erythrocyte and spleen gangliosides of horses and cattle.

Physical properties: Insoluble in nonpolar solvents; form micelles in aqueous solution.

Major bases: C_{18} and C_{20} sphingosines; minor amounts of dihydro analogues.

Fatty acids: Large amounts of 18:0 (86 to 95% in brain).

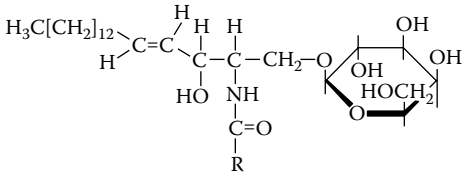
Occurrence: Mainly in grey matter of brain but also in spleen, erythrocytes, liver, and kidney. Modern analytical techniques have shown them to be present in a much wider range of tissues than preiviously realized. Main gangliosides of human brain are G_{GNT}1, 2a,2b,3a.

Taken from the IUPAC-IUB Commission on Biochemical Nomenclature (1977).

1.2.4.3 Cerebrosides

These are glycosides of *N*-acyl long-chain bases (ceramides). Galactose and glucose are the monosaccharides commonly found. The structures of two representative molecules — one from mammalian brain and the other from a higher plant — are shown in (**29**) and (**30**).

Brain cerebrosides

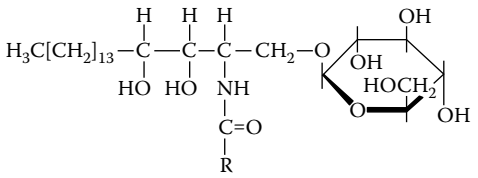


(29) *N*- Acylsphingosine-1-β-D-galactopyranoside

Where R-CO is:

lignoceryl ($C_{24}H_{47}O$) in kerasin
cerebronyl (2-hydroxylignoceryl) in phrenosin
nervonyl ($C_{24}H_{45}O$) in nervone
2-hydroxynervonyl in oxynervone

Plant cerebrosides

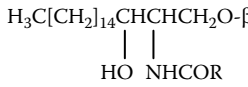


(30) *N*- (α-Hydroxy) acylphytosphingosine-1-*o*-β-glucopyranoside

Cerebrosides frequently contain large amounts of 2-hydroxy fatty acids, which are linked through the amino group of the sphingosine base. In addition, odd chain-length acids are also found. Typical fatty acids might be behenic (22:0), lignoceric (24:0) as in kerasin, nervonic (24:1) as in nervone, cerebronic (α-OH 24:0) as in phrenosin, and 2-hydroxynervonic (α-OH 24:1) as in oxynervone. The sugar composition of mammalian cerebrosides depends on the tissue source: brain cerebroside containing mainly galactose while that of blood contains mainly glucose. The myelin sheath of nerves contains particularly large amounts of cerebrosides, as well as substantial quantities found in the lung and the kidney.

1.2.4.4 Sulfatides

Sulfatides are 3-sulfate esters of galactosyl-cerebrosides (**31**). Their distribution in mammalian tissues is generally similar to that of cerebrosides and their fatty acid and base compositions are also alike.



(**31**) sulfatide

In addition to the "classical" sulfatides indicated above, there are a whole series of sulfatides in organisms ranging from mycobacteria to mammals. These include ganglio- and globo-series sulfatides from mammalian kidneys and ganglioside sulphatides from echinoderms. Details are given in Ishizuka (1997).

References

Brennan, P.J., Griffin, P.F., Losel, D.M., and Tyrrell, D. (1974). The lipids of fungi. *Prog. Chem. Fats Other Lipids,* **14,** 49–89.

Gurr, M.I., Harwood, J.L., and Frayn, K.N. (2002) *Lipid Biochemistry,* 5th ed., Oxford, U.K.: Blackwell Science.

Ishizuka, I. (1997) Chemistry and functional distribution of sulphoglycolipids. *Prog. Lipid. Res.*, **36**, 245–319.

IUPAC-IUB Commission on Biochemical Nomenclature (1977) The nomenclature of lipids. Recommendations, 1976. *Eur. J. Biochem.,* **79**, 11–21.

Kanfer, J.N. and Hakomori, S.-I. (1983) *Sphingolipid Biochemistry*, New York: Plenum.

Kates, M. (1986) *Techniques in Lipidology,* 2nd ed., Amsterdam: Elsevier.

Merrill, A.H. and Sweeley, C.C. (1996) Sphingolipids: metabolism and cell signalling. In *Biochemistry of Lipids, Lipoproteins and Membranes*, D.E. Vance and J. Vance, Eds., Amsterdam: Elsevier, pp. 309–327.

Ratledge, C. and Wilkinson, S.G. (Eds.) (1988) *Microbial Lipids,* vol. 1, London: Academic Press.

Svennerholm, L. (1963) Chromatographic separation of human brain gangliosides. *J. Neurochem.,* **10**, 613–623.

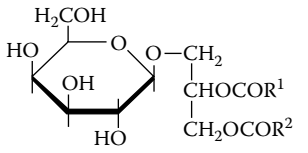
Sweeley, C.C. (1991) Sphingolipids. In: *Biochemistry of Lipids, Lipoproteins and Membranes* (D.E. Vance and J.E. Vance, Eds.), Amsterdam: Elsevier pp. 327–361.

Wiegandt, H. (1973) Gangliosides of extraneural organs. *Hoppe-Seyler's Z. Physiol.Chem.,* **354,** 1049–1056.

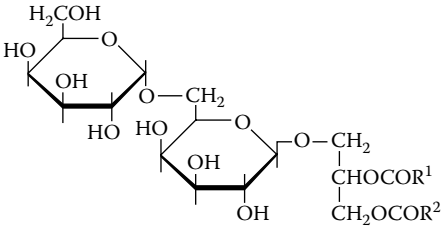
Wiegandt, H. (1992) Insect glycolipids. *Biochim, Biophys. Acta.,* **1123**, 117– 126.

1.2.5 Glycosylglycerides

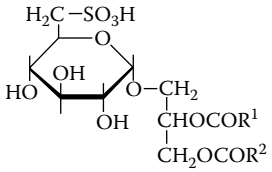
These compounds, which are especially important in the photosynthetic membranes of Cyanobacteria, algae, and higher plants, contain one to four sugars linked glycosidically to diacylglycerol. The two galactosylglycer-ides (**32**) and (**33**) contain large amounts of polyunsaturated fatty acids, e.g., >,90% α-linolenic acid in chloroplastic diacylgalactosylglycerol (monogalactosyldiacylglycerol) (Harwood, 1980). In diacyldigalactosylglycerol (digalactosyldiglyceride, often abbreviated as DGDG), the linkage between the galactose residues is 1→6. Although the additional linkages in diacyltrigalactosyl-glycerol and diacyltetragalactosylglycerol have not been fully characterized, they are also probably 1→6. These higher homologues are usually only found in small quantities and may be the result of stress to the plant. In bacteria and algae, a large number of different sugar combinations have been reported, some of those for bacteria being shown in Table 1.11. Structures of some 12 eubacterial monoglycosyldiacylglycerols and 3 polygly-cosyldiacylglycerols are given by Kates (1990). Their distribution in different bacteria is also reported.



(32) 1,2-diacyl-[β-D-galactopyranosyl-(1′→3)]-*sn*-glycerol (monogalactosyldiacylglycerol, MGDG)



(33) 1,2-diacyl-[α-D-galactopyranosyl-(1′→6′)]-β-D-galactopyranosyl-(1′→3)-*sn*-glycerol (digalactosyldiacylglycerol, DGDG)



(34) 1,2-Diacyl-[6-sulfo-α-D-quinovopyranosyl-(1′→3)-*sn*-glycerol (plant sulfolipid, sulfoquinovosyldiacylglycerol, SQDG) D-quinovose is 6-deoxy-D-glucose (note the carbon-sulfur bond)

In addition to diacylgalactosylglycerol and diacyldigalactosylglycerol, plant, algal, and cyanobacterial photosynthetic membranes also contain large amounts of diacylsulphoquinovosylglycerol (**34)** (Harwood, 1980; Harwood and Okanenko, 2003). This compound, also known trivially as the plant sulpholipid, is the only lipid with a sulphonic acid linkage that has been reported so far. It contains more saturated fatty acids (mainly palmitic) than the plant galactosylglycerides.

TABLE 1.11 Some bacterial *sn-O*-glycosyldiacylglycerols

Glyceride	Structure of Glycoside Moiety	Occurrence
Monoglucosyldiacylglycerol	α-D-Glucopyranoside	*Pneumococcus, Mycoplasma*
Diglucosyldiacylglycerol	β-D-Glucopyranosyl(1→6)-O-β-D-glucopyranoside	*Staphylococcus*
Diglucosyldiacylglycerol	α-D-Glucopyranosyl(1→2)-O-β-D-glucopyranoside	*Mycoplasma, Streptococcus*
Dimannosyldiacylglycerol	α-D-Mannopyranosyl(1→3)-O-D-mannopyranoside	*Micrococus lysodeikticus*
Galactofuranosyldiacylglycerol	α-D-Galactofuranoside	*Mycoplasma, Bacteroides*
Galactosylglucosyldiacylglycerol	α-D-Galactopyranosyl(1→2)-O-α-D-glucopyranoside	*Lactobacillus*
Glucosylgalactosylglucosyl-diacylglycerol	α-D-Glucopyranosyl(1→6)-O-α-D-galactopyranosyl(1→2)-O-α-D-glucopyranoside	*Lactobacillus*

Adapted from Kates (1972).

A number of gluco- and galactoglycerolipids have been isolated in small quantities from animal tissue. Their structures are given in Murray and Narasimhan (1990). The majority of galactoglycerolipids contain a single galactose residue, which is linked in a β-glycosidic link between the C-1 of galactose and the C-3 of glycerol. The glucoglycerolipids constitute a large number of compounds with up to eight glucose residues linked α(1→6). Alkylacyl and diacyl lipids as well as sulfated forms have been reported (Slomiany et al., 1987).

TABLE 1.12 Structures of the sulfolipids of *Ochromonas danica*

Derivative	Position of substitution in 1,14-dicosane-disulfate	Position of substitution in 1,15-tetracosane-disulfate
Monochloro-	13	14
Dichloro-	11,15	
Pentachloro-	3,3,11,13,16	2,12,14,16,17
Hexachloro-	3,3,11,13,15,16	2,2,12,14,16,17

References

Harwood, J.L. (1980) Plant acyl lipids: structure, distribution and analysis. In *Biochemistry of Plants,* vol. 4, P.K. Stumpf and E. E. Conn, Eds., New York: Academic Press, pp. 1–55.

Harwood, J.L. and Okanenko, A.A. (2003) Sulphoquinovosyl diacylglycerol — the sulpholipid of higher plants. In *Sulfur in Plants,* Y.P. Abrol and A. Ahmad, Eds., Dordrecht: Kluwer, pp. 189–219.

Kates, M. (1972) *Techniques in Lipidology,* 2nd ed., Amsterdam: Elsevier.

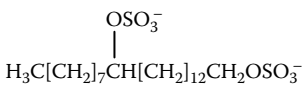
Kates, M. (1990) Glyco-, phosphoglyco- and sulfoglycoglycerolipids from bacteria. In *Handbook of Lipid Research*, vol.6, M. Kates, Ed., New York: Plenum, pp. 1– 122.

Murray, R.K and Narasimhan, R. (1990) Glycerolipids in animal tissues. In *Handbook of Lipid Research,* vol. 6, M. Kates, Ed., New York: Plenum, pp. 321–361.

Slomiany, B.L., Murty, V.L.N., Liau, Y.N., and Slomiany, A. (1987) Animal glycoglycerolipids. *Prog. Lipid Res.,* **26**, 29– 51.

1.2.6 Sulfur–containing lipids

In addition to cerebroside sulfates, other sulfur-containing sphingolipids (Section 1.2.4) and diacylsulpho-quinovosylglycerol and sulfated gluco- or galactogly-cerolipids (Section 1.2.5), various other sulfur-containing lipids have been reported. These include alkyl sulfates (Mayers et al., 1969) in microorganisms (**35**).

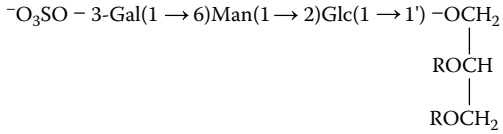


(35) (1,14*S*)-Docosanediol-1,14-disulfate

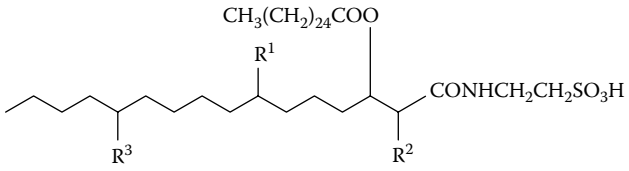
Chlorosulpholipids are found in some fungi (e.g., caldariomycin) and certain algae. *Ochromonas danica* contains particularly high amounts of chlorosulpholipids, where they represent almost half of the total membrane lipids. An entire family of compounds can be found with two sulfate ester functions and from one to six chlorines (Haines, 1973), as shown in Table 1.12. Some other structures (e.g., trichloro derivatives) have been reported but not characterized (Haines, 1973).

An unusual glycolipid sulfate ester (**36**) has been reported in extremely halophilic bacteria by Kates and coworkers (cf. Kates, 1972), and a glycolipid sulfate (2,3,6,6′-tetraacetyl-α-α-tetrahalose-2′-sulfate) in *Mycobacterium tuberculosis* (Goren, 1970). For a review of sulfated glycolipids in Archaebacteria, see Kates (1990), and for mycobacterial sulphoglycolipids, see Goren (1990). Sulfated glycolipids and sterols are minor components of animal tissues (see Murray and Narasimhan, 1990). Lactosyl sulfatide and seminolipid are examples of the former (Ishizuka, 1997).

A number of novel taurine-containing lipids have been isolated from the ciliated protozoan, *Tetrahymena*. These have the structures shown in (**37**).



(36) Glycolipid sulfate ester



(37) Taurine-containing lipids

Lipid	R^1	R^2	R^3
Taurolipid A	OH	H	H
Taurolipid B	OH	OH	H
Taurolipid C	OH	OH	OH
7-Acyltaurolipid A	$CH_3(CH_2)_{14}COO$	H	H

An alkaline-stable, taurine-containing lipid, lipo-taurine (2-(7,13-dihydroxy-2-*trans*-octadecenoylamino)ethanesulphonic acid) was also detected and probably plays a role as a metabolic intermediate. Other related compounds, such as 2-(octadecanoylamino)ethanesulphonic acid, were also identified. The isolation, characterization, and biochemistry of the taurolipids have been reviewed (Kaya, 1992).

References

Goren, M.B. (1970) Sulfolipid I of *Mycobacterium tuberculosis*, strain H37Rv. II Structural studies. *Biochim. Biophys. Acta,* **210**, 127– 138.

Goren, M.B. (1990) Mycobacterial fatty acid esters of sugars and sulfosugars. In *Handbook of Lipid Research, vol.* 6, M. Kates, Ed., Plenum, New York, pp. 363– 461.

Haines, T.H. (1973). Halogenated sulphatides. In *The Biochemistry of Lipids,* T.W. Goodwin, Ed., MTP International Reviews of Science, Biochemistry Series 1, vol. 4, Butterworths, London, pp. 271–286.

Ishizuka, I (1997) Chemistry and functional distribution of sulfoglycolipids, *Prog. Lipid Res,* **36**, 245–319.

Kates, M. (1986) *Techniques of Lipidology,* 2nd ed. Elsevier, Amsterdam.

Kates, M. (1990) Glyco-, phosphoglyco-, and sulfoglycoglycerolipids of bacteria. In *Handbook of Lipid Research,* vol. 6 (M. Kates, Ed.), Plenum, New York, pp. 1–122.

Kaya, K. (1992) Chemistry and biochemistry of taurolipids. *Prog. Lipid Res.,* **31**, 87–108.

Lederer, E. (1967). Glycolipids of mycobacteria and related microorganisms. *Chem. Phys. Lipids,* **1**, 294–315.

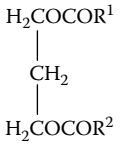
Mayers, G.M. et al. (1969). Microbial sulfolipids. III. Disulfate of (+) -1, 14-docosanediol in *Ochromonas danica*. *Biochemistry,* **8**, 2981–2986.

Murray, R.K. and Narasimhan, R. (1990) Glycerolipids of animal tissues. In *Handbook of Lipid Research,* vol. 6, M. Kates, Ed., Plenum, New York, pp. 321–361.

1.2.7 Diol lipids

Only recently has the presence of diol lipids, such as diacylpropane-1,3-diol (**38**), been confirmed for a wide variety of tissues. This is probably because techniques for the elucidation of their structures are a recent development. Small quantities of such diols are found in mammalian and fish liver, mammalian adipose tissue, egg yolk, corn seed, and yeast.

Diesters of butane-1,3-diol and butane-1,4-diol are produced by various yeasts (Ratledge and Wilkinson, 1988) and mixed acyl and alk-1-enyl derivatives of these and other simple diols (e.g., ethylene glycol) have been reported (Batrakov et al., 1974). Extracellular acyl esters of arabinitol, xylitol, or mannitol have been reported (Stodola et al., 1967) and an acylated diol phospholipid has been isolated from the yeast *Lipomyces starkeyi,* after growth on propane-1,2-diol (Suzuki and Hasegawa, 1974).



(**38**) Diacylpropane-1,3-diol

References

Batrakov, S.G. et al. (1974) Identification of *threo*-butane-2,3-diol phospholipid from *Actinomyces olivaceus.*, *Biochim. Biophys. Acta,* **337,** 29– 40.

Ratledge, C. and Wilkinson, S.G. (1988) An overview of microbial lipids. In *Microbial Lipids,* vol. 1, C. Ratledge and S.G. Wilkinson, Eds., Academic Press, London, pp. 3–22.

Stodola, F.H. et al. (1967) Extracellular lipids of yeasts. *Bacterial Rev* , **31,** 194–213.

Suzuki, T. and Hasegawa, K. (1974) Diol lipids in the phospholipid fraction of *Lipomyces starkeyi* grown in the medium containing 1,2-propanediol. *Agric. Biol. Chem.,* **38**, 613–620.

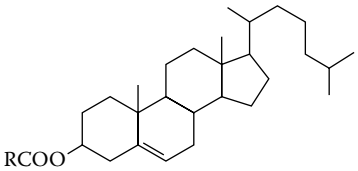
1.2.8 Other esters

A wide variety of other lipid esters have been reported. Wax esters are a typical example. Although the term "wax" should, strictly speaking, only be used for esters of long-chain fatty acids with long-chain primary alcohols, common usage, unfortunately, often equates "wax" with an entire mixture of lipids of which the true waxes are but a part. Ester waxes are found in animals and plants where they form part of the water-repellent surface coating (i.e., skin surface of animals and the leaf cuticle (cf. Section 1.2.11)). The general formula for a simple wax is shown in (**39**). The preen glands of birds, in addition, contain esters of normal alcohols with mono- or multibranched fatty acids (Odham, 1967).

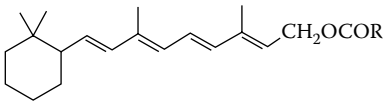
(39) A simple wax

Complex waxes are compounds where either the fatty acid or the alcohol component or both has a complex structure. For example, the waxes of *Mycobacterium* spp. are diesters of phthiocerols (C_{33}-C_{35} branched-chain diols)

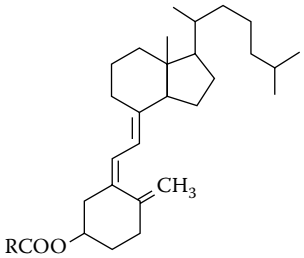
with mycocerosic acids (C_{29}-C_{32} branched-chain acids) (Asselineau, 1966; see Barry et al., 1998).



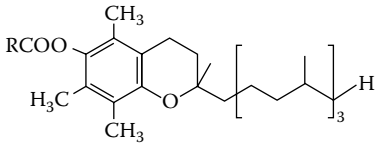
(40) Cholesterol ester



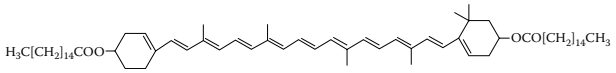
(41) Acyl retinol



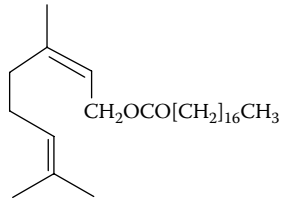
(42) Acyl cholecaldiferol



(43) Acyl-α-tocopherol



(44) Luteol dipalmitate



(45) Geranyl stearate

Animal skin-surface lipids have two types of diester waxes. In the first, a hydroxy acid has its hydroxyl group esterified to a normal fatty acid and its carboxyl group to a fatty alcohol. The second wax type consists of an alkane α, β-diol in which both hydroxyls are esterified with fatty acids (Nicolaides et al. 1970).

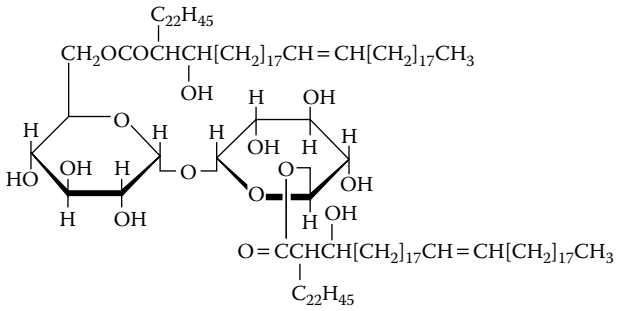


FIGURE 1.15 Cord factor from Mycobacterium smegmatis.

Esters are found in most of the commonly occurring sterols (**40**), including those from plant tissues (Mudd, 1980). The fatty acid constituents usually reflect those of the acylglycerols from the same source. Likewise, esters are found in vitamin alcohols, such as vitamin A, the D vitamins, and vitamin E. Examples are shown in (**41**) to (**43**).

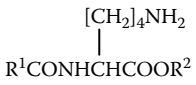
Carotenoid esters have been reported from a few plant sources (Hitchcock and Nichols, 1971). Flower pigments, for example, are known to contain saturated fatty acid esters of carotenoid alcohols. These compounds are also found in green algae. Where dihydroxy alcohols are involved, then both substitutents are usually esterified, e.g., (**44**).

In addition, acyl esters of terpenoid alcohols have been reported. For example, Dunphy and Allcock (1971) showed that 30 to 60% of the total monoterpenoid alcohol content of rose petals occurred as acyl esters with geranyl stearate **(45)** predominating.

An important example of a carbohydrate ester is the so-called cord factor from *Mycobacteria* spp. This contains an ester of the disaccharide, trehalose, with two molecules of a complex acid, mycolic acid. The latter is a general term embracing a whole series of fatty acids containing 60 to 90 carbons. They are hydroxy fatty acids that differ in their degree of unsaturation and chain branching (see Section 1.1.2.10). In the example given in Figure 1.15, the mycolic acid is the 60-carbon compound found in *Mycobacterium smegmatis*.

Various other esters have been reported in different bacteria. For example, propionibacteria contain diacyl *myo*-inositol mannosides in which the mannose is glycosidically linked to position 2 of *myo*-inositol. The 1 and 6 positions of inositol are esterified with fatty acids. Other bacteria and yeasts contain esters of glucose and certain other sugars (Lederer, 1967; Weete, 1980).

Not only are simple carbohydrate esters found in Nature, but fatty acyl derivatives of amino acids have also been reported. These include serratamic acid (*N*-(D-3-hydroxydecanoyl)-L-serine), siolipin A **(46),** and siolipin B. The latter is the ornithine analogue of siolipin A.



R^1COOH = normal, branched and β-hydroxy-branched acids

R^2OH = long-chain polyalcohols

(46) Siolipin A

The peptidolipids that occur in mycobacteria and *Nocardia* spp. are *N*-acyl oligopeptides. They often occur as glycoside derivatives (Kates, 1972).

References

Asselineau, J. (1966) *The Bacterial Lipids,* Hermann, Paris.

Barry, C.E. et al. (1998) Mycolic acids: structure, biosynthesis and physiological functions. *Prog. Lipid. Res.* **37**, 143–179.

Dunphy, P.J. and Allcock, C. (1971) quoted by Hitchcock and Nichols (1971).

Hitchcock, C. and Nichols, B.W. (1971) *Plant Lipid Biochemistry,* Academic Press, London.

Kates, M. (1986) *Techniques in Lipidology,* 2nd ed., Elsevier, Amsterdam.

Lederer, E. (1967) Glycolipids of mycobacteria and related microorganisms. *Chem. Phys. Lipids,* **1**, 294–315.

Mudd, J.B. (1980) Sterol interconversions. In *The Biochemistry of Plants,* P.K. Stumpf and E.E. Conn, Eds., vol. 4. Academic Press, New York, pp. 509–534.

Nicolaides, F., Fu, H.C., and Ansari, M.N.A. (1970) Diester waxes in surface lipids of animal skin. *Lipids,* **5**, 299–307.

Odham, G. (1967) Fatty acids in the feather waxes of some water birds. *Fette Seifen Anstrichm.* **69**, 164–172.

Weete, J.D. (1980) *Lipid Biochemistry of Fungi and Other Organisms,* Plenum, New York.

1.2.9 Glycosides

Several types of glycosides can be identified — those of hydroxy fatty acids, aromatic glycols and sterols.

Glycolipids of some microorganisms, particularly yeasts, are extracellular products. There has been increasing interest in several of these compounds as biosurfactants (Solaiman, 2005). Emulsan, a polyanionic heteropolysaccharide having acyl chains esterified to the sugar moieties, is produced by *Acinetobacter calcoaceticus.* Often the lipids contain a mono- or disaccharide glycosidically linked to a hydroxy acid. Examples would be a rhamnolipid from *Pseudomonas aeruginosa,* a sophorolipid from *Candida bombicola* (see Figure 1.16) and cellobiolipids from *Ustilago maydis* (see Ratledge and Wilkinson, 1988). Emulsan is used as a degreasing agent and detergent. Rhamnolipid is used for oil recovery, in the printing industry and in a multitude of applications as a detergent in the agrochemical, food and cosmetic industry, and as a component of germicidal solutions for food and medical uses (Solaiman, 2005).

Mycosides from mycobacteria are glycosides of methylated sugars with a long-chain, highly branched hydroxylated hydrocarbon terminated by a phenol group. The hydroxy groups of the long-chain glycol are esterified with mycocerosic and palmitic acids (Kates, 1972).

Acylated steryl glycosides of plants usually contain D-glucose attached via a β-glycosidic linkage to the 3-position of sterols, such as sitosterol, stigmasterol, campesterol, and cholesterol (see Figure 1.17). The 6-position of the glucose is esterified with fatty acids, such as palmitic, stearic, oleic, linoleic, and linolenic (Mudd and Garcia, 1975). In some tissues, such as potato tubers, acylated sterol glycosides are major components (around 20% total) (Mudd, 1980). Although glucose is the major esterified sugar, galactose, mannose, xylose, and gentiobiose have been found in isolated cases and an α-glycosidic link also reported (Harwood, 1980).

Nitrogen-fixing cyanobacteria produce heterocyst cells containing characteristic glycolipids (see Murata and Nishida, 1987). The chemical structures of major components of *Anabaena cylindrica* are shown in **(47)** and (**48**). Glycerol ester glycolipids **(49)** and **(50)** are also present (Murata and Nishida, 1987). The heterocyst lipids of *Nodularia harveyana* (a marine cyanobacterium) have been purified

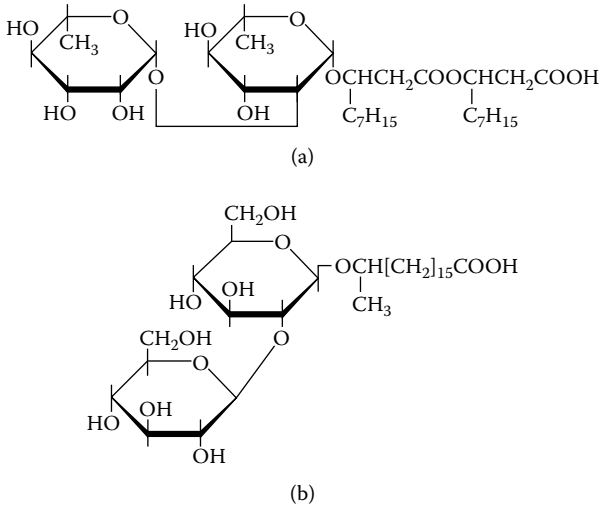


FIGURE 1.16 (a) Rhamnolipid of *Pseudomonas aeruginosa;* (b) sophorolipid (yeasts).

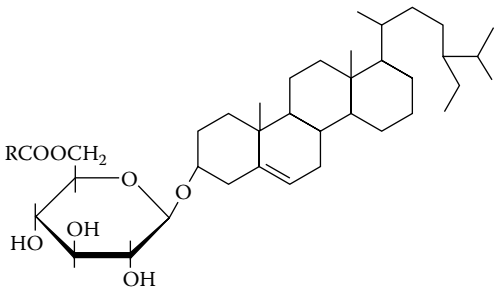
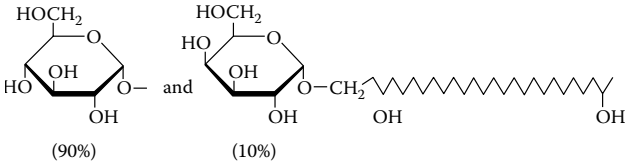


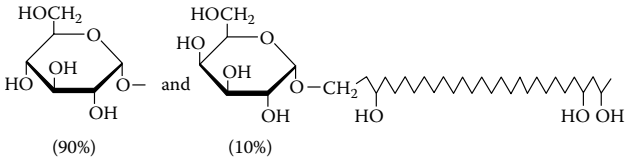
FIGURE 1.17 Acylated steryl glucoside (ASG).

recently and fully characterized as 1-(*O*-α-D-glucopyranosyl)-3*R*, 25*R*-hexacosanediol, 1-(*O*-α-D-glucopyranosyl)-3*S*, 25*R*-hexa-cosanediol, and 1-(*O*-α-D-glucopyranosyl)-3-keto-25*R*-hexacosanol (Soriente et al*.,* 1992).

Glycosidic glycolipids

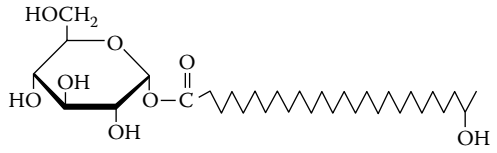


(47) 3,25-Dihydroxyhexacosanyl α-D-glycopyranoside

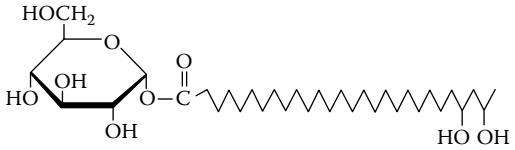


(48) 3,25,27-Trihydroxyoctacosanyl α-D-glycopyranoside

Glycosyl ester glycolipids



(49) α-D-Glucopyranosyl 25-hydroxyhexacosanate



(50) α-D-Glucopyranosyl 25,27-dihydroxyoctacosanate

References

Harwood, J.L. (1980) Plant acyl lipids: structure, distribution and analysis. In *Biochemistry of Plants,* Vol. 4, P.K. Stumpf and E.E. Conn, Eds., Academic Press, New York, pp. 1–55.

Kates, M. (1986) *Techniques in Lipidology,* 2nd ed. Elsevier, Amsterdam.

Mudd, J.B. (1980) Sterol interconversions. In *The Biochemistry of Plants*, P.K. Stumpf and E.E. Conn, Ed., vol. 4, Academic Press, New York, pp. 509–534.

Mudd, J.B. and Garcia, R.E. (1975) Biosynthesis of glycolipids. In *Recent Advances in the Chemistry and Biochemistry of Plant Lipids,* T. Galliard and E. I. Mercer, Eds., Academic Press, New York, pp. 161–201.

Murata, N. and Nishida, I. (1987) Lipids of blue-green algae (cyanobacteria). In *The Biochemistry of Plants,* vol. 9, P.K. Stumpf and E. E. Conn, Eds., Academic Press, New York, pp. 315–347.

Ratledge, C. and Wilkinson, S..G. Eds., (1988) *Microbial Lipids,* vol. 1, Academic Press, London.

Solaiman, D.K.Y. (2005) Applications of microbial biosurfactants. INFORM 16, 408–410.

Soriente, A., Sodano, G., Gambacorta, A., and Trincone, A. (1992) Structure of "heterocyst glycolipids" from the marine cyanobacterium, *Nodularia harveyana. Tetrahedron,* **48,** 5375–5384.

1.2.10 Arsenolipids

The occurrence of lipid-soluble arsenic compounds in marine organisms was first reported over 30 years ago (Lunde 1973). At the present time, more than 100 naturally occurring arsenolipids have been reported, as reviewed recently (Dembitsky and Levitsky, 2004). They are found in a wide variety of organisms ranging from lichens, fungi, and plants, to freshwater and marine algae, and invertebrates, fishes, and animals. The primary analytical technique used for separation, identification, and quantification of arsenolipids is LC coupled to various types of mass spectrometry and a full discussion is given in Dembitsky and Levitsky (2004).

Many species of bacteria seem to be active in metabolising arsenic compounds and, in particular, have been shown to be capable of producing methylated derivatives, such as trimethylarsine. These include soil organisms, such as *Flavobacterium* or *Pseudomonas* spp. (Shariatpanahi et al*.,* 1981) as well as microorganisms from the deep sea (1000 to 3500 m) where they seem important for the metabolism of arsenic compounds eventually to simpler metabolites and inorganic arsenic (Hanaoka et al*.,* 1997). Other notable microorganisms with high contents of arsenic, including arsenobetaine and arsenocholine compounds, are various halophytes (Oremland and Stolz, 2003).

Some examples of arsenolipids found in freshwater and marine algae are given in Figure 1.18. Marine brown algae have been well studied (Dembitsky and Levitsky, 2004), but green and red algae have also been noted to actively metabolise arsenic compounds. From freshwater environments, *Chlorella* spp. and C*hlamydomonas reinhardtii* have been studied. Most algal species seem well capable of methylating arsenic as part of the conversion to compounds isolated in the polar lipid fraction. A variety of arsenolipids were identified and these could accumulate in the range of 1.5 to 33.8 μg/g dry weight.

Arsenolipids have been identified in marine invertebrates (Benson, 1989), including jellyfish, crustacea, worms and molluscs. Freshwater molluscs, crustacea, and earthworms have also been studied. The major arsenic compound in marine fish and animals is arsenobetaine, first detected in lobsters (Edmonds et al*.,* 1977). Other animals studied include the sperm whale. In plants, arsenic-containing lipids have been identified in species ranging from higher plants through ferns and lichens.

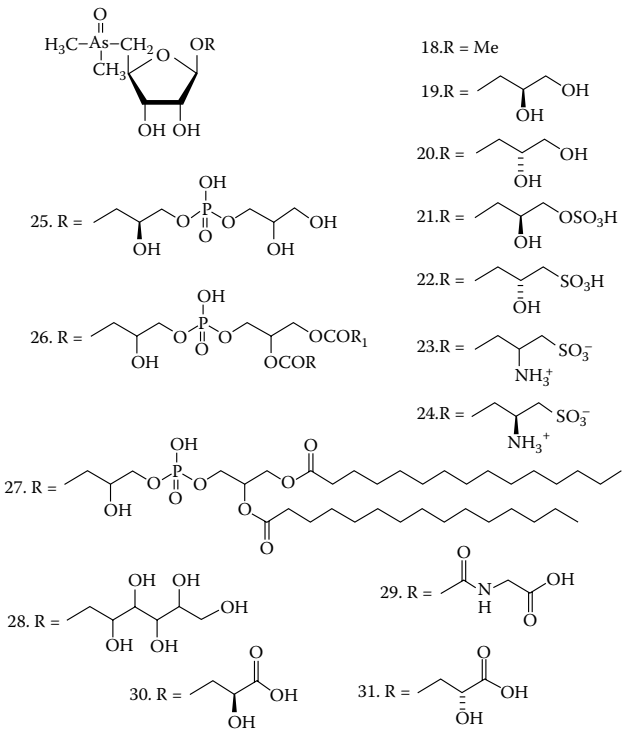


FIGURE 1.18 Arsenolipids isolated from freshwater and marine algae. (From Dembitsky, V.M. and Levitsky D.O. (2004) Arsenolipids. *Prog. Lipid Res.,* **43**, 403–448. With permission.)

Some plants are hyper-accumulators and may take up and accumulate more than 1 mmol As g^{-1} dry weight (Brooks et al., 1977).

Details of all these reports of different arsenolipids in various species and their possible metabolism are given in Dembtisky and Levitsky (2004).

References

Benson, A.A. (1989) In *Marine Biogenic Lipids, Fats and Oils*, R.G. Actaman, Ed., vol. 1, Boca Raton, FL: CRC Press, pp. 243– 250.

Brooks R.R. et al. (1977) Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants. *J. Geochem. Explor.* **7**, 49–57.

Dembitsky, V.M. and Levitsky D.O. (2004) Arsenolipids. *Prog. Lipid Res.*, **43**, 403–448.

Edmonds, J.S. et al. (1977) Isolation, crystal-structure and synthesis of arsenobetaine, arsenical constituent of western rock lobster *Panulirus longipes cygnus* George. *Tetrahedron Lett.* **18**, 1543–1546.

Hanoaka, K. et al. (1997) Arsenobetaine-decomposing ability of marine microorganisms occurring in particles collected at depths of 1100 and 3500 metres. *Appl. Organomet. Chem.* **11**, 265–271.

Lunde, G. (1973) Synthesis of fat and water-soluble arseno organic compounds in marine and limnetic algae. *Acta Chem. Scand.* **27**, 1586–1594.

Oremland, R.S. and Stolz, J.F. (2003) The ecology of arsenic. *Science,* **300**, 939–944.

Shariatpanahi, M. et al. (1981) Biotransformation of the pesticide sodium arsenate. *J. Environ. Sci. Health*, **16**, 35–47.

1.2.11 Waxes

Plant wax is the general term used to describe the lipid components of the cuticle that covers the outer surface of aerial plant tissues or is associated with the suberin matrix of underground or wound tissues. The components of plant cuticular waxes have been reviewed by Kolattukudy (1980, 1987) and Walton (1990). Major components include hydrocarbons, very long-chain fatty acids, alcohols and monoesters (Table 1.13 and Table 1.14).

Surface waxes are exposed to the environment and, therefore, are chemically rather stable. Thus, there is an absence of functional groups, which might be susceptible to attack by atmospheric agents. Furthermore, the very long carbon chains of most wax components reduces their volatility. In addition, many of compounds present in surface waxes are rather stable metabolically and are not readily susceptible to microbial degradation (Kolattukudy, 1976).

Certain general structural features of natural waxes have been described by Kolattukudy (1976), and these are summarized in Table 1.13. However, it must also be stressed that the structure and composition of surface waxes vary considerably from organism to organism. Thus, with regard to Table 1.13, the longer aliphatic chains are more abundant in plant waxes than in animal surface waxes, whereas bird waxes may contain appreciable amounts of chains of less than 16 carbons. With regard to branching, methyl branches are the most common, but, in birds, ethyl and propyl branches are found. Although polyunsaturated carbon chains are nearly always absent from surface waxes, in insects substantial proportions of di-unsaturated hydrocarbons have been found. In this case, autoxidation may be reduced by the simultaneous presence of cuticular phenolics. So far as the general composition of surface waxes is concerned, very long chain hydrocarbons are common in insects and plants, but rare in animals. Higher plant waxes contain the most complex

TABLE 1.13 General structural features of natural waxes

Chain length	Very long chains (up to C_{62}) are common
Branching	Branched carbon chains common, with methyl branches frequent
Unsaturation	Polyunsaturated chains nearly always absent; double bonds, when present, at different position from those of internal lipids
Functional types	Saturated hydrocarbons, olefins, wax esters, aldehydes, ketones, primary and secondary alcohols, and terpenoids can be present; the bulk of the surface lipid is distinctly different from the major internal lipids of the same organism

TABLE 1.14 Major classes of plant aliphatic wax components

Wax Class	Chain-Length Range in Plants	Notes for Plants in General	Major *Arabidopsis* Components	% in *Arabidopsis* Stems
n-Alkanes	C_{21}-C_{35}	Common; usually C_{29}, C_{31}	C_{29},C_{31},C_{27}	38
Secondary alcohols	C_{21}-C_{35}	About as common as ketones	C_{29},C_{31},C_{27}	10
Ketones	C_{21}-C_{35}	Not as common as alkanes	C_{29}	30
Fatty alcohols	C_{22}-C_{34}	Common, even chains predominate	C_{28},C_{30}, C_{26}	12
Fatty acids	C_{16}-C_{34}	Very common; even-chain saturated usually	C_{30}, C_{28}	3
Aldehydes	C_{21}-C_{35}	Usually minor; not as common as alcohols	C_{30}, C_{28}	6
Wax esters	C_{32}-C_{64}	Common	-	1

Source: See Kolattukudy, P.E. (1980) In *Biochemistry of Plants,* vol. 4 (P.K. Stumpf and E. E. Conn, Eds.), Academic Press, New York, pp. 571–645; Kunst, L. and Samuels, A.L. (2003) *Prog. Lipid. Res.* **42**, 51–80.

mixture of components, while insects and birds have the simplest.

The wax associated with suberin has also been examined, and very long-chain fatty acids, alcohols and terpenes have all been found. These are all typical components of cuticular wax, but certain differences have been noted. The hydrocarbons in suberin have a broader chain-length distribution with a predominance of shorter carbon chains and more even-numbered carbon chains than cuticular wax. Suberin-associated wax also contains a high proportion of free fatty acids. Free and esterified alkan-2-ols are also present (Kolattukudy, 1980).

Further details of the wax components of other organisms will be found in Section 2.5.

References

Hamilton, R.J. Ed., (1996) *Waxes: chemistry, molecular biology and functions.* The Oily Press, Dundee, Scotland.

Kolattukudy, P.E. Ed., (1976) *Chemistry and Biochemistry of Natural Waxes,* Elsevier, New York.

Kolattukudy, P.E. (1980) Cutin, suberin and waxes. In *Biochemistry of Plants,* vol. 4, P.K. Stumpf and E.E. Conn, Eds., Academic Press, New York, pp. 571– 645.

Kolattukudy, P.E. (1987) Lipid-derived defensive polymers and waxes and their role in plant-microbial interaction. In *Biochemistry of Plants,* vol. 9, P.K. Stumpf and E.E. Conn, Eds., Academic Press, New York, pp. 291–314.

Kunst, L. and Samuels, A.L. (2003) Biosynthesis and secretion of plant cuticular wax. *Prog. Lipid. Res.* **42**, 51–80.

Walton, T.J. (1990) Waxes, cutin and suberin. In *Methods in Plant Biochemistry,* vol. 4, J.L. Harwood and J.R. Boyer, Eds., Academic Press, London, pp. 105–158.

1.2.12 Cutin and suberin

In many organisms the outer envelope or covering consists of polymers of carbohydrate or amino acids. In plants, however, the covering (cuticle) consists of a hydroxy fatty acid polymer called cutin. The underground parts and healed wound surfaces of plants are covered with an analogous material, suberin. Both cutin and suberin are embedded in or associated with a complex mixture of lipids, which is termed wax (see Section 1.2.11). The structure and composition of cutin and suberin are reviewed by Kolattukudy (1980, 1987) and by Walton (1990).

Cutin contains C_{16} and C_{18} families of acids. The former is predominate in rapidly growing plants, while both are present in the thicker cuticle of slower- growing plants. The C_{16} family is based on palmitic acid, while the C_{18} family is based on oleic acid (Table 1.15) (see also Kolattukudy, 1980).

In the cutin structure, a polyester intramolecular structure exists where crosslinking is mainly influenced by the availability of secondary hydroxyl groups. Thus, cutins that contain large amounts of epoxy, oxo, and ω-hydroxy

TABLE 1.15 The major components of cutin, the cutin acids

C_{16} Family	C_{18} Family[a]
$H_3C[CH_2]_{14}COOH$	$H_3C[CH_2]_7CH = CH[CH_2]_7COOH$
$HOCH_2[CH_2]_{14}COOH$	$HOCH_2[CH_2]_7CH = CH[CH_2]_7COOH$
$HOCH_2[CH_2]_xCHOH[CH_2]_yCOOH$ ($x + y = 13$; $y = 5$-8)	$HOCH_2[CH_2]_7CH{-}CH[CH_2]_7COOH$ (epoxide O)
	$HOCH_2[CH_2]_7CHOHCHOH[CH_2]_7COOH$

[a] Δ12 unsaturated analogues also occur.

Note: For further details, see Harwood (1980) and Kolattukudy (1977).

TABLE 1.16 Typical cutin monomers and their ability to form polyesters

Cutin Monomers (Acids) Capable of Cross-Linking a Polyester Polymer	
$HO[CH_2]_6CHOH[CH_2]_8COOH$[a]	10,16-dihydroxyhexadecanoic acid (and other positional isomers)
$HOOC[CH_2]_5CHOH[CH_2]_8COOH$	7-hydroxyhexadecanedioic acid (and other positional isomers)
$OHC[CH_2]_6CHOH[CH_2]_7COOH$[a]	9-hydroxy-16-oxohexadecanoic acid (and other positional isomers)
$HO[CH_2]_8CHOHCHOH[CH_2]_7COOH$[a]	9,10,18-trihydroxyoctadecanoic acid (and its Δ12 analogue)
$HO[CH_2]_5CHOHCHOHCH_2CHOHCHOH[CH_2]_7COOH$	9,10,12,13,18-pentahydroxyoctadecanoic acid
Cutin Monomers (Acids) Capable of Forming Only a Linear Polyester	
Monobasic	
α, ω-Dibasic	
ω-Hydroxymonobasic namely	16:0, 18:0, 18:1(9), 18:2 (9,12)
$HO[CH_2]_6CO[CH_2]_8COOH$[a]	16-hydroxy-10-oxohexadecanoic acid (and other positional isomers)
$HO[CH_2]_8\overset{\diagdown\,O\,\diagup}{CH - CH}[CH_2]_7COOH$[a]	9,10-epoxy-18-hydroxyoctadecanoic acid (and its Δ 12 analogue)

[a] Major components of cutin.
Source: Adapted from Deas and Holloway (1977).

TABLE 1.17 Polymeric form of dihydroxyhexadecanoic acid and related C_{18} acids in four plant cutins

Polymeric Form	**Total Monomers (%)**			
	Tomato	**Rosehip**	**Blackcurrant leaf**	**Lemon**
Cutin-$O[CH_2]_{15}COO$-Cutin	5	7	18	14
Cutin-$OOC[CH_2]_5CHOH[CH_2]_8COO$-Cutin	1	6	-	2
Cutin-$OOC[CH_2]_5\underset{\displaystyle O-\text{Cutin}}{\underset{\displaystyle |}{CH}}[CH_2]_8COO$-Cutin	4	5	5	1
$HO[CH_2]_6CHOH[CH_2]_8COO$-Cutin	2	5	3	2
Cutin-$O[CH_2]_6CHOH[CH_2]_8COO$-Cutin	48	50	38	25
Cutin-$O[CH_2]_6\underset{\displaystyle O-\text{Cutin}}{\underset{\displaystyle |}{CH}}[CH_2]_8COO$-Cutin	36	24	30	5
$HO[CH_2]_6\underset{\displaystyle O-\text{Cutin}}{\underset{\displaystyle |}{CH}}[CH_2]_8COO$-Cutin	3	3	4	1
Cutin—O—$[CH_2]_6CO[CH_2]_8COO$—Cutin	2	1	2	51

Source: Adapted from Deas and Holloway (1977).

monomers must be predominantly linear (Table 1.16) (Deas and Holloway, 1977). Esterification appears to occur chiefly through the primary hydroxy groups of the monomers. A significant portion (up to 40%) of the monomers is also cross-linked through secondary hydroxyl groups (Table 1.16 and Table 1.17).

Considerable diversity is evident when cutins from different sources are compared in detail. It is of interest, though, that the cutin composition of delicate tissues, such as spinach leaves, is essentially similar to that of much more substantial membranes. The wax part of the epidermal layer (see Section 1.2.11) is also usually similar between species, but with differences in detail (Harwood. 1980; Kolattukudy, 1980).

The major aliphatic components of suberins are ω-hydroxy acids and dicarboxylic acids. Octadec-9-enedioic acid is the usual dicarboxylic acid, and 18-hydroxyoleic, the major hydroxy fatty acid. The proportion of very long-chain fatty acids ($>C_{20}$) is usually much greater in the ω-hydroxy acid fraction than in the dicarboxylic acid fractions. Among the α,ω-diols, fatty alcohols and fatty acids, which are often found as significant components of suberin, long chains are common. Kolattukudy has suggested some basic rules for the classification of hydroxy acid phytopolymers as cutin or suberin (Table 1.18). However, these rules must be regarded only as a guide, since the examination of individual plant species has provided exceptions. Indeed, it should be noted that, apart from species or varietal differences, environmental conditions may cause large changes in surface lipids. Thus, light, temperature, and age have all been found to affect leaf cuticular components (Harwood. 1980).

TABLE 1.18 Compositional differences between cutin and suberin

Monomer	Cutin	Suberin
Dicarboxylic acids	Minor	Major
In-chain substituted acids	Major	Minor
Phenolics	Low	High
Very long-chain (C_{20}-C_{26}) acids	Rare and minor	Common and substantial
Very long-chain alcohols	Rare and minor	Common and substantial

Source: Kolattukudy, P.E. (1975) In *Recent Advances in the Chemistry and Biochemistry of Plant Lipids* (T. Galliard and E.I. Mercer, Eds.), Academic Press, New York, pp. 203–246. With permission.

References

Deas, A.H.B. and Holloway. P.J. (1977) The intracellular structure of some plant cutins. In *Lipids and Lipid Polymers in Higher Plants,* M. Tevini and H.K. Lichtenthaler, Eds., Springer-Verlag, Berlin. pp. 293–299.

Harwood, J.L. (1980) Plant acyl lipids: structure, distribution and analysis. In *Biochemistry of Plants.* vol. 4, P.K. Stumpf and E.E. Conn, Eds., Academic Press, New York. pp. 1–55.

Kolattukudy, P.E. (1975) Biochemistry of cutin, suberin and waxes on the lipid barriers of plants. In *Recent Advances in the Chemistry and Biochemistry of Plant Lipids,* T. Galliard and E.I. Mercer, Eds., Academic Press, New York, pp. 203–246.

Kolattukudy. P.E. (1977) Biosynthesis and degradation of lipid polymers. In *Lipids and Lipid Polymers in Higher Plants,* M. Tevini and H.K. Lichtenthaler, Eds., Springer-Verlag, Berlin. pp. 271–292.

Kolattukudy, P.E. (1980) Cutin, suberin and waxes. In *Biochemistry of Plants,* vol. 4, P.K. Stumpf and E.E. Conn, Eds., Academic Press, New York, pp. 571–645.

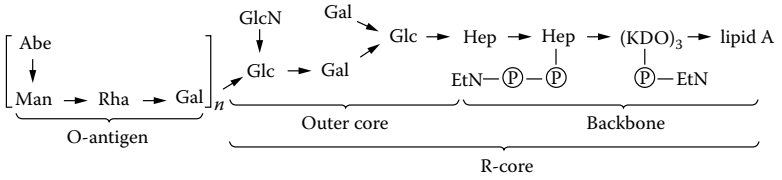
Kolattukudy, P.E. (1987) Lipid-derived defensive polymers and waxes and their role in plant-microbe interaction. In *Biochemistry of Plants,* vol. 9, P.K. Stumpf and E.E. Conn, Eds., Academic Press, New York, pp. 291–314.

Walton, T.J. (1990) Waxes, cutin and suberin. In *Methods in Plant Biochemistry,* vol. 4, J.L. Harwood and J.R. Boyer, Eds., Academic Press, London, pp. 105–158.

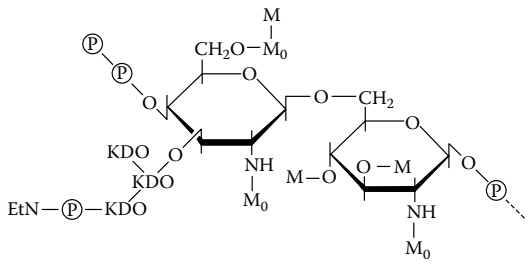
1.2.13 Bacterial-wall lipids

A full discussion of the varied lipid structures found in bacterial cell walls is beyond the scope of this book, but the reader will find detailed accounts in Rogers et al. (1980) and Goldfine (1982). More recent detailed updates on the biochemistry and distribution of the cell-wall lipids of mycobacteria and other actinomycetes and of Gram-negative bacteria will be found in Brennan (1988) and Wilkinson (I988, 1996), respectively. The structure, biosynthesis, and physiological functions of mycolic acids are reviewed in Barry et al. (1998). The unique lipid-rich cell walls of mycobacteria contribute to their resilience and contain many compounds known to increase pathogenicity. The dimycocerosate esters (also called phthiocerol diesters) are particularly important and have been reviewed recently (Onwueme et al., 2005).

Gram-negative bacteria have a cell envelope containing two membranes, with the outer membrane having lipopolysaccharide in its outer leaflet. Lipopolysaccharide is complex and consists of four parts. On the outside is the O-antigen, which is a polysaccharide of variable structure. This is attached to a core polysaccharide, which is in two parts, an outer core and a backbone. The backbone is connected to a glycolipid, called lipid A, through a short "link" usually composed of 3-deoxy-D-manno-octulosonic acid (KDO). These structures are shown in Figure 1.19. The role of lipid A as bacterial endotoxin and further details of different structures are given in Raetz and Whitfield (2002).

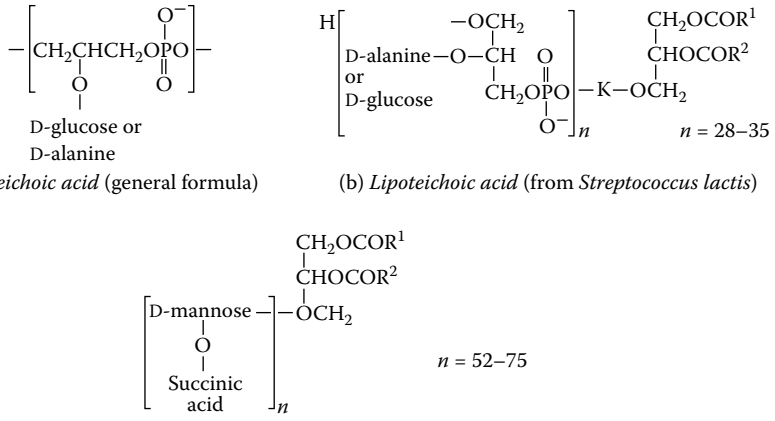


(a) *Lipopolysaccharide*



(b) *Lipid A–KDO link region*

FIGURE 1.19 Generalized structures of lipopolysaccharide and lipid A. *Abbreviations:* Abe, abequose; Man, mannose, Rha, rhamnose; Gal, galactose; Glc, glucose; Hep, heptose; KDO, 3-deoxy-D-manno-octulsonic acid; (P), phosphate; EtN, ethanolamine; M, myristate; M_0, β-hydroxymyristate. (From Harwood and Russell (1984). With permission.).



(a) *Teichoic acid* (general formula) (b) *Lipoteichoic acid* (from *Streptococcus lactis*)

(c) *Lipomannan* (from *Micrococcus lysodeikticus*)

FIGURE 1.20 Structures of some anionic polymers in bacteria. *Abbreviations:* K, kojibiose (6,*O*,β-D-glucosyl-D-glucose); R^1 and R^2, fatty acids. (From Harwood and Russell (1984) With permission.)

The cell walls and membranes of most Gram-positive bacteria contain a series of highly anionic polymers. Quantitatively, one of the most important of these is teichoic acid, which can be covalently linked to a glycolipid to give a lipoteichoic acid (Figure 1.20). An alternative type of anionic polymer. which is found in Gram-positive bacteria such as *Micrococcus lysodeikticus,* is succinylated lipomannan (Figure 1.20). Like teichoic acid, the lipomannan is embedded in the membrane by linkage to a diacylglycerol moiety.

References

Barry, C.E. et al. (1998) Mycolic acids: structure, biosynthesis and physiological function. *Prog. Lipid. Res.* **37**, 181–207.

Brennan, P.J. (1988) Mycobacterium and other actinomycetes. In *Microbial Lipids.* vol. 1, C. Ratledge and S.G. Wilkinson, Eds., Academic Press, London. pp. 203–285.

Goldfine, H. (1982) Lipids of prokaryotes — structure and distribution. *Curr. Top. Membr. Transp,* **17,** 1–43.

Harwood, J.L. and Russell, N.J. (1984) *Lipids in Plants and Microbes,* Allen and Unwin, Hemel Hempstead, U.K.

Onwueme, K.C. et al. (2005) The dimycocerosate ester polyketide virulence factors of mycobacteria. *Prog. Lipid. Res.* **44**, 259–302.

Raetz, C.R.H. and Whitfield, C. (2002) Lipopolysaccharide endotoxins. *Annu. Rev. Biochem*. **71**, 635–700.

Rogers, H.J. et al. (1980) *Microbial Cell Walls and Membranes,* Chapman & Hall, London.

Wilkinson, S.G. (1988) Gram-negative bacteria. In *Microbial Lipids.* vol. 1, C. Ratledge and S.G. Wilkinson, Eds., Academic Press, London, pp. 299–488.

Wilkinson, S.G. (1996) Bacterial lipopolysaccharides — themes and variations. *Prog. Lipid Res.* **35**, 283–343.

2

OCCURRENCE AND CHARACTERISATION OF OILS AND FATS

F. D. Gunstone and J. L. Harwood

2.1 Introduction

The first chapter in this book was concerned with the wide range of fatty acids that occur naturally and with the various natural lipids of which the acids are major constituents. This chapter is devoted to information about the natural occurrence of the lipids covering the important materials (mainly triacylglycerols) that furnish our food lipids and are the basis of the (growing) oleochemical industry and also the less common lipids, such as those occurring in leaves, in algae, etc. These may seem to be mature topics, but they are being developed in many new and important ways. The following are typical:

- Oils and fats are being produced in ever-increasing quantities. In Chapter 3 of the second edition of this book (published in 1994), it was forecast that the average annual supply of oils and fats (from 17 commodity sources) in the 5-year period of 2003 to 2007 would be 104 metric tonnes. In the harvest year 2004–2005, the supply was 136 million tonnes, with soybean oil and palm oil predominating, each at levels of 33 million tonnes.
- We now recognise a close relation between health and disease on the one hand and dietary intake of lipids on the other. Our increasing ability to modify lipid composition through seed breeding with or without genetic modification is leading to extensive changes in fatty acid composition, driven in large part by nutritional influences. These new products are considered to be healthier fats. This will be illustrated in several ways in the following section. The most dramatic example relates to the attempts — which must surely be ultimately successful — to grow plants that generate long chain PUFA (polyunsaturated fatty acids), such as eicosapentaenoic (EPA) and docosahexaenoic (DHA) in their seed lipids.
- For a long time, fats were considered to be useful only as a source of calories, but now, in addition to the recognition of essential fatty acids and to the important minor components present in oils and fats, it is recognised that many fatty acid derivatives and lipids act as signalling molecules in the complex interactions that make up life in both animals and plants.
- Gunstone (2005) has calculated the annual fatty acid production during 2004 and 2005 on the basis of the production in that year of 17 commodity oils and fats. Of the 136.4 million tonnes produced, he calculated levels for the following acids: lauric (3.4 million tonnes, 2.5% of total production), myristic (2.6, 1.9%), palmitic (27.4, 20.0%), stearic (7.2, 5.3%), oleic (47.8, 35.1%), linoleic (37.5, 27.5%), linolenic (4.5, 3.3%), and other (6.0, 4.4%). Other figures relate to oils and fats used for food purposes and show the changes resulting from industrial hydrogenation. Attention is drawn to the serious consequence of hydrogenation for the level of linolenic acid, which is the major source of omega-3 PUFA.

References

Gunstone, F.D., Fatty acid production for human consumption, *Inform*, **16**, 736–737, 2005.

2.2 Major oils from plant sources

2.2.1 Introduction

This discussion on vegetable fats is divided arbitrarily into major and minor oils, so it is necessary to consult Section 2.3 for a long list of minor oils. The oils in each section are presented in alphabetical order.

The major oils are discussed where possible in the following terms: production; harvest yields; trade (exports and imports); major components (fatty acids and triacylglycerols); minor components (phospholipids, sterols, tocols, other); major uses; and sources of information. Information is tabulated where appropriate and data for several oils may be collated in a single table. Tables at the end of this section contain information on many different oils. They include:

- Table 2.43a: Past, present, and future production of oils and fats.
- Table 2.43b: Past and present production of oils and fats.
- Table 2.44: Predicted total (million tonnes) and per capita consumption (kg per annum) on a global basis and for selected countries/regions throughout the century.
- Table 2.45: Production, consumption, imports, and exports of 17 oils and fats (million tonnes) by country/region for the calendar years 2000 to 2004 by country/region.
- Table 2.46a and Table 2.46b: Range of fatty acid composition for some major oils taken from Codex Alimentarius.
- Table 2.47: Sterols (mg/100 g oil) in a range of crude vegetable oils.
- Table 2.48: Content of tocols in selected vegetable oils, animal fats, and nuts and berries.
- Table 2.49: Some physical and chemical properties of major vegetable oils adapted from Firestone (1997).

Useful general information is available in the following sources: Rossell and Pritchard, 1991; Ucciani, 1995; Ching Kuang Chow, 2000; Gunstone, 2002 and 2006; Akoh and Min, 2002; O'Brien, 2004; Shahidi, 2005; and Murphy, 2005. Aitzetmüller and his colleagues have prepared a valuable database on seed oil fatty acids (www.bagkf.de/SOFA) and have described this in Aitzetmüller et al (2003a and 2003b). An older database is also available (www.ncaur.usda.gov/nc/ncdb).

References

Aitzetmüller, K., and Matthäus, B., Potential uses of the seed oil fatty acids database 'SOFA', *Lipid Technol. Newsl.*, **9**, 123–127, 2003a.

Aitzetmüller, K. et al. A new database for seed oil fatty acids — the database SOFA, *Eur. J. Lipid Sci. Technol.***105**, 92–103, 2003b.

Akoh, C.C. and Min, D.B. Eds. *Food Lipids — Chemistry, Nutrition and Biotechnology,* 2nd ed., Marcel Dekker, New York, 2002.

Ching Kuang Chow Ed. *Fatty Acids in Foods and Their Health Applications,* Marcel Dekker, New York, 2000.

Firestone, D. Ed. Official Methods and Recommended Practices of the American Oil Chemists' Society: Physical and Chemical Characteristics of Oils, Fats and Waxes, AOCS Press, Champaign, IL, 1997, reprinted as a book and disc, 1999.

Gunstone, F.D. Ed. *Vegetable Oils in Food Technology — Composition, Properties and Uses,* Blackwell Publishing, Oxford, U.K., 2002.

Gunstone, F.D. Ed. *Modified Foods For Use in Lipids,* Woodhead Publishing Ltd., Cambridge, U.K., 2006.

Murphy, D.J., Ed. *Plant Lipids — Biology, Utilisation and Manipulation*, Blackwell Publishing, Oxford, U.K., 2005.

O'Brien, R.D., *Fats and Oils — Formulating and Processing for Applications*, 2nd ed., CRC Press, Boca Raton, FL, 2004.

Rossell, J.B. and Pritchard, J.L.R., Ed. *Analysis of Oilseeds, Fats, and Fatty Foods*, Elsevier Applied Science, London, 1991.

Seed oil fatty acids database (SOFA) www.bagkf.de/SOFA

Shahidi, F. Ed. *Bailey's Industrial Oil and Fat Products,* 6th ed., Wiley-Interscience, Hoboken, New Jersey, USA, 2005.

Ucciani, E., *Nouveau Dictionnaire des Huiles Végétales, Compositions en acides gras,* Lavoisier Tec & Doc, Paris, 1995.

Websites (general) www.codexalimentarius.net
www.cyberlipid.org
www.fosfa.org
www.ncaur.usda.gov/currentres
www.usda.gov/nas
www.margarine.org
www.nal.usda.gov/fnic
www.oilworld.de
www.gafta.com

2.2.2 Castor oil (*Ricinus communis*)

Castor oil is unique among commodity oils in that it is rich in a hydroxy acid (ricinoleic, 12-hydroxyoleic) and is used only for industrial and cosmetic purposes. The distinct physical and chemical properties of the oil depend on the unusual chemical nature of this acid. The hydroxyl group provides additional functionality and polarity in a mid-chain position. Compared with common vegetable oils, castor oil is more viscous, less soluble in hexane, more soluble in ethanol, and is optically active. It can be converted to a range of interesting and useful materials (see Section 9.8).

The castor plant is grown mainly in India, China, and Brazil (Table 2.1). Extraction by pressing and with solvent furnishes castor oil and residual meal. The latter contains a mildly toxic alkaloid (ricinine), an extremely poisonous protein (ricin), and a heat-stable allergen.

Castor oil contains about 90% ricinoleic acid and small amounts of palmitic, stearic, oleic, linoleic, and 9,10-dihydroxystearic acids. Most of the triacylglycerols are triricinolein or glycerol esters with two ricinoleic and one other acyl chain. In contrast to some other (less common) hydroxy acid-containing oils, the hydroxyl groups in castor oil remain free and are not themselves acylated. Ricinoleic

TABLE 2.1 Major countries/regions involved in the production (from indigenous or imported seed), consumption, export, and import of castor oil in 2004/05 (1000 tonnes)

	Total (kt)	Countries/Regions
Production	522	India 316, China 106, Brazil 70
Consumption	519	China 146, EU-25 110, India 87, Brazil 65, US 39, Japan 25, Thailand 20
Exports	258	India 224
Imports	260	EU-25 110, China 45, US 39, Japan 24
Seed yield (t/ha)	0.99	India 1.07, China 0.96, Brazil 0.91

Source: Adapted from *Oil World Annual 2005*, ISTA Mielke GmbH, Hamburg, 2005.

acid is produced in nature by hydroxylation of oleic acid, probably present in a phosphatidylcholine molecule. The oil contains some sterols and some tocols, but since it is not used for food purposes these are not considered to be very important. Castor oil differs from other commodity oils and fats in that it contains high levels of ricinoleic acid (12-hydroxyoleic acid) and the oil or castor acids is a starting point for several useful chemicals (Caupin, 1997).

Sulfation converts the secondary hydroxyl group (>CHOH) to a sulfate (>$CHOSO_2OH$) with improved surfactant properties. Apart from soap, this is the earliest anionic surfactant (1874) and is still used in textile processing, leather treatment, and as an additive for cutting oils and hydraulic fluids. The sulfated hydrogenated oil has the consistency of an ointment and gives adjustable viscosity to water-based formulations with excellent skin compatibility.

Castor oil has been converted to estolides by acylation of the free hydroxyl groups with oleic acid at 175 to 250°C in the absence of any catalyst (Isbell and Cermak, 2002). Reaction with other acids has been achieved using tetrabutyl titanate as catalyst (Kulkarni and Sawant, 2003).

Dehydration of castor oil and of castor acids gives products rich in diene acids (mainly 9,11- and 9,12-18:2), some of which have conjugated unsaturation. These products are valuable alternatives to drying oils, such as tung oil, which contain conjugated trienoic acids (see Section 2.3.109).

Hydrogenated castor oil and hydrogenated castor acids, with higher melting points than the nonhydrogenated material, are used in cosmetics, coatings, and greases. Greases prepared from tallow are much improved when salts of 12-hydroxystearic acid are added.

Castor oil reacts with isocyanates to give polyurethanes, which are frequently used for wood preservation and have been developed as encapsulating materials.

Splitting ricinoleic acid with caustic soda gives C_8 and C_{10} products. At 180 to 200°C with a 1:1 caustic/castor ratio, the major products are 2-octanone and 10-hydroxydecanoic acid. At 250 to 275°C and a 2:1 ratio, the products are 2-octanol and sebacic (decanedioic) acid. The dibasic acid, when reacted appropriately, produces a nylon (polyamide) and efficient lubricants (esters).

Splitting ricinoleic acid with steam yields C_7 and C_{11} products. This splitting process has been much improved by the development of a continuous steam-cracking process. Heptanal is used in perfumes and 10-undecenoic acid shows antifungal properties and can be converted, via 11-amino-undecanoic acid, to a polyamide (Rilsan).

A new plasticiser made from fully hydrogenated castor oil and acetic acid is particularly effective with PVC and, unlike the presently used phthalates, shows no hormone disrupting effects. It is metabolised like other vegetable oils and is fully biodegradable (Anon., 2005).

For further information, see Table 2.43 to Table 2.49.

References

Anon., Danisco's plasticizer base on vegetable oil gets EU approval, *Lipid Technol.*, **17**, 51–52, 2005.

Caupin, H-J., Products from castor oil in Gunstone, F.D. and Padley, F.B. Eds. *Lipid Technologies and Applications*, Marcel Dekker, New York, 1997, pp. 787–795.

Isbell, T.A. and Cermak, S.C., Synthesis of triglyceride estolides from lesquerella and castor oils, *J. Amer. Oil Chem. Soc.*, **79**, 1227–1233, 2002.

Kulkarni, M.G. and Sawant, A.B., Some physical properties of castor oil esters and hydrogenated castor oil esters, *Eur. J. Lipid Sci. Technol.*, **105**, 214–218, 2003.

2.2.3 Cocoa butter (*Theobroma cacao*)

The commercial exploitation of cacao or cocoa beans was probably first practised by the Aztecs. The Spanish transferred the bean from Mexico to Europe in the 16th century, where it was consumed as a drink. Chocolate was developed only in the 19th century. The plant is an evergreen tree growing to 5 to 10 metres. The fruit is a large pod approximately 15 to 20 cm long and 7 cm in diameter containing 25 to 50 seeds embedded in a soft sweet edible pulp (Nickless, website).

Production figures for cocoa butter are not included in the statistics generally cited for oil and fat production, but according to information cited in www.gobi.co.uk, world consumption of cocoa butter was over 700 kt in 2003 and is growing at a rate around 2% a year. Europe is the largest consuming region accounting for 60% of world consumption and Germany, the U.S., and France are the main importing countries.

Cocoa is grown mainly in West Africa (Ghana, Ivory Coast, Nigeria), Malaysia, Brazil, Central America, India, and Sri Lanka. The composition of cocoa butter from these different sources varies somewhat as shown in Table 2.2 for cocoa butter from Ghana, Ivory Coast, Brazil, and Malaysia. Small differences in fatty acid composition are reflected in the iodine value, but more significantly in the triacylglycerol composition and, consequently, in the melting profile. The average content

TABLE 2.2 Composition and properties of cocoa butter from different countries

	Ghana	Ivory Coast	Brazil	Malaysia
Iodine value	35.8	36.3	40.7	34.2
Melting point °C	32.2	32.0	32.0	34.3
Diacylglycerols (%)	1.9	2.1	2.0	1.8
Free acid (%)	1.53	2.28	1.24	1.21
Component acids (%)				
Palmitic	24.8	25.4	23.7	24.8
Stearic	37.1	35.0	32.9	37.1
Oleic	33.1	34.1	37.4	33.2
Linoleic	2.6	3.3	4.0	2.6
Arachidic	1.1	1.0	1.0	1.1
Component triacylglycerols (%)				
Trisaturated	0.7	0.6	trace	1.3
Monounsaturated	84.0	82.6	71.9	87.5
POP	15.3	15.2	13.6	15.1
POSt	40.1	39.0	33.7	40.4
StOSt	27.5	27.1	23.8	31.0
Diunsaturated	14.0	15.5	24.1	10.9
Polyunsaturated	1.3	1.3	4.0	0.3
Solid content (pulsed NMR) — after tempering for 40 hours at 26°C				
20°C (%)	76.0	75.1	62.6	82.6
25°C (%)	69.6	66.7	53.3	77.1
30°C (%)	45.0	42.8	23.3	57.7
35°C (%)	1.1	0.0	1.0	2.6

Source: Adapted from Shukla, V.J.S., *Inform*, **8**, 152–162, 1997. The original paper contains more details along with information on cocoa butter from India, Nigeria, and Sri Lanka.

of the important SOS triacylglycerols (S = saturated, O = oleic) varies between 87% in Malaysian and 72% in Brazilian cocoa butter, with the African samples midway between these extremes (Shukla, 1995 and 1997, see also Kurvinen et al, 2002). There is, however, some evidence that the cocoa butters of different geographical origins are becoming more alike.

Harvested pods are broken open and left in heaps on the ground for about a week during which time the sugars ferment. The beans are then sun dried and are ready for transportation and storage. To recover the important components, the beans are roasted at ~150°C, shells are separated from the cocoa nib, and the latter is ground to produce cocoa mass. When this is pressed, it yields cocoa butter and cocoa powder still containing some fat (10 to 24%). Typically, 100 g of beans produce 40 g of cocoa butter by pressing, expelling, or solvent extraction; 40 g of cocoa powder; and 20 g of waste material (shell, moisture, dirt, etc.). Increasingly the beans are processed in the country where they grow and cocoa liquor, cocoa powder, and cocoa butter (usually in 25 kg parcels) are exported to the chocolate-producing countries. Cocoa butter carries a premium price and is sometimes adulterated (Crews 2002).

Cocoa butter is a solid fat melting at 32 to 35°C (Table 2.2). It is in high demand because its characteristic melting behaviour gives it properties that are significant in chocolate. At ambient temperature, it is hard and brittle giving chocolate its characteristic snap, but also it has a steep melting curve with complete melting at mouth temperature. This gives a cooling sensation and a smooth creamy texture. For example, the content of solids falls from 45 to 1% between 30 and 35°C. The hardness of cocoa butter is related to its solid fat content at 20 and 25°C. This melting behaviour is related in turn to the chemical composition of cocoa butter. The fat is rich in palmitic (24 to 30%), stearic (30 to 36%), and oleic acids (32 to 39%) and its major triacylglycerols are of the kind SOS, where S represents saturated acyl chains in the 1 and 3 positions and O represents an oleyl chain in the 2 position. There are three major components: POP, POSt, and StOSt (P = palmitic acid and St = stearic acid). These triacylglycerols have 50, 52, or 54 carbon atoms in their 3 acyl chains and the levels of these can be determined by high temperature gas chromatography (GC) with the ratio of these being used to detect adulteration of cocoa butter. (Triacylglycerol molecular species are detailed in Table 2.2.) Cocoa butter has a high content of saturated acids that raises health concerns, but it has been argued that much of this is the noncholesterolemic stearic acid. Chocolate is also a rich source of flavonoids, which are considered to be powerful antioxidants (Beckett, 1999 and 2000).

Triacylglycerol analysis of cocoa butter is generally carried out by capillary GC and the results of an interlaboratory study have been reported (Buchgruber et al., 2003). Seventeen triacylglycerol species were recognised, including POSt (39.8%), StOSt (28.0%), POP (15.6%), PLSt (3.2%), StLSt/StOO (2.9%), POO (1.9%), PLP (1.8%), OOO (1.5 %), StOA (1.0%), and seven others (total 3.3%).

The crystal structure of cocoa butter has been studied extensively because of its importance in understanding the nature of chocolate (Section 4.6.5). The solid fat has been identified in six crystalline forms designated I to VI. Some crystals show double chain length (D) and some triple chain length (T) (Sato et al 1989). The six forms have the following melting points (°C) and D/T structure: I (17.3, D), II (23.3, D), III (25.5, D), IV (27.3, D), V (33.8, T), and VI (36.3, T). Form V is the one preferred for chocolate. This crystalline form gives good molding characteristics and has a stable gloss and favourable snap at room temperature. It is desirable to promote the formation of form V and to inhibit its conversion to form VI. Form V is usually obtained as a result of extensive tempering (putting molten chocolate through a series of cooling and heating processes), which have been found to optimise production of the appropriate polymorph. Alternatively, molten chocolate can be seeded with cocoa butter already crystallised in form V.

Transition from form V to the more stable form VI leads to the appearance of white crystals of fat on the surface of the chocolate. This phenomenon ("bloom") is promoted

by fluctuations in temperature during storage and by migration of liquid oils from nut centres. This change is undesirable because it detracts from the appearance of the chocolate and may be mistaken for microbiological contamination. Bloom can be inhibited by the addition of a small amount of 2-oleo 1,3-dibehenin (BOB), milk fat, or other form V stabilisers to the cocoa butter. This phenomenon is discussed in more detail by Padley, 1997; Smith, 2001; Timms, 2003; and Longchampt and Hartel, 2004.

Minor components include phospholipids (0.05-0.13%), tocopherols (~200 ppm, — mainly γ-tocopherol), sterols, 4-methylsterols, and triterpene alcohols. Cocoa butter is also used in cosmetics (Section 9.4).

For further information, see Table 2.43 to Table 2.49. For information on cocoa butter replacers and cocoa butter substitutes, see the appropriate minor vegetable oils and the section on chocolate.

References

Beckett, S.T., Ed. *Industrial Chocolate Manufacture and Use*, Blackwell Science, Oxford, 1999.

Beckett, S.T., *The Science of Chocolate*, The Royal Society of Chemistry, Cambridge, 2000.

Buchgruber, M. et al. Capillary GC: a robust method to characterise the triglyceride profile of cocoa butter — results of an intercomparison study, *Eur. J. Lipid Sci. Technol.,* **105**, 754–760, 2003.

Crews, C., Authenticity of cocoa butter, in *Oils and Fats Authentication*, Jee, M. Ed. Blackwell Publishing, Oxford, U.K., 2002.

Kurvinen, J.-P. et al. Rapid MS method for analysis of cocoa butter TAG, *J. Amer. Oil Chem. Soc.,***79**, 621–626, 2002.

Longchampt, P.L. and Hartel, R.W., Fat bloom in chocolate and compound coatings, *Eur. J. Lipid Sci. Technol.,* **106**, 241–274, 2004.

Nickless, H., Cocoa butter quality, Guest contribution: www.britanniafood.com

Padley, F.B., Chocolate and confectionery fats, in *Lipid Technologies and Applications,* Gunstone, F.D. and Padley, F. B. Eds. Marcel Dekker, New York, 1997, pp. 391–432.

Sato, K. et al., Polymorphism of POP and SOS. I Occurrence and polymorphic transformation, *J. Am. Oil Chem. Soc.,* **66**, 664–674, 1989.

Shukla, V.J.S., Chocolate — the chemistry of pleasure, *Inform*, **8**, 152–162, 1997.

Shukla, V.J.S., Cocoa butter — properties and quality, *Lipid Technol.,* **7**, 54–57, 1995.

Smith, K.W., Cocoa butter and cocoa butter equivalents, in *Structured and Modified Lipids,* Gunstone, F.D. Ed. Marcel Dekker, New York, 2001.

Timms, R.E., *Confectionery Fats Handbook — Properties, Production, and Application,* The Oily Press, Bridgwater, England, 2003.

2.2.4 Coconut oil (*Cocos nuciferus*)

Coconut oil and palm kernel oil differ from other commodity oils and are known collectively as lauric oils because of their high content of lauric acid. Some other lauric oils occur among the minor oils (Section 2.3). This oil has been reviewed by Pantzaris and Yusof Basiron, 2002; O'Brien, 2004; Canapi et al., 2005; and Gervajio, 2005.

Coconuts grow in coastal regions between 20° N and 20° S of the equator. The trees bear fruit after 5 or 6 years and for up to 60 years thereafter. The shell is split open and allowed to dry. The "meat" on the inside of the shell is called copra and is the source of coconut oil in a yield of ~65%. The oil is extracted by pressing, usually followed by solvent extraction.

As indicated in Table 2.3, coconut oil is produced mainly in the Philippines, Indonesia, and India and exported from the first two countries to EU-25 and U.S., in particular. Both lauric oils are used for a similar range of food and nonfood purposes. They are used to make soaps and other surface-active products and in the production of spreads and other food products. They are also the source of the C_8 and C_{10} acids required to make MCT (medium chain triglycerides). These liquid products are used as lubricants in food-making equipment and, because they are easily metabolised, they appear in food preparations for invalids and athletes. Both oils can be fractionated into oleins and stearins, and hydrogenated to modify their properties and extend their range of uses. Palm kernel stearin is used as a chocolate substitute fat. Coconut stearin is somewhat softer and is used as a confectionery filling fat.

The two lauric oils differ slightly from one another mainly in that coconut oil is the richer in the 6:0 to 10:0 acids and palm kernel oil is the richer in unsaturated C_{18} acids (Table 2.4). This is reflected in the triacylglycerol composition usually expressed in terms of carbon number (the sum of the carbon atoms in the three acyl groups and ignoring the three glycerol carbon atoms). The C_{36} triacylglycerols, dominant in both oils, will be mainly, but not

TABLE 2.3 Major countries/regions involved in the production (from indigenous or imported seed), consumption (food and nonfood uses), export, and import of coconut oil in 2004/05 (million tonnes)

	Total (mt)	Countries/Regions
Production	3.01	Philippines 1.27, Indonesia 0.74, India 0.40, Mexico 0.11
Consumption	2.99	EU-25 0.69, India 0.43, US 0.36, Philippines 0.28, Indonesia 0.20, China 0.12, Mexico 0.12
Exports	1.86	Philippines 0.98, Indonesia 0.56, Malaysia 0.13
Imports	1.87	EU-25 0.72, US 0.38, Malaysia 0.17, China 0.12
Seed yield (t/ha)	0.52	Philippines 0.90, Indonesia 0.46, India 0.35, Mexico 1.36

Source: Adapted from *Oil World Annual 2005*, ISTA Mielke GmbH, Hamburg, 2005.

TABLE 2.4 Fatty acid composition (% weight) of coconut oil and palm kernel oil

	Coconut oil			Palm kernel oil		
	Mean (b)	**Range (b)**	**Range (c)**	**Mean (d)**	**Range (d)**	**Range (c)**
6:0	0.4	0–0.6	0–0.7	0.2	0–0.8	0–0.8
8:0	7.3	4.6–9.4	4.6–10.0	3.3	2.1–4.7	2.4–6.2
10:0	6.6	5.5–7.8	5.0–8.0	3.5	2.6–4.5	2.6–5.0
12:0	47.8	45.1–50.3	45.1–53.2	47.8	43.6–53.2	45.0–55.0
14:0	18.1	16.8–20.6	16.8–21.0	16.3	15.3–17.2	14.0–18.0
16:0	8.9	7.7–10.2	7.5–10.2	8.5	7.1–10.0	6.5–10.0
18:0	2.7	2.5–3.5	2.0–4.0	2.4	1.3–3.0	1.0–3.0
18:1	6.4	5.4–8.1	5.0–10.0	15.4	11.9–19.3	12.0–19.0
18:2	1.6	1.0–2.1	1.2–2.5	2.4	1.4–3.3	1.0–3.5
18.3			0–0.2			
20:0	0.1	0–0.2	0–0.2			
20.1			0–9.2			
IV (a)	8.5	6.3–10.6	6.3–10.6	17.5	14.1–21.0	14.1–21.0
SMP (°C)	24.1	23.0–25.0		26.4	24.0–28.3	

Source: Adapted from Pantzaris, T.P. and Yusof Basiron, in Gunstone, F.D. (Ed.) *Vegetable Oils in Food Technology — Composition, Properties and Uses,* Blackwell Publishing, Oxford, pp 157–202, 2002.
(a) Iodine value calculated from fatty acid composition (b) Leatherhead Food Research Association (LFRA) survey, 35 samples
(c) Codex Alimentarius values (d) LFRA survey, 71 samples
Values in the original references cited as trace and as not detected have been replaced by 0 in this table.

entirely, trilaurin because of the very high level of this acid. Careful study of Table 2.5 shows small differences in triacylglycerol composition between the two oils reflecting the differences in fatty acid composition referred above.

Interesting results reported by Caro et al. (2004) show that the *sn*-2 position is enriched in lauric acid and the two unsaturated C_{18} acids, and that the remaining saturated acids are enriched at the *sn*-1/3 positions. Other work cited by Caro et al. (Table 2.6) shows that the 1 and 3 positions differ in their fatty acids with 6:0, 8:0, and 10:0 occurring particularly at *sn*-3.

Coconut oil is highly saturated with an iodine value between 7 and 10 and this is probably associated with the low levels of tocols in the oil. These have been cited at a mean level of 10 ppm (ranging between 0 and 44 ppm) with α-tocopherol and α-tocotrienol the major members. Among the sterols (mean 836 ppm, range 470 to 1140 ppm) β-sitosterol, Δ^5-avenasterol, stigmasterol, and campesterol predominate and account typically for 46, 27, 13, and 9% of total sterols, respectively.

Polycyclic aromatic hydrocarbons (PAH) are usually present at levels of 150 ppb in crude vegetable oils and <80 ppb after refining. But these values do not hold for crude coconut oil where copra has been dried with combustion gases. Values of around 3000 ppb are

TABLE 2.5 Triacylglycerol composition by carbon number (% weight) of coconut oil and palm kernel oil

	Coconut oil		Palm kernel oil	
	Mean (a)	**Range (a)**	**Mean (b)**	**Range (b)**
C_{28}	0.8	0.5–1.0	0.6	0.3–2.2
C_{30}	3.5	2.6–5.0	1.4	0.9–2.6
C_{32}	13.4	10.8–17.5	6.5	4.8–8.0
C_{34}	17.1	15.6–20.1	8.5	6.2–10.0
C_{36}	19.1	18.3–20.6	21.6	16.6–24.1
C_{38}	16.5	15.1–18.0	16.4	13.2–17.6
C_{40}	10.2	8.4–11.9	9.8	8.3–10.5
C_{42}	7.3	5.5–8.8	9.1	8.2–9.8
C_{44}	4.1	2.8–4.7	6.6	5.5–7.4
C_{46}	2.5	1.6–3.0	5.4	4.1–6.5
C_{48}	2.1	1.2–2.6	6.1	4.7–7.6
C_{50}	1.5	0.7–2.0	2.6	1.6–5.8
C_{52}	1.2	0–2.0	2.7	1.5–7.8
C_{54}	0.8	0–1.7	2.7	1.7–7.9

Source: Adapted from Pantzaris, T.P. and Yusof Basiron, in Gunstone, F.D. (Ed.) *Vegetable Oils in Food Technology — Composition, Properties and Uses,* Blackwell Publishing, Oxford, pp. 157–202, 2002.
(a) LFRA survey 1989, 34 samples (b) LFRA survey 1989, 66 samples
LFRA, see previous table
Values in the original references cited as trace and as not detected have been replaced by 0 in this table.

TABLE 2.6 Fatty acid composition (mol %) of coconut oil — triacylglycerols (TAG), *sn*-2 and *sn*-1/3 positions

	6:0	**8:0**	**10:0**	**12:0**	**14:0**	**16:0**	**18:0**	**18:1**	**18:2**
TAG	0.6	8.6	6.4	47.9	18.2	8.7	2.5	5.7	1.4
sn-2	0.1	2.9	1.1	78.2	10.2	-	-	5.9	2.0
sn-1/3	0.9	11.4	9.1	32.7	22.2	13.2	4.0	5.6	1.1

Source: Caro, Y. et al., *Eur. J. Lipid Sci. Technol.,* **106**, 503–512, 2004.

observed with crude oil, though this can be reduced to a normal value through charcoal treatment.

Hydrogenated coconut oil has an iodine value between 0 and 2 (down from 7 to 10) and a slip melting point of 32 to 34º (up from 24 to 26º). Coconut stearin, resulting after fractionation of coconut oil, has an IV of 4 to 7 and a SMP of 28º.

For further information, see Table 2.43 to Table 2.49. See also the entry for palm kernel oil.

References

Canapi, A.C. et al., Coconut oil, in Shahidi, F. (Ed.) *Bailey's Industrial Oil and Fat Products,* 6th ed., vol. 2, 123–148, Wiley Interscience, New York, 2005.

Caro, Y. et al., Enzymatic synthesis of medium-chain triacylglycerols by alcoholysis and interesterification of copra oil using a crude lipase preparation. *Eur. J. Lipid Sci. Technol.,* **106**, 503–512, 2004.

Gervajio, G.C., Fatty acids and derivatives from coconut oil, in Shahidi, F., Ed. *Bailey's Industrial Oil and Fat Products,* 6th ed., vol. 6, 1–56, Wiley Interscience, New York, 2005.

O'Brien, R.D., *Fats and Oils — Formulating and Processing for Applications*, 2nd ed., CRC Press, Boca Raton, FL, 2004.

Pantzaris, T.P. and Yusof Basiron, The lauric (coconut and palm kernel) oils, in Gunstone, F.D. Ed. *Vegetable Oils in Food Technology — Composition, Properties and Uses,* Blackwell Publishing, Oxford, pp 157–202, 2002.

Websites (coconut oil): www.cocofef.org.ph
www.coconut board.nic.in
www.apcc.org.sg
and some sites listed for palm and palm kernel oils.

2.2.5 Corn (maize) oil (*Zea mays*)

Corn oil is isolated from corn germ (seed) by expelling and solvent extraction. The germ seeds are isolated during wet milling — a process designed to isolate starch from corn kernels. Corn oil, thus, is a by-product of the starch industry. As shown in Table 2.7, more than half the world supply of corn oil is produced in the U.S. The kernel contains 3 to 5% of oil with ~80% of this in the germ.

Corn oil is marketed as a healthy oil, low in saturated acids, rich in linoleic acid, with virtually no linolenic acid, and having high oxidative stability. With growing concern over the omega-6/omega-3 ratio, the low level of linolenic assumes a different significance. Moreau (2002, 2005) has summarised several reports on fatty acid composition. Typically the oil contains 16:0 (10.9%), 18:0 (2.0%), 18:1 (25.4%), 18:2 (59.6%), and 18:3 (1.2%). (For Codex ranges, see Table 2.46.) Not surprisingly, the major triacylglycerols contain two or more linoleic acid chains and three analyses summarised by Moreau (2002) have five major triacylglycerol species: LLO (20, 21, and 23%), LLL (18, 25, and 23%), LLP (14, 15, and 15%), OOL (12, 11, and 11%), and PLO (11,10, and 10%). Variations may result from differences in the fatty acid composition of the oil analysed and from the method of triacylglycerol analysis.

Shen et al. (1999) have described five corn oils with saturated acids in the range 13 to 17%. In these oils, oleic acid varies between 22 and 32% and linoleic acid between 52 and 62% (Table 2.8). Laakso and Christie (1990) reported the stereospecific distribution of fatty acids in corn oil by separation of 1,3-, 1,2-, and 2,3-diacylglycerols as their naphthylethyl urethanes (Table 2.9). (Information on the regiospecific distribution of fatty acids is also available in Table 2.17.)

Corn oil contains 1.3 to 2.3% of unsaponifiable material, including free and esterified sterols, tocopherols, and some squalene (~ 0.2%). Total sterols are mainly β-sitosterol (55 to 67%), campesterol (19 to 24%), Δ^5-avenasterol (4 to 8%), stigmasterol (4 to 8%), and other minor members. The oil is rich in tocols with RBD oil containing four tocopherols (α 23-573, β 0-356, γ 268-2468 and δ 23-75) and three tocotrienols (α 0-239, γ 0-450 and δ 0-20) at the ppm levels shown.

There are two different photosynthetic mechanisms in higher plants. In the "C_3" route, a three-carbon intermediate

TABLE 2.7 Major countries/regions involved in the production (from indigenous or imported seed), consumption (food and nonfood uses), export, and import of corn oil in 2004/05 (million tonnes)

	Total (mt)	Countries/Regions
Production	2.05	US 1.10, EU-25 0.23, Japan, 0.10
Consumption	2.06	US 0.74, EU-25 0.23, Japan 0.10, Turkey 0.10
Exports	0.72	US 0.40
Imports	0.72	Turkey 0.09

Source: Adapted from *Oil World Annual 2005*, ISTA Mielke GmbH, Hamburg, 2005.

TABLE 2.8 Fatty acid composition of five corn oils containing 13 to 17% of saturated acids

Corn oil	16:0	18:0	18:1	18:2	18:3
P3394	11.7	1.4	26.4	59.6	0.9
TS143	12.6	2.1	32.3	52.2	0.7
TS43-45	13.1	2.5	22.0	61.7	0.7
TS42-44	13.5	2.4	22.1	61.4	0.6
TS86	14.5	2.6	27.9	54.5	0.6

Source: Shen, N., Duvick, S., White, P., and Pollack, L., *J. Am. Oil Chem. Soc.,***76**, 1425–1429, 1999.

TABLE 2.9 Stereospecific analysis of the major fatty acids in maize oil

	16:0	18:1	18:2
sn-1	15.3	34.9	48.2
sn-2	0.5	33.7	62.9
sn-3	19.2	35.1	51.5

Source: Laakso, P. and Christie W.W., *Lipids*, **25**, 349–353, 1990. The original paper also has results for the minor acids.

is the first product derived from atmospheric carbon dioxide and in the "C_4" route, this is a four-carbon compound. C_3 plants (such as most cereals, potatoes, and sugar beets) have a lower $^{13}C/^{12}C$ ratio than C_4 plants (such as maize and sugar cane). Because corn oil is the only commodity oil derived from a C_4 plant, it can be distinguished from other oils by its $^{13}C/^{12}C$ ratio. This provides a method for checking the authenticity of corn oil (Rossell 1999).

For further information, see Table 2.43 to Table 2.49.

References

Laakso, P. and Christie W.W., Chromatographic resolution of chiral diacylglycerol derivatives: potential in the stereospecifiic analysis of triacyl-*sn*-glycerols, *Lipids*, **25**, 349–353, 1990.

Moreau, R.A., Corn oil, in Gunstone, F.D., Ed., *Vegetable Oils in Food Technology — Composition, Properties and Uses,* Blackwell Publishing, Oxford, pp. 278–296, 2002.

Moreau, R.A., in Shahidi, F., Ed., *Bailey's Industrial Oil and Fat Products,* 6th ed., vol. **2**, 149–172, Wiley Interscience, New York, 2005.

Rossell, J.B., Origins and properties of edible vegetable oils, in Rossell, J.B., Ed., *LFRA Oils and Fats Handbook Series, Volume 1, Vegetable Oils and Fats,* Leatherhead Food RA, Leatherhead, England, pp. 0–21, 1999.

Shen, N. et al. Oxidative stability and aromascan analyses of corn oil with altered fatty acid content, *J. Am. Oil Chem. Soc.,* **76**, 1425–1429, 1999.

2.2.6 Cottonseed oil (Gossypium hirsutum and G. barbadense)

Cottonseed oil is a by-product of the production of cotton. Prior to World War II, it was the dominant vegetable oil and many early refining technologies were developed for this oil. Now it is of less significance, coming 9th out of the 17 oils and fats listed in *Oil World* publications. It comes even lower among trade figures as it is used almost entirely in the country of origin. This is apparent from the figures detailed in Table 2.10, which show that only 5% of world production of cottonseed oil is exported/imported and by the fact that consumption in the producing countries is virtually the same as production.

TABLE 2.10 Major countries/regions involved in the production (from indigenous or imported seed), consumption (food and nonfood uses), export, and import of cottonseed oil in 2004/05 (million tonnes)

	Total (mt)	Countries/Regions
Production	5.00	China 1.55, India 0.69, Pakistan 0.51, US 0.42, CIS 0.44, Brazil 0.28
Consumption	4.93	China 1.28, India 0.68, Pakistan 0.51, CIS 0.41, US 0.36, Brazil 0.22
Exports	0.21	Brazil 0.06, US 0.06
Imports	0.21	Iran 0.04. Canada 0.03
Seed yield (t/ha)	1.25	China 2.06, India 0.81, US 1.44, CIS 1.15, Pakistan 1.54, Brazil 2.04

Source: Adapted from *Oil World Annual* 2005, ISTA Mielke GmbH, Hamburg, 2005.

The cotton plant is grown for its fibre. Cotton is attached to the seed and a single cotton boll may contain up to 40 seeds. When separated, the seeds can furnish oil (16%), meal (45%), hull (26%), and residual cotton (linters, up to 9%). The oil is a strong yellow colour because of the presence of gossypol (for structure see Dictionary entry). This tetracyclic phenolic compound is found mainly in the meal. Low levels remaining in the crude oil are removed during refining — mainly through neutralisation (O'Brien, 2002; O'Brien et al., 2005). Cottonseed may appear as a liquid or a solid or as a mixture. The temperature at which it becomes wholly liquid is between 10 and 16°C.

Cottonseed oil contains three major acids: palmitic (23%), oleic (17%), and linoleic (56%) at the typical levels shown. This is reflected in its iodine value ranging from 99 to 113. The remaining 4% of fatty acids include myristic, stearic, arachidic, behenic, palmitoleic, and linolenic. The crude oil also contains two unusual acids having cyclopropene rings. These [malvalic (C_{18}) and sterculic (C_{19})] together reach 1.0% at most. They are present at higher levels in kapok seed oil (~13%), and at still higher levels in other malvaceous seed oils. (For structures, see Chapter 1, Dictionary, or the SOFA website.) The cyclopropene acids have undesirable physiological properties and, when present in meal fed to chickens, they reduce egg yield and lead to poor hatching and to pink egg whites. However, the cyclopropene acids in crude cottonseed oil are removed during refining and in hydrogenation. These cyclopropene acids are known to inhibit the Δ-9 desaturase enzyme. (For information on the distribution of fatty acids between the *sn*-2 and *sn*-1/3 positions, see Table 2.17 and Table 2.27.)

Cottonseed has been genetically modified to produce oils with changed fatty acid composition, but there is no evidence that these are being grown commercially. In general, they contain more oleic acid and less linoleic acid or they have more palmitic and stearic acid so that they could be used in spreads without partial hydrogenation.

The fatty acid composition of cottonseed oil is reflected in the triacylglycerols, which are dominated by compounds with two or three linoleic chains at almost 60% (Table 2.11).

In addition to gossypol and other similar molecules (up to 0.5% total) that are removed during refining, crude cottonseed oil contains phospholipids and tocols. The major phospholipids in glandless cottonseed oil are PC, PE, and PI. The tocols in crude cottonseed oil are mainly α - (~350 ppm) and γ-tocopherols (~500 ppm), but these levels are reduced through high temperature physical refining to a combined level below 100 ppm.

The oil is used widely in the food industry mainly in the production of cooking fats and spreads. Partially

TABLE 2.11 Triacylglycerols in cottonseed oil determined by GLC and by HPLC procedures

TAG species[a]	GLC	HPLC
PLL	25.7	27.5
LLL	16.1	19.0
POL	14.0	14.0
OLL	12.9	12.5
PLP	8.7	7.1
LOO	4.4	3.1
POO	3.3	3.1
POP	2.5	2.2
OOO	2.4	1.6
LLS	2.4	1.4
PLS	2.1	1.5
SOL	1.5	1.3

[a] The TAG species include all isomeric members containing the three acids indicated. The symbols P, S, O, and L represent palmitic, stearic, oleic, and linoleic acids, respectively.

Source: Adapted from O'Brien, R.D., in Gunstone, F.D. (Ed.) *Vegetable Oils in Food Technology — Composition, Properties and Uses,* Blackwell Publishing, Oxford, pp 203–230, 2002.

hydrogenated cottonseed oil, with its significant level of palmitic acid, promotes crystallisation in the β′ form.

For further information, see Table 2.43 to Table 2.49.

References

National Cottonseed Processors Association, www.cotton seed.com

O'Brien, R.D., Cottonseed oil, in Gunstone, F.D., Ed., *Vegetable Oils in Food Technology — Composition, Properties and Uses,* Blackwell Publishing, Oxford, pp. 203–230, 2002.

O'Brien, R.D. et al., Cottonseed oil, in Shahidi, F., Ed., *Bailey's Industrial Oil and Fat Products,* 6th ed., vol. **2**, 173–280, Wiley Interscience, New York, 2005.

2.2.7 Groundnut (peanut) oil (*Arachis hypogaea*)

Groundnut oil is also known as peanut oil, earth oil, and arachis oil. The nuts are enclosed in shells attached to the roots and grow underground. Many of the nuts are used for snack foods and for animal feed and only about one-half are crushed for oil. The oil content is generally 40 to 50%. If stored inadequately, the nuts are prone to infestation by *Aspergillus* species, which produce afflatoxin (see Dictionary Section). This potential carcinogen remains in the meal after extraction, but is absent from refined oil. People with an allergy to products containing peanuts must avoid them. Usually, the presence or possible presence of peanuts is indicated on food labels.

It is clear from Table 2.12 that China and India are the main producers, where the seed is grown and oil is extracted. Only a limited share of the nuts has the oil extracted, as most are eaten as snacks.

Groundnut oil is rich in oleic and linoleic acids — typically 43 and 36%, respectively, but the proportion of these two acids can vary with iodine values in the range of 86 to 107. The level of linolenic acid is low (0 to 0.3%), but the oil contains palmitic, stearic, and a range of saturated and unsaturated C_{20}, C_{22}, and C_{24} acids (total of long-chain acids is 7 to 8%). Stereospecific analyses, showing that the saturated acids are enriched at the *sn*-1 and 3 positions, but not equally at these two positions, are rather old and not included here (Sanders, 2002). Table 2.13 shows the fatty acid composition of three varieties grown and harvested in Alabama (U.S.) and of a typical commercial sample.

TABLE 2.12 Major countries/regions involved in the production (from indigenous or imported seed), consumption (food and nonfood uses), export, and import of groundnut oil in 2004/05 (million tonnes)

	Total (mt)	Countries/Regions
Production	4.50	China 2.05, India 1.13, Nigeria 0.32
Consumption	4.55	China 2.03, India 1.15, Nigeria 0.32
Exports	0.20	
Imports	0.20	EU-25 0.12
Seed yield (t/ha)	1.05	China 2.13, India 0.62, Nigeria 0.69

Source: Adapted from *Oil World Annual 2005*, ISTA Mielke GmbH, Hamburg, 2005.

The major triacylglycerols in a sample of groundnut oil examined by HPLC-GC-EIMS (Singleton and Pattee, 1987) were reported to be OLL 26.1%, OOL 21.5%, POL 13.4%, PLL 8.4%, LLL 5.8%, POO 5.6%, OOO 4.5%, SOO 4.4%, OOA 4.2%, and other 6.1%. (O, L, P, S, and A represent oleic, linoleic, palmitic, stearic, and arachidic acyl chains and a three-letter unit includes all the isomeric triacylglycerols containing those three chains). Dorschel (2002) has examined the triacylglycerols of peanut oil using HPLC and tandem MS and identified 168 different molecular species of which LLL, LLO, OLO, PLO, OOO, POO, and StOO are the major members (order of letters representing acyl groups is not significant).

A high-oleic variety developed by conventional seed breeding contains oleic, linoleic and palmitic acids at levels of 80 ± 2, 2–3, and 9 ± 1% respectively.

The phospholipids (500 mg/100 g) were shown by HPLC analysis to contain PA 2.2%, PG 2.5%, PE 13.3%, PI 15.7%, and PC 66.4% (Singleton and Stikeleather, 1995).

Crude groundnut oil generally contains 20 to 130 mg of tocopherols per 100 g of oil, equally divided between α- and γ-tocopherols. Having examined oils from Argentina, China, and the U.S. in 1986, 1987, and 1988, Sanders (1992) found tocopherol levels of 103 to 244 mg/100 g oil, varying with geographical origin and harvest year. Typically the sterols in groundnut oil (337 mg sterols/100 g oil) are β-sitosterol (217), campesterol (49), stigmasterol (36), and Δ^5-avenasterol (26) along with other minor compounds (Sanders, 2002).

TABLE 2.13 Fatty acid composition of three varieties of groundnut grown and harvested in Alabama (U.S.) and of a typical commercial sample

	16:0	18:0	18:1	18:2	20:0	20:1	22:0	24:0
Florigiant	10.8	2.9	53.1	27.3	1.8	1.0	1.9	1.1
Florunner	11.4	2.1	50.9	29.1	1.6	1.1	2.4	1.3
Starr	14.2	3.3	43.3	33.0	1.8	1.1	2.7	0.7
KS[a]	10	2.4	43	36	1		3	2

[a] Also 18:3 0.3% 20:1 and 20:2 together 1.5%

Source: Sanders, T.H., in Gunstone, F D., (Ed.) *Vegetable Oils in Food Technology — Composition, Properties and Uses,* Blackwell Publishing, Oxford, pp. 231–243, 2002. Lidefelt, J-O., (Ed.) *Handbook — Vegetable Oils and Fats*, Karlshamns, Karlshamn, Sweden, 2002.

For further information, see Table 2.43 to Tables 2.49 and in the review by Pattee, 2005.

References

Dorschel, C.A., Characterization of the TAG of peanut oil by electrospray LC-MS-MS, *J. Am. Oil Chem. Soc.,* **79**, 749–753, 2002.

Lidefelt, J-O., Ed., *Handbook — Vegetable Oils and Fats*, Karlshamns, Karlshamn, Sweden, 2002.

Pattee, H.E., Peanut oil, in Shahidi, F., Ed., *Bailey's Industrial Oil and Fat Products,* 6th ed., vol. **2**, 431–464, Wiley Interscience, New York, 2005.

Sanders, T.H. et al., Quality factors in exported peanuts from Argentina, China and the United States, *J. Am. Oil Chem. Soc.,* **69**, 1032–1035, 1992.

Sanders, T.H., Groundnut (peanut) oil, in Gunstone, F D., Ed., *Vegetable Oils in Food Technology — Composition, Properties and Uses,* Blackwell Publishing, Oxford, pp. 231–243, 2002.

Singleton, J.A. and Pattee, H.E., Characterization of peanut oil triacylglycerols by HPLC, GLC and EIMS, *J. Am. Oil Chem. Soc.,* **64**, 534–538, 1987.

Singleton, J.A. and Stikeleather, L.F., High-performance liquid chromatography analysis of peanut phospholipids, II, Effect of postharvest stress on phospholipid composition, *J. Am. Oil Chem. Soc.,* **72**, 485–488, 1995.

2.2.8 Linseed (flaxseed) oil (*Linum usitatissimum*)

The flax plant has a long history and is grown to yield both fibre (linen) and oil (linseed, flaxseed). Different strains of this plant are grown depending on whether the major interest is fibre or oil. Over several decades, production of the seeds and oil have declined to the present levels indicated in Table 2.14. At one time, Argentina, Canada, and the USSR were significant producers of this oil.

Typically linseed oil has the following fatty acid composition with range values in parentheses: palmitic 6.0% (5–7), stearic 2.5% (2–6), oleic 19.0% (14–40), linoleic 24.1% (14–29), and linolenic acid 47.4% (35–60). Information on the regiospecific distribution of linseed fatty acids is available in Table 2.27. The fatty acid composition of flax and solin has been measured by NIR spectroscopy (Siemens and Daun, 2005).

Using MALDI-TOF (matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry), Ayorinde (2000) identified 10 major triacylglycerols in flaxseed oil of which the most dominant were those containing only linolenic and/or linoleic acids (LnLnLn, LnLnL, LnLL, and LLL).

Based on its high level of unsaturation, linseed oil is used mainly for technical purposes as in drying oils for paints and floor coverings (linoleum) and in the production of epoxidised oil (Section 8.3). With the growing recognition of the importance of dietary omega-3 acids, linseed is being used increasingly for food purposes and for blending with other oils to produce healthy oils often provided in capsules. It is also consumed as flaxseed as a convenient source of the oil, fibre, and lignans all of which are reported to display health benefits. As a dietary component, the oil is generally obtained by cold pressing and mild refining and is traded as flaxseed oil since the name linseed oil is strongly associated with industrial use.

Linseed oil (Table 2.15) contains 440 to 588 mg/kg of tocopherols, which is almost entirely the γ-compound. Sterols (4200 mg/kg) are mainly β-sitosterol (46%), campesterol (29%), Δ^5-avenasterol (13%), and stigmasterol (9%). Australian and Canadian scientists have produced a modified form of flax with a very different fatty acid composition (Green and Dribnenki, 1994). This is now grown in Canada, Europe, and Australia and traded as linola or solin. The oil is used as an alternative to sunflower oil as a linoleic-rich oil in spreads. A detailed composition is given in Table 2.15.

TABLE 2.14 Major countries/regions involved in the production (from indigenous or imported seed), consumption (food and nonfood uses), export, and import of linseed oil in 2004/05 (thousand tonnes)

	Total (kt)	Countries/Regions
Production	603	EU-25 154, USA 130, China 127, India 63
Consumption	607	EU-25 151, China 134, US 85, India 67
Exports	109	US 46, EU-25 29,
Imports	107	EU-25 22, Japan 13, China 6
Seed yield (t/ha)	0.70	EU-25 0.95, China 0.84, US 1.27, India 0.32

Source: Adapted from *Oil World Annual 2005*, ISTA Mielke GmbH, Hamburg, 2005.

TABLE 2.15 Composition of linseed and linola oils

	Linseed (typical)	Linola (crude)	Linola (RBD)
Iodine value	170–203	142	144
Fatty acid composition (%)			
16:0	6.0	5.6	5.6
18:0	2.5	4.0	4.0
18:1	19.0	15.9	15.9
18:2	24.1	71.8	71.9
18:3	47.4	2.0	2.0
Other	1.0	0.7	0.6
Tocopherols (ppm)			
Total	440–588	507	172
γ-Tocopherol	430–575	471	172
Sterols (ppm)			
Total	4200	3095	2324
β-Sitosterol	1932	1608	1251
Campesterol	1218	801	530
Δ^5-Avenasterol	546	492	430
Stigmasterol	378	164	96

Source: Adapted from Kochhar, S.P., in Gunstone, F.D. (Ed.) *Vegetable Oils in Food Technology — Composition, Properties and Uses,* Blackwell Publishing, Oxford, pp. 297–326, 2002.

For further information, see Table 2.43 to Table 2.49. Thompson and Cunnane (2003) have edited a monograph on flaxseed.

References

Ayorinde, F.O., Determination of the molecular distribution of triacylglycerol oils using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry, *Lipid Technol.*, **12**, 41–44, 2000.

Green, A.G. and Dribnenki, J.C.P., Linola — a new premium polyunsaturated oil, *Lipid Technol.*, **6**, 29–33, 1994.

Kochhar, S.P., Sesame, rice-bran and flaxseed oils, in Gunstone, F.D., Ed., *Vegetable Oils in Food Technology — Composition, Properties and Uses,* Blackwell Publishing, Oxford, pp. 297–326, 2002.

Siemens, B.J. and Daun, J.K., Determination of fatty acid composition by NIR spectroscopy, *J. Am. Oil Chem. Soc.,* **82**, 153–157, 2005.

Thompson, L. and Cunnane, S., Ed., *Flaxseed in Human Nutrition* 2nd ed., AOCS Press, Champaign, IL, 2003.

Websites: www.ngfa.org/trygrams-flaxseed
www.unm.edu/flaxseeds

2.2.9 Olive oil (*Olea europaea*)

Olive oil is a major vegetable oil obtained from the mesocarp of the fruits of the olive tree. The oil has a long history with many biblical references, and the trees bear fruit for over 100 years.

Annual production is 2.5 to 2.8 million tonnes, with commercial cultivation of the tree confined almost entirely to the Mediterranean countries of Italy, Greece, Spain, Turkey, and Tunisia (Table 2.16). Olive oil is an important part of the Mediterranean diet and appears to be associated with low incidence of coronary heart disease. Its oxidative stability and unique flavour are linked to the fatty acid composition of the oil, particularly its high level of oleic acid, and to its many minor components still present in virgin (i.e., unrefined) oil.

Virgin olive oil is produced from the first pressing of olives. Other grades of lower quality are produced subsequently. Crushed olives produce oil, water, and solid residue, which is separated mainly by centrifugation. The oil is recovered without the use of a solvent and the highest quality oil is used without further refining. The quality and processing of several grades of olives are defined by Codex Alimentarius and by EU Commission Regulations (European Communities Commission, 1991, 1998, and 2001). The regulations provide defined value ranges for physical and chemical properties and for composition of minor fatty acids and sterols. These are well summarised by Boskou (2002), Bianchi (2002), and Stewart (2002).

Olive oil is characterised by a high level of oleic acid with *Codex* ranges of 8 to 20% for palmitic acid, 55 to 83% for oleic acid, and 4 to 21% for linoleic acid. Other acids present in trace amounts include myristic, palmitoleic, heptadecanoic, heptadecenoic, linolenic, and C_{20}, C_{22}, and C_{24} saturated acids. A mean fatty acid composition of 78 Greek olive oils detailed by Boskou (2002) indicates the presence of palmitic (10.5%), stearic (2.6%), oleic (76.9%), and linoleic acids (7.5%), in addition to nine other acids each present at a level <1.0%. The fatty acid composition depends on, among other factors, the geographical location where olive trees are grown, and attempts are being made to identify this from the composition of the oil (Angerosa et al., 2003 and Bianchi et al., 2003).

The positional distribution of fatty acids in a number of vegetable oils, including olive, is given in Table 2.17. As expected, the *sn*-2 position is occupied mainly by oleic acid

TABLE 2.16 Major countries/regions involved in the production, consumption (food and nonfood uses), export, and import of olive oil in 2004/05 (million tonnes)

	Total (mt)	Countries/Regions
Production	2.73	Spain 1.00, Italy 0.56, Greece 0.45, Syria 0.20, Tunisia 0.13
Consumption	2.96	EU-25 2.02, US 0.23, Syria 0.15, Morocco 0.07, Tunisia 0.06
Exports	0.63	Italy 0.20, Spain 0.11, Tunisia 0.10
Imports	0.62	US 0.25, EU-25 0.11

Source: Adapted from *Oil World Annual 2005*, ISTA Mielke GmbH, Hamburg, 2005.

TABLE 2.17 Positional distribution of fatty acids (mol %) for olive and other vegetable oils

	16:0	18:0	16:1	18:1	18:2	18:3
Olive						
1,2,3	12.6	2.9	0.9	74.6	8.4	0.7
1,3	18.2	3.4	1.0	69.3	7.4	0.5
Corn						
1,2,3	11.6	2.0	0.1	26.0	59.6	0.8
1,3	18.3	2.8	0.1	28.1	49.6	0.9
Soybean						
1,2,3	11.9	4.3	0.1	22.5	54.4	6.9
1,3	17.7	6.8	0.1	21.2	46.1	7.0
Sunflower						
1,2,3	7.1	4.4	0.1	22.7	65.7	0.1
1,3	10.9	6.0	0.1	21.8	60.9	0.1
Cottonseed						
1,2.3	25.3	2.4	0.6	17.3	54.2	0.2
1,3	38.8	3.6	0.7	15.0	41.6	0.1

Method: Reaction of oil with butanol in presence of a 1,3-stereospecific lipase (Lipozyme IM) with isolation and GC analysis of the butyl esters. Fatty acids in the *sn*-2 position can be calculated from the data in this table. Subtract 2/3 of the 1,3 value from the 1,2,3 value and multiply the result by 3. Alternatively, subtract twice the 1/3 figure from 3 times the 1,2,3 figure.
Source: adapted from Dourtoglou, V.G. et al., *J. Am. Oil Chem. Soc.,* **80**, 203–208, 2003. The original paper also contains a second analysis for each oil.

and the polyunsaturated C_{18} acids, while the saturated acids are mainly in the 1/3 positions (Dourtoglou et al., 2003). Information on the regiospecific distribution of fatty acids is also available in Table 2.27.

The major triacylglycerols are: OOO (40–59%), LOO (12–20%), POO (12–20%), POL (6–7), and StOO (3–7%). Smaller amounts of POP, POSt, OLnL, LOL, OLnO, PLL, PLnO, and LLL may also be present. The oil also contains some mono- and di-acylglycerols, some phospholipids (40–135 mg/kg), and traces of iron (0.5–3.0 ppm) and copper (0.001–0.2 ppm). Chromium, manganese, tin, nickel, and lead are present at levels not exceeding a few ppb.

The oil is characterised by a range of unsaponifiable constituents (total 0.5 to 1.5%), some of which confer high oxidative stability. They include tocopherols (usually 100 to 300 mg/kg and almost entirely α-tocopherol) and squalene, which may be 40 to 50% of total unsaponifiable material. This acyclic C_{30} hydrocarbon is the biological precursor of the sterols and is present at a higher level (700 to 12000 mg/kg of oil) in olive oil than in other vegetable oils (usually only 50 to 500 mg/kg). It can be recovered from olive oil deodorizer distillate. Other minor components include carotenoids, chlorophylls responsible for the greenish colour of virgin olive oil, sterols (desmethylsterols, 4α-methylsterols, 4,4-dimethylsterols or triterpene alcohols, triterpene dialcohols, and hydroxyterpenic acids), fatty alcohols and waxes, polyphenols, and volatile and aroma compounds. Several of these show antioxidant properties and add to the nutritional value of this oil. For more details, see Table 2.18 and Boskou (2002).

As a highly-regarded product sold at a premium price, olive oil is sometimes adulterated with cheaper materials. An adulterant frequently used because of its similar fatty acid composition is hazelnut oil. Typical of many reports is the paper by Gordon et al. (2001) describing the detection of hazelnut oil in virgin olive oil by HPLC analysis

TABLE 2.18 Minor components identified in virgin olive oil

Minor components by type	Concentration in virgin olive oil	Major components within the category
Carotenes	1–20 mg/kg, usually <10	β-carotene, lutein (3,3′-dihydroxy- β,ε-carotene)
Chloropylls	10–30 mg/kg	pheophytin-a
Desmethylsterols	100–200 mg/100 g oil	β-sitosterol, Δ^5-avenasterol, campesterol
4α-Methylsterols	20–70 mg/100 g oil	obtusifolol, cycloeucalenol gramisterol, citrostadienol
4,4-Dimethylsterols (triterpene alcohols)	100–150 mg/100 g oil	β-amyrin, butyrospermol, cycloartenol 24-methylene-cycloartenol
Triterpene dialcohols	1–20 mg/100 g oil	erythrodiol, uvaol
Fatty alcohols	<35 mg/100 g oil	22:0 to 26:0
Waxes	<35 mg/100g oil	C_{36} to C_{46} saturated and unsaturated esters and esters of nonfatty alcohols
Polyphenols		A range of aromatic compounds, mainly carboxylic acids or alcohols, with one or more phenolic groups
Volatile compounds		Mainly short-chain saturated and unsaturated aldehydes and alcohols

Note: concentrations relate to virgin olive oil. Different values, which may be higher or lower, are present in other grades of olive oil.
Source: Adapted from Boskou, D., in Gunstone, F.D. (Ed.) *Vegetable Oils in Food Technology — Composition, Properties and Uses,* Blackwell Publishing, Oxford, pp. 244–277, 2002, where further details and structures are given. Some structures are available in the Dictionary section. Tocopherols and squalene are covered in the text.

of polar components and a method based on the 4,4′-dimethylsterols present in these two oils (Damirchi et al., 2005). Adulteration down to levels of 2 to 4% can be detected. Compositional data (fatty acids, triacylglycerols, and sterols) are used to distinguish between oil from different cultivars and geographical location. Attempts are also being made to achieve this by ^{13}C NMR spectroscopy (Brescia et al., 2003).

Several papers on olive oil have been presented together (Sanchez, 2002). Boskou (1996) has written a monograph on olive oil, Salas et al. (2000) have reviewed the biochemistry of lipid metabolism in olive fruits, and Firestone (2005) has reviewed this oil. Figures for the use of olive oil as food and for industrial purposes are given in Table 2.24 and further information is given in Table 2.43 to Table 2.49.

References

Angerosa. F. and Basti, C., The volatile composition of samples from the blend of monovarietal olive oils and from the processing of mixtures of olive fruits, *Eur. J. Lipid Sci. Technol.,* **105**, 327–332, 2003.

Bianchi, G., Authentication of olive oil, in Jee, M., Ed., *Oils and Fats Authentication*, Blackwell Publishing, Oxford, 2002, pp. 25–65.

Bianchi, G. et al., Chemometric criteria for the characterisation of Italian Protected Denomination of Origin (DOP) olive oils from their metabolic profiles, *Eur. J. Lipid Sci. Technol.*, **103**, 141–150, 2003.

Boskou, D., *Olive Oil Chemistry and Technology*, AOCS Press, Champaign, IL, 1996.

Boskou, D., Olive oil, in Gunstone, F.D., Ed., *Vegetable Oils in Food Technology — Composition, Properties and Uses,* Blackwell Publishing, Oxford, pp. 244–277, 2002.

Brescia, M.A. et al., Chemometric classification of olive cultivars based on compositional data of oils, *J. Am. Oil Chem. Soc.,* **80**, 945–950, 2003.

Damirchi, S.A. et al., Sterol fractions in hazelnut and virgin olive oils and 4,4-dimethylsterols as possible markers for detection of adulteration in virgin olive oil, *J. Am. Oil Chem. Soc.,* **82**, 717–725, 2005.

Dourtoglou, V.G. et al., Detection of olive oil adulteration using principal component analysis applied on total and region FA content, *J. Am. Oil Chem. Soc.,* **80**, 203–208, 2003.

European Communities Commission (1991) Regulations (EEC) No 2568/91 on the characteristics of olive and olive residue oil and on the relevant methods of analysis, *Official Journal of the European Communities,* L248, 5.9.1991, p. 1.

European Communities Commission (1998) Regulations (EEC) No 2815/98 concerning marketing standards for olive oil, *Official Journal of the European Communities,* L349, 24.12.1998, p. 8.

European Communities Commission (2001) Regulations (EEC) No 455/2001 amending Regulation 2568/91 on the characteristics of olive and olive residue oil and on the relevant methods of analysis, *Official Journal of the European Communities,* L65, 6.43.2001, p. 9.

Firestone, D., Olive oil, in Shahidi, F., Ed., *Bailey's Industrial Oil and Fat Products,* 6th ed., vol. 2, 303–332, Wiley Interscience, New York, 2005.

Gordon, M.H. et al., Detection of pressed hazelnut oil in admixtures with virgin olive oil by analysis of polar components, *J. Am. Oil Chem. Soc.,* **78**, 621–624, 2001.

Salas, J.J. et al., Biochemistry of lipid metabolism in olive and other fruits, P*rogress in Lipid Research*, **39**, 151–180, 2000.

Sanchez, J., Editor of a collection of 12 papers related to olive oil, *Eur. J. Lipid Sci. Technol.* **104**, 559–705, 2002.

Stewart, C., Authenticity of edible oils and fats: the legal position in Jee, M., Ed., *Oils and Fats Authentication*, Blackwell Publishing, Oxford, 2002, pp. 181–205.

Website: www.unctad.org.olive

2.2.10 Palm oil (*Elaeis guinensis*)

The oil palm originated from East Africa. The extensively cultivated *tenera* hybrid is a cross between *dura* and *pisifera* varieties. It gives the highest yield of oil per hectare of any oil crop. In Southeast Asia, oil palms yield on average four tonnes of palm oil per hectare, in addition to 0.5 tonnes of palm kernel oil and 0.5 tonnes of palm kernel meal. These are average values, and yields of around 10 tonnes/hectare are reported under the most favourable conditions. In contrast, soybeans grown in the U.S. yield about 2.5 tonnes of beans per hectare and these furnish 0.5 tonnes of oil and 2 tonnes of meal.

The oil palm grows in tropical regions of Asia, Africa, and the Americas, but is cultivated most extensively in Malaysia and Indonesia (Table 2.19). Palm oil is now produced at virtually the same level as soybean oil and is traded at levels above those for soybean, even after making allowance for the oil equivalent of traded soybeans. Its range of use is greatly extended by fractionation (Section 4.4) and palm oil is available as such and in a range of oleins, stearins,

TABLE 2.19 Major countries/regions involved in the production, consumption (food and nonfood uses), export, and import of palm oil in 2004/05 (million tonnes)

	Total (mt)	Countries/Regions
Production	32.50	Malaysia 15.16, Indonesia 12.60, Nigeria 0.80, Thailand 0.71, Colombia 0.65
Consumption	31.86	EU-25 4.30, China 4.17, Indonesia 3.44, India 3.37, Malaysia 1.95, Pakistan 1.36, Nigeria 1.02
Exports	25.46	Malaysia 13.70, Indonesia 9.01
Imports	25.25	EU-25 4.42, China 4.20, India 3.32, Pakistan 1.45, Egypt 0.77, CIS 0.65, Bangladesh 0.62, Malaysia 0.53, Japan 0.49
Oil yield (t/ha)	3.60	Malaysia 4.20, Indonesia 3.54

Source: Adapted from *Oil World Annual 2005*, ISTA Mielke GmbH, Hamburg, 2005.

and mid-fractions. All of these are further provided as crude or as RBD (refined, bleached, and deodorised) oils. Indeed the largest export of palm oil from Malaysia is in the form of palm olein.

Palm trees bear fruit in the third year after planting and continue producing for about 25 years. Fruit bunches of 4 to 20 kg contain 200 to 2000 individual fruits and are harvested throughout the year. The fruit bunches are transported to palm oil mills where crude palm oil (20 to 24%) is produced by mechanical and physical extraction processes along with palm kernels from which a further 2 to 4% of palm kernel oil (based on weight of palm fruits) can be produced. Oil quality is maintained by careful harvesting of fruits at the optimum stage of ripening, by minimal handling during transport to reduce bruising, and with proper processing conditions during oil extraction. Palm trees of lower height have been developed through seed breeding, which makes harvesting easier and extends the productive life of the trees. Other breeding targets include higher yields, a more unsaturated oil, and a higher proportion of the more valuable kernel.

Oil is obtained from palm fruits by steaming at 95°C to loosen the fruits in the fruit bunch and to deactivate the lipase present in the fruits, followed by centrifugation or hydraulic pressing to release the oil. This may be bleached with earths and deodorised with steam at 240 to 270°C. At the upper temperature there may be some interesterification.

Because palm oil and its fractions are mixtures of solid and liquid triacylglycerols, their melting behaviour is important. The slip melting point (SMP) is a measure of the temperature at which a fat becomes sufficiently fluid to slip or run (Table 2.21). Of greater importance is the solid fat content (SFC) now usually measured by pulse NMR (Section 4.2.5.2). Typical SFC values are listed in Table 2.20. They show the marked changes occurring through fractionation. Palm mid-fractions with steep melting curves and complete melting by 35°C are suitable replacements for cocoa butter.

TABLE 2.20 Solid fat content of palm oil and palm oil fractions determined by pulsed NMR

Temperature (°C)	Palm oil	Palm olein	Super olein	Palm stearin	PMF soft	PMF hard
Samples[a]	244	238	32	205		
10	53.7	38.3	17.5	76.0	75	95
15	39.1	19.9	0.9	68.9	64	93
20	26.1	5.7		60.2	45	90
25	16.3	2.1		50.6	11	78
30	10.5			40.4		47
35	7.9			34.3		6
40	4.6			28.1		
45				22.4		
50				12.5		
55				0.6		

[a] Columns 2 to 5 are mean values for the number of samples indicated. The original publication also gives range values.

Note: PMF = palm mid-fraction

Source: Siew Wai Lin, in Gunstone, F.D., (Ed.) *Vegetable Oils in Food Technology – Composition, Properties and Uses,* Blackwell Publishing, Oxford, pp. 59–97, 2002.

Information on the fatty acid and triacylglycerol composition of palm oil and its fractions is collated in Table 2.21 and Table 2.22. Palm oil contains almost equal quantities of saturated (palmitic 44% and stearic acid 4%) and unsaturated acids (oleic 39% and linoleic acid 11%) and this is reflected in an iodine value generally in the range 51 to 55. Fractionation leads to useful materials in which iodine values are raised or lowered. The main fractionation products are palm olein, palm stearin, and palm mid-fraction, but further fractionation gives products with iodine values as high as 70 to 72 and as low as 10 to 15. The changes in levels of palmitic, oleic, and linoleic acids in these fractions are apparent in Table 2.21. Minor acids also present in palm oil include lauric, myristic, arachidic, hexadecenoic, *cis*-vaccenic, and linolenic (see Table 2.46).

The triacylglycerols present in palm oil and its fractions are frequently expressed as groups having the same carbon numbers (the sum of the carbon atoms in three acyl chains) and are easily measured by the gas chromatographic study of the oil. There are four main triacylglycerol groups with 48, 50, 52, and 54 carbon atoms corresponding to triacylglycerols with three, two, one, or no 16:0 chains and the balance being saturated or unsaturated C_{18} chains. The C_{50} and C_{52} groups predominate and the ratio of these change in the fractionated products since 16:0 levels rise in the stearins and fall in the oleins.

Information on triacylglycerol molecular species is summarised in Table 2.22. The major species are POP and POO (around 30 and 20%, respectively) followed by PLO and PLP (each around 10%). Tripalmitin at around 5% in palm oil rises in the stearin fractions and reaches almost 60% in palm stearin of IV 11. Even higher concentrations are obtained through solvent fractionation. Palm oil contains more saturated acid in the *sn*-2 position than is normal for vegetable oils and fats. Typically a palm oil with 51% (total) of saturated acids in its triacylglycerols has 13% in the *sn*-2 position. This means, for example, that the two major triacylglycerols POO and POP will contain more OPO and PPO than would otherwise have been expected. (Information on the regiospecific distribution of fatty acids is also available in Table 2.27.)

Palm oil is also characterised by the presence of higher levels of diacylglycerols than in other vegetable oils. This is normally ~5% with higher and lower values in the olein and stearin fractions, respectively. (See Table 2.22.)

Palm oil is rich in several valuable minor components (Goh et al., 1985) and attention must be given to what happens to these during refining and fractionation (Rossi et al., 2001). Crude Malaysian palm oil is reported to contain only 5 to 130 ppm of phospholipids and a higher level of glycolipids (1000 to 3000 ppm, mainly monogalactosyldiacylglycerols). The phospholipids are mainly phosphatidylcholines (36%), phosphatidylethanolamines

TABLE 2.21 Composition of fatty acids (% wt) and triacylglycerols (by carbon number) for palm oil and its fractions

	Palm oil[a]	Palm olein[b]	Super olein[b]	Top olein	Stearin	Soft stearin	Palm mid-fraction
				Fatty acids (wt%)			
12:0	0.2	0.3	0.3	-	0.1–0.6	0.1	0–0.3
14:0	1.1	1.1	1.0	1.0	1.1–1.9	1.1	0.8–1.4
16:0	44.1	40.9	35.4	28.8	47.2–73.8	49.3	41.4–55.5
16:1	0.2				0.05–0.2	0.1	
18:0	4.4	4.2	3.8	2.5	4.4–5.6	4.9	4.7–6.7
18:1	39.0	41.5	45.1	52.0	15.6–37.0	34.8	32.0–41.2
18:2	10.6	11.6	13.4	14.6	3.2–9.8	9.0	2.6–11.5
18:3	0.3	0.4	0.3	0.4	0.1–0.6	0.2	0–0.2
20:0	0.2	0.4	0.3	0.2	0.1–0.6	0.4	0–0.6
				Triacylglycerols[c]			
46	1.2	0.8	0.2		0.5–3.3	1.2	0–1.6
48	8.1	3.3	1.9		12.2–55.8	15.3	1.4–11.3
50	39.9	39.5	30.8		33.6–49.8	42.7	45.5–73.9
52	38.8	42.7	53.4		5.1–37.3	33.4	19.4–42.0
54	11.4	12.8	13.6		Tr–8.4	7.4	1.7–8.5
56	0.6	0.7	0.2		ND	ND	0–0.9
Iodine value	52.1	56.8	61.9	70–72	22–49	46.7	34–55
SMP[d]	36.7	21.5	15.1		44–56	47.7	24–45

[a] Average of 215 Malaysian samples examined in 1990 and not differing significantly from a similar study conducted in 1981. Siew Wai Lin also cites the range of values producing these means.
[b] Range values are also given.
[c] The numbers in column 1 refer to the sum of the carbon atoms in the three acyl chains. For example, 48 represents almost entirely tripalmitin.
[d] Slip melting point (°C).
Source: Adapted from appropriate tables in Siew Wai Lin, in Gunstone, F.D., (Ed.) *Vegetable Oils in Food Technology — Composition, Properties and Uses,* Blackwell Publishing, Oxford, pp. 59–97, 2002. Further data is provided by Sambanthamurthi, R. et al., *Prog.Lipid Res.*, **39**, 507–558, 2000, and Noor Lida, H.M.D. et al., *J. Am. Oil Chem. Soc.,* **79**, 1137–1144, 2002.

TABLE 2.22 Triacylglycerols (molecular species, wt%) in palm oil and its fractions

	Palm oil	Palm olein	Palm olein	Palm olein	Palm stearin	Palm stearin	Palm stearin
IV		<60	60–64	65–67	46	38	11
OLL	0.5	<0.1	0.7	0.8	0.5	0.3	0.1
PLL	2.5	2.8	3.4	3.7	2.3	1.8	0.4
MLP	0.6	0.6	0.7	0.6	0.5	0.4	-
OLO	1.7	2.3	2.6	3.0	1.7	1.3	0.2
PLO	9.9	11.8	13.6	15.4	8.4	7.1	1.7
PLP	9.5	9.9	9.8	8.4	9.4	8.3	3.5
OOO	4.3	4.5	5.1	6.1	2.7	2.3	3.8
POO	22.8	26.8	30.2	34.5	18.4	16.7	5.0
POP	29.0	26.6	19.1	12.8	30.9	29.8	13.6
PPP	5.4	ND	ND	ND	12.5	18.6	59.6
StOO	2.5	3.3	4.2	4.5	–	–	–
POSt	5.1	4.7	3.6	2.5	5.4	4.8	2.4
PPSt	1.0	0.1	0.2	0.2	2.7	3.6	8.0
StOSt	0.5	0.2	0.4	0.2	0.6	0.6	–
DAG	4.9	5.3	6.4	7.1	4.0	4.5	1.1

Note: Symbols, such as PLO, represent all the triacylglycerols containing the three acyl chains indicated. P, St, O, and L represent palmitic, stearic, oleic, and linoleic acyl chains respectively. ND = not detected.
Source: Adapted from Siew Wai Lin, in Gunstone, F.D., (Ed.) *Vegetable Oils in Food Technology — Composition, Properties and Uses,* Blackwell Publishing, Oxford, pp. 59–97, 2002.

(24%), phosphatidylinositols (22%), phosphatidylglycerols (9%), and other (9%). The low levels of phospholipids means that palm oil can be physically refined without prior degumming.

The dark red colour of crude palm oil is due to the presence of carotenoids at concentrations of 500 to 700 ppm in the tenera variety. These are mainly β-carotene (~56%) and α-carotene (~35%) (for structures, see Dictionary section) along with many other carotenes at lower levels. After dry fractionation, crude palm olein (600 to 760 ppm) and crude palm stearin (380 to 540 ppm), as well as the residual oil remaining in the fibre after pressing (4000 to 6000 ppm), contain carotenoids at the levels indicated. These compounds are completely removed during bleaching and physical refining (Rossi et al., 2001), but about 80% of these valuable components are retained in a product called "red palm" resulting from a modified refining procedure. This is marketed as a valuable source of carotenoids with pro-vitamin A activity and is being used to combat vitamin A deficiency in Africa.

Palm oil also contains other isoprenoid hydrocarbons including C_{15} and C_{20} terpenes and squalene (C_{30}). This last has been reported at levels of 980 and 790 ppm in crude and refined oils, respectively, and 5000 to 8000 ppm in palm fatty acid distillate (PFAD, a by-product of refining). Squalene and its perhydro derivative (squalane), used in cosmetic preparations, are also available in shark oils (see Section 2.4.5) and in amaranthus and olive oils.

Tocols (combined tocopherols and tocotrienols) are important minor components in crude palm oil (710 to 1140 ppm). Their levels (ppm) in other materials are indicated in parentheses: refined palm oil (380–890), crude and refined palm olein (880–1130 and 560–900), crude and refined palm stearin (430–550 and 350–380), and still higher levels in PFAD (750–8000) (Siew Wai Lin, 2002). Palm tocols differ from those in most other vegetable oils in that they are 70 to 80% tocotrienols (γ-, α-, and δ-). Concentrates can be recovered from PFAD. Important physiological properties are claimed for the tocotrienols in respect of certain cancers and of coronary heart disease (Sambanthamurthi et al., 2000).

Crude palm oil contains 200 to 600 ppm of sterols (mainly β-sitosterol, campesterol, and stigmasterol). These compounds are also present in palm olein and palm stearin, but levels are reduced during refining. Higher levels have been recorded for PFAD (1500 to 20,000 ppm).

Palm oil and/or one of its fractions is used extensively as a frying oil (particularly palm olein), in the production of margarines, vanaspati, and shortenings; as a cocoa butter equivalent (palm mid-fraction); as a source of hard stock (palm stearin); and as a vegetable source of saturated acids (palm stearin). Figures for the use of palm oil as food and for industrial purposes are given in Table 2.24.

For more information on palm oil and its fractions see Salas et al. (2000), Sambanthamurthi et al. (2000), Berger (2001), Siew Wai Lin (2002), and Yusof Basiron (2005). For further information, see Table 2.43 to Table 2.49.

References

Berger, K.G., Palm oil, in Gunstone, F.D., Ed., *Structured and Modified Lipids*, Marcel Dekker, New York, pp. 119–153, 2001.

Goh, S.H. et al., Minor constituents of palm oil, *J. Am. Oil Chem. Soc.,* **62**, 237–240, 1985.

Noor Lida, H.M.D. et al., TAG composition and solid fat content of palm oil, sunflower oil, and palm kernel oil blends before and after chemical interesterification, *J. Am. Oil Chem. Soc.,* **79**, 1137–1144, 2002.

Rossi, M. et al., The effect of bleaching and physical refining on color and minor components of palm oil, *J. Am. Oil Chem. Soc.,* **78**, 1051–1055, 2001.

Salas, J.J. et al., Biochemistry of lipid metabolism in olive and other oil fruits, *J. Lipid Res.*, **39**, 151–180, 2000.

Sambanthamurthi, R. et al., Chemistry and biochemistry of palm oil, *Prog.Lipid Res.*, **39**, 507–558, 2000.

Siew Wai Lin, Palm oil, in Gunstone, F.D., Ed., *Vegetable Oils in Food Technology — Composition, Properties and Uses,* Blackwell Publishing, Oxford, pp. 59–97, 2002.

Yusof Basiron, Palm oil, in Shahidi, F., Ed., *Bailey's Industrial Oil and Fat Products,* 6th ed., vol. **2**, 333–430, Wiley Interscience, New York, 2005.

Websites (palm and palm kernel oils)
mpob.gov.my
www.palmolis.mpob.gov.my
www.palms.org
www.mpopc.org.my
www.acidchem-international.com
www.agroindonesia.com

2.2.11 Palm kernel oil (*Elaies guineensis*)

The oil palm bears fleshy fruits yielding palm oil when pressed and a kernel giving palm kernel oil (~45%) when subsequently pressed and solvent-extracted. These two oils differ from each other and are produced in a ratio of about 8:1 at levels around 4.0 and 0.5 tonnes/hectare. Palm kernel oil and coconut oil (see Section 2.2.4) are the two commercial lauric oils. Coconut was long the dominant member of the pair, but with the steady increase in oil palm production, the concomitant output of palm kernel oil (now ~3.5 million tonnes) exceeds that of coconut oil (~3.1 million tonnes) and this is likely to continue. Production, consumption, and trade in this oil is summarised in Table 2.23 and its fatty acid and triacylglycerol composition is given in Table 2.4 and Table 2.5. A comparison of the two lauric oils is explained in the section on coconut oil.

Palm kernel oil contains only around 10 ppm of tocols (median value from a survey of 33 samples, ranging between 0 and 260 ppm), and is mainly β-tocopherol. Sterols total around 1000 ppm (Codex range 700 to 1400 ppm) and are mainly β-sitosterol (~68% of total sterols), stigmasterol (~14%), and campesterol (~10%).

Palm kernel oil has an iodine value of 14 to 21 and can be hydrogenated to give products with IV down to 2 and slip melting points (SMP) up to 34°C. Such material is

used, for example, in the production of nondairy creamers. Alternatively, the oil can be fractionated into an olein (IV 21-26, SMP 22-26ºC) and a stearin (IV 6-8, SMP 31-33ºC) and each of these may be hydrogenated. The fractionated products differ particularly in their levels of lauric acid and oleic acid. PK stearins are used in substitute chocolates and other confectionery.

The two lauric oils are widely used in the production of surface-active compounds where chain length is important and they have several food uses, including margarine and spreads, shallow frying, cocoa butter substitutes, filling creams, ice cream, nondairy whipping creams, filled milks. It is also a source of medium chain triglycerides.

Because of their low unsaturation, both lauric oils show high oxidative stability despite their low level of natural antioxidant. However, they are prone to hydrolytic rancidity and lauric acid imparts a soapy flavour. Further, the C_{12} acid can be oxidised and decarboxylated to undecan-2-one $[CH_3(CH_2)_8COCH_3]$, a phenomenon described as ketonic rancidity.

As indicated in Table 2.24, in EU-25 in 2003 coconut and palm kernel oils were used mainly for food purposes (61 and 83%, respectively), but a significant portion (37 and 16%, respectively) were used for nonfood purposes.

TABLE 2.23 Major countries/regions involved in the production (from indigenous or imported kernels), consumption (food and nonfood uses), export, and import of palm kernel oil in 2004/05 (million tonnes)

	Total (mt)	Countries/Regions
Production	3.80	Malaysia 1.83, Indonesia 1.31, Nigeria 0.21
Consumption	3.74	Malaysia 1.05, EU-25 0.66, Indonesia 0.39, US 0.27, Nigeria 0.21, China 0.19
Exports	1.98	Indonesia 0.92, Malaysia 0.87
Imports	1.98	EU-25 0.67 US 0.27, China 0.19, Malaysia 0.15, India 0.11
Seed yield (t/ha)	0.94	Malaysia 1.10, Indonesia 0.83, Nigeria 1.23

Note: The high consumption of palm kernel oil in Malaysia is related to its rapidly growing oleochemical industry.

TABLE 2.24 Consumption (kt and %) of selected seed oils in EU-25 during 2003 for industrial purposes (including biodiesel), for food, and as feed, seeds, and waste (FSW)

	Industrial use		Food		FSW		Total
Oil	kt	%	kt	%	kt	%	kt
Rape	1327	32.8	2720	67.2	2	0	4049
Palm	286	9.6	2453	82.0	252	8.4	2991
Sunflower	85	3.4	2433	96.6	–	–	2518
Soybean	263	12.4	1746	82.4	111	5.2	2120
Olive	37	1.9	1932	98.1	–	–	1969
Coconut	300	37.3	492	61.2	12	1.5	804
Palm kernel	90	15.8	473	83.3	5	0.9	568

Source: USDA GAIN Report July 2004: www.fas.usda.gov/gainfiles

Further details about palm kernel oil are to be found in Pantzaris (2002), in Yusof Basiron et al. (2005), and in appropriate websites (see Palm oil). See also Table 2.43 to Table 2.49.

References

Pantzaris, T.P. and Yusof Basiron, The lauric (coconut and palm kernel) oils in Gunstone, F.D. (Ed.) *Vegetable Oils in Food Technology — Composition, Properties and Uses,* Blackwell Publishing, Oxford, pp. 157–202, 2002.

USDA GAIN Report, July 2004: www.fas.usda.gov/gainfiles

Yusof Basiron et al., *Palm Kernel Products —Characteristics and Applications*, Malaysian Palm Oil Board, Luala Lumpur, Malaysia, 2005.

Websites: see Palm oil.

2.2.12 Rapeseed (canola oil) (*Brassica napus*, *B. rapa*, and other *Brassica* species)

Brassica oilseeds have been cultivated for thousands of years and are one of the few edible oilseeds capable of being grown in cool temperate climates. They are closely related to the mustard condiments used for flavouring and for their reputed medicinal properties. Records indicate early cultivation of vegetable forms of the crop in India 1500 years ago and in China more than 1000 years ago. Cultivation extended across Europe in the Middle Ages and, by the 15th century, rapeseed was being grown in the Rhineland as a source of lamp oil and also for cooking fat. Demand for rapeseed oil grew significantly in the developed world during the 20th century with concurrent improvements in agronomic techniques, processing methods, and seed varieties (Gunstone, 2004).

Several rapeseed species are grown in the major oilseed rape-producing areas of the world. *Brassica rapa* (turnip rape) is the most cold-hardy species and currently accounts for half of all crops grown in Western Canada, largely because of its early maturity. Ecotypes of this species are also grown in the Indian subcontinent. *B. napus* (swede rape) is the most commonly grown rapeseed in Europe, Canada, and China. Both spring- and winter-sown varieties are available, although winter types dominate due to their higher yields in favourable growing conditions. *B. juncea* is well adapted to drier growing conditions and is widely grown in northern India and China. This species has been introduced into parts of Australia and is used in mainstream breeding programmes of *B. napus* for drier climates. *B.carinata* is less widely grown than the other species and is largely restricted to Ethiopia and surrounding countries in East Africa.

Breeding developments, first in Canada and then elsewhere in the 1960s, led to the production of rapeseed low in both erucic acid (22:1) in the oil and glucosinolates in the meal. These are the so-called "double-low" varieties. The trade name Canola™ was established with the licensing of the first double-low variety in Canada in 1974. The

canola trademark is held by the Canola Council in Canada and may be used to describe rapeseed with less than 2% erucic acid in the oil and less than 30 μM/g glucosinolates in the meal. The term "canola" is used in many English-speaking countries, such as the U.S. and Australia, but in Europe, "rapeseed" continues to be used in reference both to double-low types and to other quality types, such as high-erucic acid rapeseed for industrial purposes. The terms LEAR and HEAR are used to designate low- and high-erucic acid rapeseed oils. Shatter-resistant varieties were also developed.

Among oilseeds, rapeseed is now second only to soybean and among vegetable oils it is third after soybean oil and palm oil. Production of rapeseed oil peaked in 1999 and 2000 at 14.5 million tonnes after which it fell back to 12.4 million tonnes 3 years later. Since then it has risen and was expected to be around 16.1 million tonnes in 2004/05. These figures do not distinguish the various types of rapeseed oil, but are likely to refer mainly to the low-erucic (canola) oil. (Production and trade data for rapeseed oil are given in Table 2.25.) It must be remembered that oil production may be based on seeds produced locally or imported. Japan, for example, is wholly dependent on imported seed, while China is both grower and importer of rapeseeds. Canada, with only a modest population, is a significant grower of seed and an important exporter of both seed and oil. Northern Europe and India are also important locations for production of seed and oil. Seed yields are highest in Europe, somewhat lower in Canada, and lower still in India. There is a growing interest in cold-pressed edible oil, especially in Germany (Matthäus and Brühl, 2004).

Low-erucic rapeseed oil (canola) contains less saturated acid (~6%) than any other commodity oil. It is rich in oleic acid (>60%) and contains linoleic (~22%) and linolenic (~10%) in a favourable omega-6/omega-3 ratio (Table 2.26). A large number of minor fatty acids have been identified in canola oil (Ratnayake and Daun, 2004), including acids with branched chains, acids with an odd number of carbon atoms, a series of n-7 monounsaturated acids, acids with a sulfur-containing ring system, and acids with *trans* unsaturation. Typical fatty acid composition data are collected in Table 2.26 for high-erucic oil (45% 22:1, along with 10% 20:1), low-linolenic oil (around 2%, with concomitant increase in linoleic acid), and high-oleic rapeseed oils (~80%, with a decrease in both linoleic and linolenic acids). This table also contains data on canola oils enriched in medium-chain acids and in γ-linolenic acid, respectively. See also Dolde et al. (1999) and Vlahakis and Hazebroek (2000).

TABLE 2.25 Major countries/regions involved in the production (from indigenous or imported seed), consumption (food and nonfood uses), export, and import of rapeseed oil in 2004/05 (million tonnes).

	Total	Countries/Regions
Production	16.11	EU-25 5.47, China 4.55, India 2.08, Canada 1.35, Japan 0.95
Consumption	15.92	EU-25 5.21, China 4.84, India 2.07, Japan 0.99, US 0.71
Exports	1.45	Canada 0.92, EU-25 0.22
Imports	1.42	US 0.54, China 0.30
Seed yield (t/ha)	1.73	China 1.78, EU-25 3.40, India 0.91, Canada 1.58

Note: These figures do not distinguish the various types of rapeseed oil, but probably refer mainly to the low-erucic (canola) oil.
Source: Adapted from *Oil World Annual 2005*, ISTA Mielke GmbH, Hamburg, 2005.

TABLE 2.26 Fatty acid composition of a range of rapeseed oils

	LEAR[c]	HEAR[d]	LLCAN[e]	HOCAN[f]	LTCAN[g]	GLCAN[h]
Saturated acids[a]	6.3	7.1	6.6	7.7	48.1	9.9
16:0	3.6	4.0	3.9	3.4	2.7	4.2
18:0	1.5	1.0	1.3	2.5	1.6	3.7
Monunsaturated acids[b]	62.4	69.7	63.1	79.9	34.3	25.5
18:1	61.6	14.8	61.4	77.8	32.8	24.4
20:1	1.4	10.0	1.5	1.6	0.8	0.8
22:1	0.2	45.1	0.1	0.1	0.5	0.1
Polyunsaturated acids	31.3	23.2	30.2	12.4	17.6	64.6
18:2	21.7	14.1	28.1	9.8	11.3	26.1
18:3	9.6	9.1	2.1	2.6	1.3	1.8

[a] Also 14:0, 20:0, 22:0, and 24:0 all at low levels.
[b] Also 16:1.
[c] Low-erucic rapeseed oil (canola).
[d] High-erucic rapeseed oil.
[e] Low-linolenic rapeseed oil.
[f] High-oleic rapeseed oil.
[g] Rapeseed oil enriched in lauric acid (also contains 12:0 38.8%, 14:0 4.1%).
[h] Rapeseed oil enriched in γ-linolenic acid (also contains γ-linolenic acid 37.2%).
Source: Adapted from Ratnayake, W.M.N. and Daun, J K., in Gunstone, F.D. (Ed.) *Rapeseed and Canola Oil — Production, Processing, Properties and Uses*. Blackwell Publishing, Oxford, pp. 37–78, 2004. Data are also available for oils from seeds grown in Virginia, U.S. (Hamama et al, 2003).

TABLE 2.27 The distribution of fatty acids in Australian canola oil and some other oils between the *sn*-2 and *sn*-1/3 positions

	16:0	18:0	18:1	18:2	18:3
Canola					
1,2,3-position	4.7	2.1	58.1	20.8	10.2
1,3-position	6.6	2.8	60.9	16.4	6.9
2-position	1.4	0.7	52.7	29.5	13.7
Olive					
1,2,3-position	12.9	2.7	71.5	8.8	0.7
1,3-position	17.7	3.6	67.1	7.6	0.1
2-position	3.5	1.0	81.1	11.2	1.9
Sunola[a]					
1,2,3-position	5.0	4.5	77.2	12.1	0.4
1,3-position	5.8	5.2	75.4	11.6	0.8
2-position	3.3	3.1	80.9	13.1	Tr
Sunflower					
1,2,3-position	6.7	3.9	28.2	60.3	0.4
1,3-position	8.0	4.7	28.3	57.0	0.7
2-position	4.1	2.5	27.9	65.0	Tr
Cottonseed					
1,2,3-position	24.9	2.3	14.8	56.8	0.2
1,3-position	37.8	3.5	13.9	43.4	0.3
2-position	Tr	Tr	16.4	83.5	Tr
Palm					
1,2,3-position	48.8	4.8	36.0	9.1	0.1
1,3-position	61.0	6.0	25.7	5.8	0.2
2-position	24.5	2.5	56.5	15.6	Tr
Flaxseed					
1,2,3-position	5.9	3.9	14.9	15.6	59.1
1,3-position	9.3	6.0	13.9	13.0	57.0
2-position	Tr	Tr	16.9	20.9	63.3
Sesame					
1,2,3-position	9.4	6.6	42.6	40.4	0.3
1,3-position	16.9	9.4	38.1	34.1	0.3
2-position	Tr	0.9	49.2	48.3	0.3

[a] Sunola is high-oleic sunflower.

TABLE 2.28 Stereospecific analysis of the major fatty acids in HEAR and soybean oil by chiral HPLC

	16:0	18:0	18:1	18:2	18:3	20:1	22:1
				Soybean oil			
sn-1	16.7	5.4	24.3	46.4	6.4		
sn-2	2.2	0.3	23.4	68.1	5.7		
sn-3	16.1	4.6	24.6	47.0	7.0		
				HEAR			
sn-1	5.6	1.9	16.5	1.2	3.3	17.4	50.2
sn-2	1.6	0.4	33.4	39.3	21.3	1.9	0.8
sn-3	4.5	1.7	5.6	7.4	1.3	12.0	61.3

Source: Takagi, T. and Ando, Y., *Lipids*, **26**, 542–547, 1991. The original paper gives details for minor acids also.

Oilseed rape has proved amenable to genetic modification and many breeding programmes are underway to modify traits and introduce new quality characteristics, principally with *B. napus*. Oilseed rape, or canola, is the third most widely grown, commercial genetically modified crop after soybeans and maize, with an area equivalent to that of cotton. Tolerance to one of several broad-spectrum herbicides was one of the first characteristics to be incorporated in crops using genetic modification and varieties tolerant to either glyphosate or glufosinate have been developed. These are widely grown in North America, but no genetically modified oilseed rape is yet being grown in Europe (at the end of 2005). Rapeseed genetically modified to produce lauric acid within the oil was grown commercially in North America, but was later discontinued on commercial grounds. Attempts are being made to increase the level of erucic acid beyond that normally present in HEAR (Sasongko and Möllers, 2005). Further developments with *Brassica* breeding are being facilitated by new techniques in biotechnology and are discussed below.

TABLE 2.29 Major triacylglycerols (wt %) in rapeseed oil

	CO[a]	LLCO[b]	HOCO[c]
LnLO	7.6	1.7	1.5
LLO	8.6	11.0	1.1
LnOO	10.4	2.6	8.6
LnOP	2.1	0.5	1.1
LOO	22.5	28.4	12.7
LOP	5.7	4.2	2.2
OOO	22.4	32.8	49.5
POO	4.6	4.8	7.7
StOO	2.6	2.4	5.0
PPP	0.1	1.4	2.8
LLP	1.4	1.1	0.8
LOSt	1.6	1.9	1.0
LLL	1.3	1.6	0.2
LnLL	1.4	0.0	0.3
LnLnO	1.7	0.4	0.1
Others	6.0	5.2	5.4

[a] Canola oil.
[b] Low-linolenic canola oil.
[c] High-oleic canola oil.
Three-letter symbols refer to all triacylglycerols containing the three acyl chains indicated. L, Ln., O, P, and St represent linoleic, linolenic, oleic, and stearic chains respectively.
Source: Adapted from Kallio, H. and Currie, G., *Lipids,* **28**, 207–215, 1993.

The distribution of fatty acids in Australian canola oil between the *sn*-2 and *sn*-1/3 positions given by Richards et al. in a lecture in 2002 and cited by Ratnayake and Daun (2004) are given in Table 2.27. In line with what is expected of vegetable oils, the saturated acids are mainly at positions 1/3, linoleic and linolenic acids concentrate at position 2, and oleic acid is slightly concentrated at this position. This distribution pattern must be partly the consequence of the very high levels of unsaturated acids (~90%). Values for HEAR have been given by Takagi and Ando (1991) using chiral HPLC (Table 2.28).

The major triacylglycerols are detailed in Table 2.29 for canola oil and for the low-linolenic and high-oleic varieties.

The dominant molecular species for canola oil are LOO (22%), OOO (22%), LnOO (10%), LLO (9%), LnLO (8%), LOP (6%), and POO (5%). Similar, but not identical, results reported by Neff et al. in 1997 are cited by Ratnayake and Daun (2004).

Levels of phosphorus are given as 529, 242, and 12 mg/kg for solvent-extracted oil, expeller oil, and degummed oil, and the phospholipids are mainly phosphatidylcholines, phosphatidic acids, phosphatidylinositols, and phosphatidylethanolamines. Full details are given by Przybylski and Mag (2002), along with the fatty acid composition of each phospholipid class. Hamama et al. (2003) give phospholipid levels of 2.1 to 2.8% for oil from 11 canola cultivars grown in the U.S.

The sterols present in canola oil range from 0.7 to 1.0% and are distributed between free and esterified compounds (Table 2.47). The major sterols are β-sitosterol (52%), campesterol (28%), and brassicasterol (14%). This last sterol is unique to *brassica* oils and its presence is used as an indicator of the occurrence of rapeseed oil in blends. The values in parentheses are typical values for canola oil and relate to the percent of total sterols (6.9 g/kg of which 4.2 comes from esterified sterols). Sterol levels in canola oil are higher than those in soybean oil, but lower than those in corn oil. Codex Alimentarius gives range values of 4.5 to 11.3, 1.8 to 4.5, and 7.0 to 22.1 mg/kg for these three oils. Similar data for modified canola oils are given by Przybylski and Mag (2002). After refining, sterol levels are reduced by about 40% and structures may be modified through high temperature dehydration. Similar data are collated by Ratnayake and Daun (2004) and are reported by Hamama et al. (2003) for canola oils grown in the U.S. Dolde et al. (1999) and Vlahakis and Hazebroek (2000) have provided details of fatty acid composition, sterols, and tocopherols from several canola, sunflower, and soybean lines.

Canola oil contains tocopherols at a level around about 770 mg/kg oil — mainly α- (270) and γ- (420) tocopherol. High-erucic rapeseed oil shows very similar values of 790, 270, and 430 mg/kg, respectively. Gogolewski et al. (2000) have described the changes in tocopherol during refining. See also Dolde et al. (1999) and Vlahakis and Hazebroek (2000).

Crude canola oil frequently contains chlorophyll and chlorophyll derivatives, such as pheophytins a and b and pyropheophytins a and b. The level of these undesirable materials is higher in oil extracted from immature seed. Top grade crude canola oil should have less than 30 mg/kg of chlorophyll pigments, but this is not always achieved. RBD (refined, bleached, deodorised) oil is expected to have levels below 25 ppb.

Canola oil contains traces of carotenoids (around 130 ppm), which are ~90% xanthophylls, but this is reduced to ~10 ppm after refining. Also present are traces of sulfur compounds and of metals (iron, copper, lead, and arsenic), but the levels of all of these are reduced after refining.

Low-erucic oil, with its low level of saturated acids, high level of oleic acid, and its favourable ratio of linoleic to linolenic acid (before hydrogenation), is a healthy oil and has many food uses, These include salad oils, cooking oils, frying oils, and for the production of spreads and shortenings (McDonald, 2004). Figures for the use of rapeseed oil as food and for industrial purposes are given in Table 2.24.

In Europe, rapeseed oil is the major oil used to make rapeseed methyl esters. These are mixed with mineral oil to form biodiesel (Komers et al., 2001; Walker, 2004). It is also used as a lubricant (Wu et al., 2000). High-erucic oil is used for industrial purposes, mainly as a source of erucic (22:1) and behenic acids (22:0). Erucic acid is converted to erucamide, which is used in the processing of polymers (Temple-Heald, 2004). (See also Chapter 9 on nonfood uses.)

For further information, see Table 2.43 to Table 2.49.

References

Dolde, D. et al., Tocopherols in breeding lines and effects of planting location, fatty acid composition, and temperature during development, *J. Am. Oil Chem. Soc.,* **76**, 349–355, 1999.

Gogolewski, M. et al., Changes of the tocopherol and fatty acid contents in rapeseed oil during refining, *Eur. J. Lipid Sci. Technol.* **102**, 618–623, 2000.

Gunstone, F.D., Ed., *Rapeseed and Canola Oil — Production, Processing, Properties and Uses.* Blackwell Publishing, Oxford, 2004.

Hamama, A.A. et al. Genotype and growing location effects on phytosterols in canola oil, *J. Am. Oil Chem. Soc.* **80**, 1121–1126, 2003.

Kallio, H. and Currie, G., Analysis of low erucic acid turnip rapeseed oil (*Brassica campestris*) by negative ion chemical ionisation tandem mass spectrometry. A method giving information on the fatty acid composition in positions *sn*-2 and *sn-1/3* of triacylglycerols*, Lipids,* **28**, 207–215, 1993.

Komers, K. et al., Biodiesel from rapeseed oil, methanol and KOH, *Eur. J. Lipid Sci. Technol.,* **103**, 359–362 and 363–371, 2001.

McDonald, B.E., Food uses and nutritional properties, in Gunstone, F.D. (Ed.) *Rapeseed and Canola Oil — Production, Processing, Properties and Uses,* Blackwell Publishing, Oxford, pp. 131–153, 2004.

Matthäus, M. and Brühl, L., Cold-pressed edible rapeseed oil production in Germany, *Inform*, **15**, 266–268, 2004.

Möllers, C., Potential and future prospects from rapeseed oil, in Gunstone, F.D., Ed., *Rapeseed and Canola Oil — Production, Processing, Properties and Uses,* Blackwell Publishing, Oxford, pp. 186–217, 2004.

Neff, W. E. et al., Oxidative stability as affected by triacylglycerol composition and structure of purified canola oil triacylglycerols from genetically modified normal and high stearic and lauric acid canola varieties, *Lebensmittelwissenschaft und Technologie,* **30**, 793–799,1997.

Przybylski, R. and Mag, T.M., Canola/rapeseed oil in Gunstone, F.D., Ed., *Vegetable Oils in Food Technology — Composition,*

Properties and Uses, Blackwell Publishing, Oxford, pp. 98–127, 2002.

Ratnayake, W.M.N. and Daun, J K., Chemical composition of canola and rapeseed oils, in Gunstone, F.D., Ed., *Rapeseed and Canola Oil — Production, Processing, Properties and Use*s. Blackwell Publishing, Oxford, pp. 37–78, 2004.

Rousseau, D., Chemical and physical properties of canola and rapeseed oil, in Gunstone, F.D., Ed., *Rapeseed and Canola Oil — Production, Processing, Properties and Use*s, Blackwell Publishing, Oxford, pp. 79–110, 2004.

Sasongko, N.D. and Möllers, C., Towards increasing erucic acid content in oilseed rape (*Brassica napus* L.) through the combination with genes for high oleic acid, *J. Am. Oil Chem. Soc.,* **82**, 445–449, 2005.

Takagi, T. and Ando, Y., Stereospecific analysis of triacyl-*sn*-glycerols by chiral HPLC, *Lipids*, **26**, 542–547, 1991.

Temple-Heald, C., High-erucic oil: its production and uses, in Gunstone, F.D., Ed., *Rapeseed and Canola Oil — Production, Processing, Properties and Use*s, Blackwell Publishing, Oxford, pp. 111–130, 2004.

Vlahakis, C. and Hazebroek, J., Phytosterol accumulation in canola, sunflower, and soybean oils: effect of genetics, planting location, and temperature, *J. Am. Oil Chem. Soc.,* **77**, 49–52, 2000**.**

Walker, K., Non-food uses, in Gunstone, F.D., Ed., *Rapeseed and Canola Oil — Production, Processing, Properties and Use*s. Blackwell Publishing, Oxford, pp. 154–185, 2004.

Wu, X. et al. The study of epoxidised rapeseed oil used as a potential biodegradable lubricant, *J. Am. Oil Chem. Soc.,* **77**, 561–563, 2000.

2.2.13 Rice bran oil (*Oryza sativa*)

Rice is the principal staple food of about half the world's population. According to Gopala Krishna (2000) annual production of rice in 1999 was about 600 million tonnes. China (200 million tonnes) and India (131 million tonnes) were the largest producers, along with seven other countries where annual production exceeded 10 million tonnes (Indonesia, Vietnam, Bangladesh, Thailand, Myanmar [Burma], Japan, and the Philippines). To produce white rice, the hull is removed and the bran layer is abraded to make 8 to 10% of the rice grain as bran, which includes the testa, cross cells, aleurone cells, part of the aleurone layer, and the germ. The bran contains almost all the oil of the rice coreopsis. Rice bran has 18 to 24% oil and 4 to 6% free acids in addition to fibre, protein, and carbohydrates. Crude rice bran oil is usually obtained by hexane extraction. Lipases liberated from the testa and the cross cells promote rapid hydrolysis of the oil and, therefore, the bran should be extracted within hours of milling. There is probably a potential for 5 to 8 million tonnes of rice bran oil per annum, but present production is only about 0.7 to 1.0 million tonnes and not all of this is of food grade. India (500,000 tonnes), China (120,000 tonnes), and Japan (80,000 tonnes) are the major countries producing rice bran oil. With the growing demand for imported oils in these countries, it seems possible that local production of rice bran oil will increase (Arumughan, 2004).

Refining rice bran oil presents some difficulties which have been discussed by Gingras, 2000; Gopala Krishna, 2002; and Narayana et al., 2002. Roy et al. (2002) have discussed the merits of enzymic degumming to reduce phosphorus levels prior to physical refining.

Crude rice bran oil contains 90 to 96% of saponifiable lipids comprising triacylglycerols (83 to 86%) along with diacylglycerols, monacylglycerols, free acids, waxes (3 to 4%), glycolipds (6 to 7%), and phospholipids (4 to 5%). Unsaponifiable lipids generally account for ~4% of the oil.

The major acids in rice bran oil are palmitic (12 to 28%, typically 20%) oleic (35 to 50%, typically 42%), and linoleic acid (29 to 45%, typically 32%), accompanied by stearic acid (2 to 4%), linolenic (0.5 to 1.8%), and by 14:0, 16:1, 20:0, 20:1, and 22:1 acids (all at levels below 1%). Kochhar (2002) reports that the major triacylglycerols are those represented as OOO, PLO, and PLL, but it is surprising that LLO, OOL, and OOP are not included in this list.

Rice bran oil contains waxes, generally at a level of 2 to 4%, but sometimes up to 8%. Gingras (2000) reports these to be esters of saturated fatty acids (C_{16} to C_{26}) and saturated alcohols (C_{24} to C_{30}) (see also Arumughan, 2004). The wax has been used as a convenient source of octacosanol and triacontanol. An efficient method of isolating the wax has been described and its composition determined (Val et al., 2005).

Crude rice bran oil is reported to contain desmethylsterols (3225 mg/100 g, reduced to 1055 mg/100 g in refined oil), monomethylsterols (420 mg/100 g), and dimethylsterols or triterpene alcohols (1176 mg/100 g) making a total of 4.8 g/100 g. Kochhar (2002) has collated information about the major members of each class. Many of these sterols are present as esters of ferulic acid (3-methoxy-4-hydroxycinnamic acid) and are known collectively as oryzanols (115 to 787 mg/100 g). These are powerful antioxidants and show physiological properties. The phytosterols have also been detailed by Jiang and Wang 2005. Kochhar also provides information on the tocols in commercially refined rice bran oil. Typical values (cited as mg/kg) total 915 and the major components are α-, γ, and δ-tocopherols (347, 89, 42, respectively) and α-, γ-, and δ-tocotrienols (126, 301, 10, respectively). This makes rice bran oil a powerful source of tocotrienols. (See also palm oil as another source of tocotrienols).

It is not surprising that, through the combined effects of the tocols and the oryzanols, rice bran oil has high oxidative stability and it has been added to frying oils at levels around 5% to extend the time before these need to be discarded. The refined oil is an excellent salad oil and frying oil and is used as a coating oil for biscuits and nuts.

Rice bran oil is reported to lower serum cholesterol by reducing LDL and VLDL without changing the level of

HDL, though this claim has been questioned (Stanley, 2002). This effect seems not to be related to fatty acid or triacylglycerol composition, but to the unsaponifiable fraction and, in particular, to the oryzanols.

For further information, see Table 2.43 to Table 2.49.

References

Arumughan, C., et al., Rice bran oil: an untapped health food, *Inform.,* **15**, 706–707, 2004.

Gingras, L., Refining of rice bran oil, *Inform.*, **11**, 1196–1203, 2000.

Gopala Krishna, A.G., Nutritional components of rice bran oil in relation to processing, *Lipid Technol.*, **14**, 80–84, 2002.

Jiang, Y., and Wang, T., Phytosterols in cereal by-products, *J. Am. Oil Chem. Soc.,* **82**, 439–444, 2005.

Kochhar, S.P., Sesame, rice-bran and flaxseed oils, in Gunstone, F.D., Ed., *Vegetable Oils in Food Technology — Composition, Properties and Uses,* Blackwell Publishing, Oxford, pp. 297–326, 2002.

Narayana, T., et al., Origins of problems encountered in rice bran oil processing, *Eur. J. Lipid Sci. Technol.,* **104**, 203–211, 2002.

Roy, S.K., et al., Enzymic degumming of rice bran oil, *J. Am. Oil Chem. Soc.,* **79**, 845–846, 2002.

Stanley, J., How good is the evidence for effects of rice bran oil and rice bran oil components on blood lipid concentrations in humans? *Lipid Technol.*, **14**, 109–112, 2002.

Val, S.R., et al., A process for the preparation of food-grade rice bran wax and the determination of its composition, *J. Am. Oil Chem. Soc.,* **82**, 57–64, 2005.

2.2.14 Safflower (*Carthamus tinctorius*)

The safflower plant has a long history in large part because its florets produce red (carthamin) and yellow coloring material used as a food colorant and as a dye. The whole seed contains oil (38 to 48%), protein (15 to 22%), and fibre (11 to 22%), and after removal of hulls the kernel contains oil (53 to 63%), protein (23 to 29%), and fibre (1 to 3%). The oil is normally rich in linoleic acid (75%), accompanied by oleic (14%) and saturated acids (10%). Because of the high level of linoleic acid and the absence of linolenic acid, it has been extensively used as a nonyellowing drying oil, especially after pretreatment to conjugate the diene acid. In more recent times, it has been favoured as a starting point for the preparation of conjugated 18:2 acids (CLA).

High-oleic varieties (~74%) with much reduced levels of linoleic acid (16%) have also been developed (Ucciani, 1995; Smith, 1996; Firestone, 1997). Attempts are being made to introduce GLA into this oil by genetic modification (see borage oil) (Anon., 2004).

In 2003/04, world production of safflower seed was 575,000 tonnes, mainly in Mexico, India, and the U.S. This suggests an oil production of about 250,000 tonnes, and about 60 to 70 kt of the seeds are imported and exported annually.

References

Anonymous, GLA-enriched safflower oil to be produced, *Inform.*, **15**, 526, 2004.

Firestone, D. (Ed.) *Official Methods and Recommended Practices of the American Oil Chemists' Society: Physical and Chemical Characteristics of Oils, Fats and Waxes*, AOCS Press, Champaign, IL, 1997, reprinted as a book and disc, 1999.

Smith, J.R., *Safflower*, AOCS Press, Champaign, IL, 1996.

Ucciani. E., *Nouveau Dictionnaire des Huiles Végétales, Compositions en acides gras,* Lavoisier Tec & Doc, Paris, 1995.

2.2.15 Sesame oil (*Sesamum indicum*)

Sesame (*Sesamum indicum* L.) is thought to have originated in Africa and may be the oldest oilseed crop known to man. It a source of edible oil and is also widely used as a spice. The seed contains 50 to 60% oil with excellent oxidative stability due to the presence of natural antioxidants, such as sesamolin, sesamin, and sesamol in addition to tocols. The fatty acid composition of sesame oil varies considerably among the different cultivars worldwide. Sesame oil has a total production of 774,000 tonnes. It is produced and consumed mainly in China, India, Myanmar (Burma), Sudan, and Japan, though many countries use the oil in small amounts (Table 2.30). Transnational trade in both sesame seeds and sesame oil is quite small.

Refined sesame oil contains palmitic (typically 9.2%, range 7.2 to 12.0%), stearic (5.8, 3.5 to 6.0), oleic (40.6, 33.5 to 50.0), linoleic (42.6, 35.5 to 50.0), and several minor acids (total ~2%) (Kamal-Eldin and Appelqvist, 1994;, Kochhar, 2002; Hwang, 2005). (Information on the regiospecific distribution of fatty acids is available in Table 2.26.)

Sesame oil contains sterols (0.51 to 0.76%) of which 35% are present in esterified form. They are mainly desmethylsterols (85 to 89%) with lower levels of monomethylsterols (9 to 11%) and triterpene alcohols (2 to 4%). The major desmethylsterols are sitosterol (60 to 67%), campesterol (15 to

TABLE 2.30 Major countries/regions involved in the production (from indigenous or imported seed), consumption (food and nonfood uses), export, and import of sesame oil in 2004/05 (thousand tonnes)

	Total	Countries/Regions
Production	774	China 198, India 145, Myanmar 109, Sudan 52, Japan 41
Consumption	774	China 203, India 145, Myanmar 108, Sudan 49, Japan 40
Exports	33	
Imports	32	
Seed yield (t/ha)	0.43	China 1.07, India 0.37, Myanmar 0.30, Sudan 0.18

Source: Adapted from *Oil World Annual 2005*, ISTA Mielke GmbH, Hamburg, 2005.

20%) and Δ^5-avenasterol (6 to 11%) and stigmasterol (5 to 8%) (Kamal-Eldin and Appelqvist 1994b, Kochhar 2002).

Sesame seed oil generally contains 400 to 700 ppm of tocols, which is 96 to 98% γ-tocopherol in addition to the lignans — sesamin (0.02 to 1.13%), sesamolin (0.02 to 0.59%), and others. Sesamolin is not itself an antioxidant, but during acid bleaching it is converted to several related phenolic antioxidants. Refining procedures have been described that retain much of these antioxidants in the refined oil. (Kochhar 2002).

For further information, see Table 2.43 to 2.49.

References

Hwang, L.S., Sesame oil, in Shahidi, F., Ed., *Bailey's Industrial Oil and Fat Products,* 6th ed., vol. **2**, 537–576, Wiley Interscience, New York, 2005.

Kamal-Eldin, A. and Appelqvist, L.A., Variation in fatty acid composition of the different acyl lipids in seed oils from four *Sesamum* species, *J. Am. Oil Chem. Soc*., **71**, 135–139, 1994a.

Kamal-Eldin, A. and Appelqvist, L.A., Variations in the composition of sterols, tocopherols and lignans in seed oils from the different acyl lipids in seed oils from four *Sesamum* species, *J. Am. Oil Chem. Soc*., **71**, 149–156, 1994b.

Kochhar, S.P., Sesame, rice-bran and flaxseed oils, in Gunstone, F.D., Ed., *Vegetable Oils in Food Technology — Composition, Properties and Uses,* Blackwell Publishing, Oxford, pp. 297–326, 2002.

2.2.16 Soybean oil (*Glycine max*)

At 32.6 million tonnes per annum (Table 2.31), soybean oil is produced in a greater amount than any other oil; however, palm oil is now very close. Soybeans were grown first in China, then increasingly over the past 60 years in the U.S., and now more and more in Argentina and Brazil. When extracted, the beans provide oil (18%) and high-quality protein meal (79%). The soybean complex includes beans, oil, and meal with considerable trade in all three commodities.

TABLE 2.31 Major countries/regions involved in the production (from indigenous or imported seed), consumption (food and nonfood uses), export, and import of soybean oil in 2004/05 (million tonnes)

	Total	Countries/Regions
Production	32.57	US 8.60, Brazil 5.74, Argentina 5.03, China 5.11, EU-25 2.63. India 0.81, Mexico 0.67
Consumption	32.23	US 7.77, China 7.45, Brazil 3.04, EU-25 1.92, India 2.42, Iran 0.82, Mexico 0.78, Japan 0.66
Exports	9.85	Argentina 4.70, Brazil 2.73, EU25 0.79, US 0.65
Imports	9.80	China 2.25, India 1.68, Iran 0.78
Seed yield (t/ha)	2.30	EU-25 2.96, US 2.86, Argentina 2.73, Brazil 2.21, China 1.80, India 0.77

Source: Adapted from *Oil World Annual 2005*, ISTA Mielke GmbH, Hamburg, 2005.

Crude soybean oil is mainly triacylglycerols (95 to 97%) with lower levels of phospholipids (1.5 to 2.5%), unsaponifiable matter (1.6%), and traces of metals. After refining, the level of triacylglycerols is in excess of 99%.

Table 2.32 to Table 2.34 contain information on fatty acid composition. Several different analyses are reported for soybean oil. These are similar but not identical. The oil is made up almost entirely of palmitic (~11%), stearic (~4%), oleic (22 to 25%), linoleic (52 to 55%), and linolenic acids (7 to 8%), along with very low levels of 14:0, 16:1, 20:0, and 22:0 (total <1%). Information on the regiospecific distribution of fatty acids is available in Table 2.17, Table 2.28, and Table 2.34. Triacylglycerol data are appended to Table 2.34. As expected almost half the triacylglycerols contain two or three linoleic acid chains. Table 2.35 shows what happens to fatty acid composition when soybean oil is partially hydrogenated from its normal iodine value of ~132 to various levels down to 65. Depending on the hydrogenation procedure employed and the extent of hydrogenation, partially hydrogenated vegetable oils contain a wide range of *trans* 18:1 acids (Δ4 to Δ16, but mainly Δ6 to Δ14 with the Δ10 and Δ11 acids predominating at ~60% of the total *trans* acids in one study). Ruminant milk fats contain the same range of *trans* acids, resulting from biohydrogenation of dietary linoleic and linolenic acids, but the distribution pattern is quite different with the Δ11 acid (vaccenic) dominant at almost 50% of the *trans* acids (Wolff et al., 1998). *Trans* isomers of the PUFA, especially linolenic acid, are formed during high temperature deodorisation of soybean oil.

Table 2.33 gives the fatty acid composition for soybean oils that have been modified for nutritional or technological reasons by conventional seed breeding or by genetic modification. When these become available on a commercial scale, good IP (identity preservation) systems will have to be in place so that there is no mixing of the regular commodity and the premium product. The low-linolenic oil is of particular interest and its production is expected to be significant by 2010. However, many of these modified oils

TABLE 2.32 Fatty acid composition of soybean oil

	16:0	18:0	18:1	18:2	18:3
Soybean oil (a)	11.0	4.0	23.4	53.2	7.8
Soybean oil (b)	11.5	4.3	25.4	52.0	6.9
sn-1	19.5	7.6	22.0	43.1	7.9
sn-2	2.8	1.1	22.7	66.3	7.1
sn-3	13.0	5.1	31.7	44.8	5.4

Source: Wang, T., in Gunstone, F.D. (Ed.) *Vegetable Oils in Food Technology — Composition, Properties and Uses,* Blackwell Publishing, Oxford, pp. 18–58, 2002.

TABLE 2.33 Fatty acid composition of soybean oil and selected modified oils

Type	16:0	18:0	18:1	18:2	18:3	Reference
Commodity oil	11	4	23	54	8	
Low-saturated						
	3	1	31	57	9	Liu KeShun, 1999
	4	3	28	61	3	Reske et al., 1997
High-palmitic						
	25	4	16	44	10	Neff and List, 1999
	23	5	21	47	4	Shen et al., 1997
High-stearic						
	9	26	18	39	8	Neff and List, 1999
	11	21	63	1	3	Wang, 2002
High-palmitic and stearic						
	24	19	9	38	10	Neff and List, 1999
	22	18	9	41	10	Wilson, 1999
Low-linolenic						
	10	5	41	41	2	Fehr, 1992
	15	6	32	45	2	Fehr, 1992
High-oleic						
	6	1	86	2	2	Liu KeShun, 1999
	9	3	79	3	6	Wilson, 1999

Note: Other analyses of low linolenc soybean oils are given by Caiping Su et al., 2003 and Warner et al., 2003.

TABLE 2.34 Fatty acid composition, regiospecific distribution, and triacylglycerol composition of soybean oil

	16:0	18:0	18:1	18:2	18:3
sn-1,2,3	10.0	4.2	25.4	53.4	7.0
sn-2	–	–	24.6	69.3	6.2
sn-1,3	15.0	6.3	25.9	45.4	7.4

Note: Major triacylglycerols (determined by RP-HPLC): LLO 15.8%, LLL 15.6%, LLP 12.4%, LOP 9.5%, LOO 8.2%, LnLL 6.6%, LnLO 5.6%, LnLP 3.6%, LOS 3.3%, OOO 3.2%, POO 2.9%, LLSt 2.7%, LnOP 2.0%, other 8.6%. These three-letter symbols represent all the triacylglycerols containing the designated fatty acids.
Source: Adapted from Neff, W.E. and List, R., *J. Am. Oil Chem. Soc.,* **76**, 825–831, 1999.

TABLE 2.35 Iodine value, content of *trans* acids, and fatty acid composition of soybean oil before and after hydrogenation

	IV	% trans	16:0	18:0	18:1	18:2	18:3
sbo	132.0	0	11.2	3.7	22.1	55.0	6.8
(a)	112.6	8.7	11.4	4.7	40.3	40.5	3.0
(b)	109.5	9.1	10.5	4.4	42.0	40.0	2.9
(c)	97.2	13.3	12.8	6.5	48.4	30.3	1.9
(d)	81.5	31.8	11.3	5.1	72.6	11.0	0
(e)	64.7	39.7	11.3	13.6	75.2	0	0

Note: (a) hydrogenated soybean oil, (b) hydrogenated and winterised soybean oil, (c) stearin, (d) shortening oil, (e) margarine oil. These are soybean oils hydrogenated to a different degree under different conditions and for different purposes.
Source: Adapted from List, G.R., *Food Technol.* **56**, pp. 23–31, 2004.

face an uncertain future because of the high cost of gaining regulatory approval for crops that are unlikely to be grown on a commodity scale. These restrictions will increase production costs.

Soybean oil contains minor components that are largely removed during refining. Because they are valuable materials, they are generally trapped in an appropriate side stream and recovered for further use. These include phospholipids removed during degumming, free acids removed through neutralisation, and sterols and tocols present in the deodorizer distillate (Clark, 1996).

The phospholipid concentrate removed from crude seed oils by degumming is usually referred to as lecithin. Soybean oil lecithin (~3%) is the largest source of commercial phospholipids. Crude soybean lecithin (Gunstone, 2001 and Wang, 2002) is generally coloured through the presence of carotenoids, brown pigments, and porphyrins. It can be bleached by treatment with hydrogen peroxide or benzoyl peroxide. It is finally dried under reduced pressure at 60 to 70°C/30 to 60 mm for 3 to 5 hours or with a thin film evaporator at 80 to 105°C/25 to 300 mm for 1 to 2 minutes. The crude product is mainly a mixture of phospholipids (~50%) and triacylglycerols (~34%), along with glycolipids, carbohydrates, and other components at levels around 7, 7, and 2%, respectively. It is possible to remove most of the triacylglycerols by "de-oiling" with acetone to give a lecithin with about 90% of mixed phospholipids (mainly PC 29%, PE 29% and PI and glycolipids together 32%) and this mixture can be separated further into an alcohol-soluble portion rich in PC and PE and an alcohol-insoluble portion rich in PI and PE (Table 2.36). These products can then be fractionated to give concentrates of a single phospholipid class, be subjected to transphosphatidylation to exchange the phosphorus-containing head group, or be chemically modified by partial deacylation or partial hydroxylation (of some unsaturated centres) to change the hydrophilic lipophilic balance (Szuhaj, 1989 Schneider, 1997; Gunstone, 2001). Lecithin of appropriate composition is used in animal feed, in chocolate/confectionery, in a range of other food products, and in cosmetic and pharmaceutical preparations. These uses

TABLE 2.36 Phospholipid composition of oil-free lecithin, and the alcohol-soluble and alcohol-insoluble fractions

	Oil-Free	Alcohol-Soluble	Acohol-Insoluble
PC	29	60	4
PE	29	30	29
PI and glycolipid	32	2	55
Other	10	8	12
Emulsion type favoured	W/O and O/W	O/W	W/O

Source: Adapted from Wang, T., in Gunstone, F.D. (Ed.) *Vegetable Oils in Food Technology — Composition, Properties and Uses,* Blackwell Publishing, Oxford, pp. 18–58, 2002.

take advantage of the amphiphilic properties of phospholipids. Wendel (2000) has provided an interesting historical account of the lecithin industry.

Soybeans are reported to be a relatively rich source of sphingolipids, though little is known about how these are related to variety and to refining procedures, and they remain an unexploited minor component. These compounds are reported to have many important physiological properties (Gutierrer et al., 2004).

Soybean deodorizer distillate, a valuable by-product of soybean refining, typically contains 11% of tocopherols (from 0.15 to 0.21% in the oil) and 18% of sterols (from ~0.33% in the oil). It is the most important commercial source of these materials (Clark, 1996). The sterol blend (Table 2.41) is a by-product of the isolation of tocopherols, which carry most of the cost. The potential supply is estimated at 5 to 6 kt. Another potential source are rapeseed sterols from the production of bio-fuel. Deodoriser distillate (especially from physical refining) also contains free acids that may be more easily separated from the other components after conversion to methyl esters.

Dolde et al. (1999) and Vlahakis and Hazebroek (2000) have provided details of fatty acid composition, sterols, and tocopherols from several canola, sunflower, and soybean lines. Crude soybean oil is reported to contain 235 to 405 mg/100 g of sterols that are mainly β-sitosterol (125 to 236), campesterol (62 to 131), and stigmasterol (47 to 77). (See Table 2.47) These are now used for dietary purposes since phytosterols and their esters are claimed to have cholesterol-lowering properties. They are also modified chemically to produce ~75% of the world production of pharmaceutical steroids.

Solvent-extracted soybean oil contains 1370 mg/kg of tocols consisting of α- (10%), β- (1%), γ- (64%), and δ-tocopherol (25%). This mixture is a powerful antioxidant and is frequently supplied as acetate that acts as an antioxidant only after it has been hydrolysed *in vivo.* Antioxidant activity requires a free phenolic group. For use as vitamin E, where the α compound has the greatest physiological effect, the product from soybean deodoriser distillate is usually per-methylated to convert mono- and di-methyl compounds to the trimethyl form (Clark, 1996 Netscher, 1998). Evans et al. (2002) have discussed the relative antioxidant activities of α-, γ-, and δ-tocopherols and recommend optimal concentrations to inhibit soybean oil oxidation. They conclude that the optimal levels for antioxidant activity in soybean oil are ~100 ppm for α-tocopherol and ~300 ppm for γ-tocopherol. Mixed soybean oil tocopherols exert optimal influence at levels of 300 to 600 ppm.

Soap stock resulting from neutralisation is the least valuable of the by-products recovered during soybean oil refining. Acidification yields crude fatty acids, which have been used for the most part in animal feed. The low cost of this product, however, makes it attractive as a starting point for biodiesel production, though some difficulties have to be overcome before the product meets the specifications laid down for this material. (Haas, 2004).

Soybean oil, as such or after partial hydrogenation, is widely used for food purposes (cooking and salad oils, spreads and shortenings, mayonnaise and salad dressings). Nonfood uses (see Chapter 9) include its use as a semi-drying oil, as a plasticiser after epoxidation, in inks and lubricants, and as biodiesel after conversion to methyl esters. Figures for the use of soybean oil as food and for industrial purposes are given in Table 2.24.

For further information, see the review of Hammond et al., 2005 and Table 2.43 to Table 2.49.

References

Caiping Su. et al., Oxidative and flavor stabilities of soybean oils with low- and ultra-low-linolenic acid composition, *J. Am. Oil Chem. Soc.,* **80**, 171176, 2003.

Clark, J.P., Tocopherols and sterols from soybeans, *Lipid Technol.*, **8**, 111–114, 1996.

Dolde, D. et al., Tocopherols in breeding lines and effects of planting location, fatty acid composition, and temperature during development, *J. Am. Oil Chem. Soc.,* **76**, 349–355, 1999.

Evans, J.C. et al., Optimal tocopherol concentrations to inhibit soybean oil oxidation, *J. Am. Oil Chem. Soc.,* **79**, 47–51, 2002.

Fehr, W.R. et al., Inheritance of reduced linolenic acid content in soybean genotypes A16 and A17, *Crop Sci.*, **32**, 903–906, 1992.

Gunstone, F.D., Phospholipids, in Gunstone, F.D., Ed., *Structured and Modified Lipids,* Marcel Dekker, New York, 2001, pp. 241–250.

Gutierrer, E. et al., Quantification of sphingolipids in soybeans. *J. Am. Oil Chem. Soc.,* **81**, 737–742, 2004.

Hammond, E.G. et al., Soybean oil, in Shahidi, F., Ed., *Bailey's Industrial Oil and Fat Products,* 6th ed., vol. 2, 577–654, Wiley Interscience, New York, 2005.

Haas, M.J., The interplay between feedstock quality and esterification technology in biodiesel production, *Lipid Technol.*, **16**, 7–11, 2004.

Liu K.-S., Soyoil modifications: products, applications, *Inform.,* **10**, 86–875, 1999.

List, G.R., Decreasing *trans* and saturated fatty acid content in food oils, *Food Technol.,* **56**, pp. 23–31, 2004.

Neff, W E. and List, R., Oxidative stability of natural and randomised high-palmitic- and high-stearic-acid oils from genetically modified soybean varieties, *J. Am. Oil Chem. Soc.,* **76**, 825–831, 1999.

Netscher, T., Synthesis and production of vitamin E, in Gunstone, F.D., Ed., *Lipid Synthesis and Manufacture*, Sheffield Academic Press, Sheffield, U.K., 1998.

Reske, J. et al., Triacylglycerol composition and structure in genetically modified sunflower and soybean oils, *J. Am. Oil Chem. Soc.,* **74**, 989–998, 1997.

Schneider, M., Phospholipids, in Gunstone, F.D. and Padley, F.B., Eds., *Lipid Technologies and Applications,* Marcel Dekker, New York, 1997, pp. 51–78.

Shen, N. et al., Oxidative stabilities of soybean oils with elevated palmitate and reduced linolenate contents, *J. Am. Oil Chem. Soc.,* **74**, 299–302, 1997.

Szuhaj, B.F., Ed., *Lecithins: Sources, Manufacture, and Uses*, AOCS Press, Champaign, IL, 1989.

Vlahakis, C. and Hazebroek, J., Phytosterol accumulation in canola, sunflower, and soybean oils: effect of genetics, planting location, and temperature, *J. Am. Oil Chem. Soc.,* **77**, 49–52, 2000**.**

Wang, T., Soybean oil, in Gunstone, F.D., Ed., *Vegetable Oils in Food Technology — Composition, Properties and Uses,* Blackwell Publishing, Oxford, pp. 18–58, 2002.

Warner, K. and Gupta, M., Frying quality and stability of low- and ultra-low-linolenic acid soybean oils, *J. Am. Oil Chem. Soc.,* **80**, 275–280, 2003.

Wendel, A., Lecithin: the first hundred years, *Inform.*, **11**, 885–892 and 992–997, 2000.

Wilson, R.F., Alternative to genetically modified soybean — The Better Bean Initiative, *Lipid Technol.*, **10**, 107–110, 1999.

Wolff, R. L. et al, Occurrence and distribution profiles of *trans*-18:1 acids in edible fats of natural origin in Sbdio, J.L. and Christie, W.W., Eds., *Trans Fatty Acids in Human Nutrition*, The Oily Press, Dundee, Scotland, 1998, pp. 1–34.

Websites: www.unitedsoybean.org
www.asa-europ.org
www.soyatech.com
www.soystats.com

2.2.17 Sunflower oil (*Helianthus annuus*)

The sunflower first grew in the Americas and was taken to Europe by the Spaniards in the 14th century. From there it spread to Russia, which along with the Ukraine is now a dominant production area.

Sunflower seed oil lies fourth after soybean, palm, and rape/canola, and comes immediately ahead of three land animal fats (tallow, lard, and butter) in terms of annual production and consumption. Production peaked at 9.6 million tonnes in 1999 to 2000, after which it fell back somewhat. There are four major producing countries/regions, namely EU-25, Russia, Ukraine, and Argentina. Europe, as the major consumer, is also the largest importer of oil since it does not produce enough for its own needs. Russia is also a significant producer and importer of sunflower seed oil. Argentina and Ukraine are the dominant exporters (Table 2.37).

Traditional sunflower seed oil, obtained in 40 to 50% yield by solvent extraction, is a linoleic-rich oil with virtually no linolenic acid. It also contains oleic acid (~20%), palmitic acid (~6%), and stearic acid (~5%) (Table 2.38 to Table 2.40). It is widely used as a cooking oil and is valued as an important component of soft spreads, particularly in Europe. Figures for the use of sunflower oil as food and for industrial purposes are given in Table 2.24.

Two variants of sunflower oil, differing in fatty acid composition, have been developed by traditional seed breeding methods. One is rich in oleic acid. With 80 to 90% oleic acid, it trades as Sunola or Highsun and is prized as a high-quality, s table oil. A third oil (Nusun) with intermediate levels of oleic acid has been developed in the U.S. It is hoped that this variety will become the commodity oil in that country, but it is evident from Table 2.37 that the U.S. produces less than 2.5% of the world supply of sunflower seed oil. The oils with less linoleic

TABLE 2.37 Major countries/regions involved in the production (from indigenous or imported seed), consumption (food and nonfood uses), export, and import of sunflower seed oil in 2004/05 (million tonnes)

	Total	Countries/Regions
Production	9.08	CIS 3.20, EU-25 1.76, Argentina 1.41, India 0.43, Turkey 0.39, South Africa, 0.27, China 0.20, US 0.14
Consumption	9.13	CIS 2.56, EU-25 2.38, Turkey 0.49, India 0.46, South Africa 0.33, Argentina 0.29, China 0.22, US 0.14
Exports	2.52	Argentina 1.10, CIS 0.95
Imports	2.53	EU-25 0.72, CIS 0.32, Algeria 0.15, Egypt 0.13, Turkey 0.11
Seed yield (t/ha)	1.21	CIS 0.96, EU-25 1.89, Argentina 1.84, India 0.62, Turkey 1.68, South Africa, 1.34, China 1.79, US 1.34

Note: CIS = Commonwealth of Independent States (former Soviet Union).
Source: Adapted from *Oil World Annual 2005*, ISTA Mielke GmbH, Hamburg, 2005.

TABLE 2.38 Fatty acid composition (% wt) of sunflower oil, high-oleic sunflower oil (HO), and high-oleic, high-palmitic sunflower oil (HOHP)

	Sunflower Oil			HO		HOHP
Reference	a	b	c	b	c	b
16:0	6.3	6.4	6.4	4.7	4.0	27.8
18:0	3.7	5.0	4.7	3.8	4.3	1.8
18:1	24.3	29.3	21.0	80.2	72.4	57.7
18:2	65.3	58.3	67.7	9.5	16.8	2.3
Other	0.4	1.0	0.2	1.8	2.5	10.4[a]

[a] Including 16:1 7.3%.
Source: (a) Noor Lida et al., 2002; (b) Guinda et al., 2003; (c) Martin-Polvillo, 2004.

acid and more oleic acid have enhanced oxidative stability (see AOM figures in Table 2.39).

The triacylglycerol composition of the commodity oil reflects its fatty acid composition. Linoleic acid-rich sunflower oil is rich in triacylglycerols containing two or three linoleic chains (LLL, LLO, and LLP). Fuller details are given in Table 2.40, where information is also provided for variants of the commodity oil. Information on the regiospecific distribution of fatty acids is available in Table 2.17 and Table 2.26. Guinda et al. (2003) have provided information on a sunflower oil with enhanced levels of oleic (58%) and palmitic (28%) acid.

Gupta (2002) reports that the tocopherols (530 to 700 ppm) are almost entirely the α-compound. This makes the seeds and the crude oil a good source of vitamin E in contrast to most other commodity oils (Muller-Mulot, 1976). Sterols (0.26 to 0.30%) are present as free sterols (72%) and as sterol esters (28%). Sunflower deodoriser distillate contains about 6% of tocopherols and 5% of sterols (Mareira et al., 2004). See also Dolde et al. (1999) and Vlahakis and Hazebroek (2000). The crude oil also contains phospholipids (0.72 to 0.86%) and carotenoids (1.1 to 1.6 ppm).

TABLE 2.39 Fatty acid composition of three types of sunflower seed oil

	Traditional	High-Oleic	Mid-Oleic-
Saturated acids	11–13	9–10	<10
Oleic acid	20–30	80–90	55–75
Linoleic acid	60–70	5–9	15–35
Iodine value (approx)	128	79	108
AOM (hours)	10–12	40–50	25–35

Note: Linolenic acid is below 1% in all the oils.
Source: Adapted from Gupta, M.K., in Gunstone, F.D. (Ed.) *Vegetable Oils in Food Technology — Composition, Properties and Uses,* Blackwell Publishing, Oxford, p.p 128–156, 2002.

TABLE 2.40 Triacylglycerols of sunflower oil (SO), high-oleic sunflower oil (HOSO), and high-oleic, high-palmitic sunflower oil (HOHPSO)

	SO (1)	SO (2)	HOSO	HOHPSO
LLL	27.2	26.4	5.6	
OLL	29.5	25.6	7.5	
PLL	9.6	10.9	2.2	
OLO	11.0	8.1	9.5	1.3
PLO/StLL	10.0	12.9	4.0	3.2
OOO	3.0	1.7	51.1	12.6
OOPo				8.5
POO/StOL	3.5	4.7	8.7	35.5
POPo				11.9
POP	0.5			18.5
Other	5.7	9.7		9.1

Note: Po = palmitoleic acid. Each three-letter group includes all triacylglycerols containing the three acyl groups indicated.
Source: SO (1) Noor Lida, H.M.D. et al., *J. Am. Oil Chem. Soc.,* 79, 1137–1144, 2002. SO (2) and HOSO — Martin-Polvillo, M. et al., *J. Am. Oil Chem. Soc.,* 81, 577–583, 2004. HOHPSO — Guinda, A. et al., *Eur. J. Lipid Sci. Technol.,* 105, 130–137, 2003.

Most samples of crude sunflower oil contain wax (typically 300 to 600 ppm in the U.S. oils). This comes mainly from the seed hulls, which have ~10,000 ppm of wax. The wax causes a haze in the refined oil and is usually removed by winterisation (cooling and filtering). The wax contains phytyl and geranylgeranyl esters of long-chain acids as well as wax esters that are almost entirely saturated. These last are C_{42} to C_{60} esters (mainly C_{44} to C_{52}) from saturated acids (mainly C_{20} and C_{22}) and saturated alcohols (mainly C_{22} to C_{28}) (Henon et al., 2001; Reiter et al., 2001; and Carelli, 2002).

Sunflower seeds are used extensively as birdseed and also as a component of mixed breakfast cereals and on breads.

For further information, see the review of Grompone (2005) and Table 2.43 to Table 2.49.

References

Carelli, A.A. et al., Wax composition of sunflower seed oils, *J. Am. Oil Chem. Soc.,* **79**, 763–768, 2002.

Dolde, D. et al., Tocopherols in breeding lines and effects of planting location, fatty acid composition, and temperature during development, *J. Am. Oil Chem. Soc.,* **76**, 349–355, 1999.

Grompone, M.A. Sunflower oil, in Shahidi, F., Ed., *Bailey's Industrial Oil and Fat Products,* 6th ed., vol. **2**, 655–530, Wiley Interscience, New York, 2005.

Guinda, A. et al., Chemical and physical properties of a sunflower oil with high levels of oleic and palmitic acids, *Eur. J. Lipid Sci. Technol.,* **105**, 130–137, 2003.

Gupta, M.K., Sunflower oil, in Gunstone, F.D., Ed., *Vegetable Oils in Food Technology — Composition, Properties and Uses,* Blackwell Publishing, Oxford, pp. 128–156, 2002.

Henon, G. et al., Wax analysis of vegetable oils using liquid chromatography on a double absorbent layer of silica gel and silver nitrate-impregnated silica gel. *J. Am. Oil Chem. Soc.,* **78**, 401–410, 2001.

Mareita, E.A., and Baltanas, M.A., Recovery of phytosterols from sunflower oil deodorizer distillate, *J. Am. Oil Chem. Soc.,* **81**, 161–167, 2004.

Martin-Polvillo, M. et al., Oxidative stability of sunflower oils differing in unsaturation degree during long-term storage at room temperature, *J. Am. Oil Chem. Soc.,* **81**, 577–583, 2004.

Muller-Mulot, W., Rapid method for quantitative determination of individual tocopherols in oils and fats, *J. Am. Oil Chem. Soc.,* **53**, 732–736, 1976.

Noor Lida, H.M.D. et al., TAG composition and solid fat content of palm oil, sunflower oil, and palm kernel oil blends before and after chemical interesterification. *J. Am. Oil Chem. Soc.,* **79**, 1137–1144, 2002.

Reiter, B. and Lorbeer, E., Analysis of wax ester fraction of olive oil and sunflower oil by gas chromatography and gas chromatography-mass spectrometry, *J. Am. Oil Chem. Soc.,* **78**, 881–888, 2001.

Vlahakis, C. and Hazebroek, J., Phytosterol accumulation in canola, sunflower, and soybean oils: effect of genetics, planting location, and temperature, *J. Am. Oil Chem. Soc.,* **77**, 49–52, 2000.

Website (sunflower): www.sunflowernsa.com

2.2.18 Tall oil

The term tall oil comes from the Swedish word for pine oil (*tallolja*). Tall oil fatty acids are a by-product of the wood pulp industry and result when pine wood chips are digested, under pressure, with an alkaline solution of sodium sulfate or an acidic solution of sodium sulfite. A mixture of resin acids and fatty acids is produced when the aqueous alkaline solution is acidified with sulfuric acid. However, the quantity of sulfuric acid required for this purpose can be reduced and the quality of the product improved if the aqueous layer is treated with carbon dioxide, thereby changing the pH of the solution from 12 to 8 prior to acidification with sulfuric acid.

Tall oil is produced mainly in North America (~250,000 tonnes) and Scandinavia (~90,000 tonnes) (figures of 10 years ago provided by Hase et al., 1994), but the products from these two sources differ in composition because of the differences in wood species being pulped. The crude product is distilled to separate fatty acids (with less than 2% of resin acids) from resin acids (with less than 2% of fatty acids). The former is a good and cheap source of an oleic-linoleic acid mixture (75 to 80%) (Table 2.41). However, tall oil fatty acids contain sulfur compounds that interfere with catalytic processes, so the acids are not usually converted to alcohols or to nitrogen-containing compounds by reactions that require catalytic processes. They are used instead to prepare dimer acids, alkyds and coatings, detergents, and lubricants (in reactions that do not require a catalyst), and are being examined for use as solvents, in inks, and for biodiesel production.

TABLE 2.41 Fatty acid composition of tall oil

Source	Sat (a)	18:1	18:2	(b)	(c)	(d)
American	2.5	46	36	2	9	1–5
Scandinavian	2.5	30	45	9	5	1–5

Note: (a) 16:0 + 18:0, (b) pinolenic acid, 5*c*9*c*12*c*-18:3, (c) conjugated diene acids, (d) rosin acids and unsaponifiable.
Source: Hase, A. and Pojakkala, S., *Lipid Technol.*, 6, 110–114, 1994.

TABLE 2.42 Average sterol composition of commercial plant sterols from wood and from vegetable oils

	Wood	Vegetable Oil
Sitosterol	72.0	45.0
Campesterol	8.2	26.8
Stigmasterol	0.3	19.3
Brassicasterol	–	1.6
Sitostanol	15.3	2.1
Campestanol	1.6	0.8
Other sterols	2.6	4.4

Source: Adapted from Salo, P. et al., in Gunstone, F.D. (Ed.) *Lipids for Functional Foods and Neutraceuticals,* The Oily Press, Bridgwater, England, 2003, pp. 183–224.

Tall oil pitch is a valuable source of phytosterols (Table 2.42). These are hydrogenated and acylated for use in cholesterol-lowering spreads.

References

Hase, A. and Pojakkala, S., Tall oil as a fatty acid source, *Lipid Technol.*, **6**, 110–114, 1994.

Salo, P. et al., Phytosterols, in Gunstone, F.D., Ed., *Lipids for Functional Foods and Neutraceuticals,* The Oily Press, Bridgwater, England, 2003, pp. 183–224.

2.2.19 Supplementary Tables (Table 2.43a/b to Table 2.49)

Comment on Tables 2.43 and 2.44. Table 2.43a contains information on the production of 17 oils and fats (including animal fats). They are presented as average annual production levels (million tonnes) for selected five-year periods including forecasts made in 2002 for the later periods. The average figures cited will be close to the actual value recorded for the mid-year in the 5-year period, i.e., levels for 1978, 1988, 1998, 2008, and 2018. The final column represents the figures for 2004 reported in May 2005. Careful consideration of these figures suggests that levels of soybean oil and palm oil have risen even more than predicted. In 1998 (mid-year of the period) the combined levels of these two oils was 41.8 million tonnes (39.8%). This figure was predicted to rise to 65.0 million tonnes (44.3%) in 2008, but had already reached 61.3 million tonnes (46.8%) in 2004. This suggests that these two oils are increasingly dominant. This is even truer of transnational trade in these commodities. In 2004, exports of soybeans at 55.7 million tonnes represent 82.9% of all oilseed exports and exports of soybean oil and palm oil at 9.1 and 24.1 million tonnes, respectively, represent 19.3% and 51.3% of total oil and fat exports.

The figures in Table 2.43b cover virtually 100 years, with figures for pre-World War I, pre-World War II, and post-World War II. They show that changes in the first half of the century were modest and gradual with animal fats and "other vegetable oils" predominating. In the second half of the century, changes were dominated by the very large increase in soybean oil and palm oil and, to a lesser extent, in rapeseed and sunflower oils. Over the 100 years there was a 10-fold increase in total production.

Fry (cited by Gunstone, 2002) reported that, in the quarter century 1976 to 2000, consumption of oils and fats increased at an average rate of 3.7%, equivalent to a doubling every 20 years or so. For animal and marine fats, the increase was only 1.4% and for vegetable oils 4.5%. The four major oils have increased at average rates of 8.3% for palm oil, 7.3% for rapeseed oil, 4.5% for sunflower oil, and 4.1% for soybean oil. These increases result from a combination of higher yields and of larger areas devoted to their production. The very large increase in palm oil has come mainly from the increase in area and only to a minor extent from a rise in

TABLE 2.43a Past, present, and future production of oils and fats. Figures are average annual production (million tonnes) for selected 5-year periods between 1976 and 1980 and 2016 and 2020. Those for 2006 to 2010 and 2016 and 2020 are forecasts.

	76/80	86/90	96/00	06/10	16/20	2004
World total	52.6	75.7	105.1	146.7	184.8	131.1
Soybean	11.2	15.3	23.1	33.6	41.1	30.7
Palm	3.7	9.2	18.7	31.4	43.4	30.6
Rapeseed	3.0	7.5	12.6	17.7	22.7	14.9
Sunflower	4.2	7.2	9.1	12.4	17.0	9.5
Lauric oils (a)	3.3	4.3	5.3	7.5	9.8	6.6
Other veg oils (b)	10.0	12.3	14.8	18.6	21.7	16.0
Animal fats (c)	17.2	19.8	21.3	25.4	29.0	23.1

Note: (a) coconut and palm kernel; (b) cottonseed, groundnut, corn, olive, sesame, linseed, and castor; (c) butter, fish oil, lard, and tallow.
Source: Adapted from *The Revised Oil World 2020 — Supply, Demand and Prices*, ISTA Mielke GmbH, Hamburg, Germany; revised details published in 2002. Figures for 2004/05 are adapted from *Oil World Annual 2005*, ISTA Mielke GmbH, Hamburg, 2005. (In this Table, some figures have been grouped. They are available in full in the original publication.)

TABLE 2.43b Past and present production of oils and fats. Figures are average annual production (million tonnes and % of total) for selected periods in the 20th century compared with those for 2004/05.

	1909/13	1935/39	1958/62	2004/05
Animal fats	6.5 (49%)	8.5 (42%)	11.8 (40%)	23.2 (17%)
Soybean and palm	0.6 (5%)	1.9 (9%)	4.6 (15%)	65.1 (48%)
Rape and sunflower	1.2 (9%)	1.8 (9%)	3.1 (10%)	25.2 (18%)
Lauric oils	0.9 (7%)	2.3 (12%)	2.3 (8%)	6.8 (5%)
Other vegetable oils	3.9 (30%)	5.7 (28%)	8.0 (27%)	16.1 (12%)
Total	13.1	20.2	29.8	136.4
Per capita (kg/person/year)	7.7	9.6	10.3	21.0

Source: Robbelen, G., Downey, R.K., and Ashri, A., (Eds.) *Oil Crops of the World,* McGraw-Hill, New York, 1989, except for figures for 2004/05 that are adapted from *Oil World Annual 2005*, ISTA Mielke GmbH, Hamburg, 2005.

TABLE 2.44 Predicted total (million tonnes) and per capita consumption (kg per annum) on a global basis and for selected countries/regions throughout the 21st century

	2000	2020	2040	2060	2080	2100
Total	114	219	364	542	736	971
Vegetable oils	92	190	328	498	685	914
Population (billions)	6.08	7.90	9.23	9.99	10.18	10.35
Consumption per person						
World	19	28	39	54	72	94
USA	52	68	85	100	113	124
EU-15	47	62	77	90	102	112
China	13	28	49	74	99	122
India	12	21	33	48	66	84

Source: Fry, J., in Gunstone, F.D. (Ed.) *Vegetable Oils in Food Technology — Composition, Properties and Uses,* Blackwell Publishing, Oxford, 2002.

yield, while the three oilseed crops show significant increases in yield as well as area under cultivation.

Extrapolation of figures for the past 40 years over the next 100 produces ridiculous conclusions with population increasing 6-fold to 36 billion, consumption per person of oils and fats increasing to a world average of 110 kilos per annum, and world production of 4 billion tonnes in 2100. More reasonably, it is now widely accepted that the world population will level out half way through the century at around 10 billion and Fry has made other assumptions about growth in personal GDP (gross domestic product) and the link between income and fat consumption. On this basis, he has calculated the production of total oils and fats and of vegetable oils and disappearance on a world basis and for the four major countries/regions — the U.S., EU-15, China, and India (Table 2.44). Vegetable oil production is expected to exceed 900 million tonnes by the end of the present century. It is not suggested that dietary consumption will reach these high levels. In the second half of the century, levels of oils and fats used for oleochemical purposes, including the preparation of methyl esters for use as biofuels, are expected to rise considerably. Reaney et al. have written, "If present trends continue, biodiesel will grow to become the largest market for triglyceride oil, expanding in size beyond current markets for food, feed, and industrial products. In spite of the diversion of food crops to energy production, it is anticipated that technology development will enable agricultural production to meet future needs".

Fry considers that oil-bearing plants could meet these requirements on the basis of an annual increase in yield of 1.50%. The present yield of 0.59 tonnes/hectare will increase to 2.03 tonnes/hectare (4.41-fold) and the required area of cultivation will rise from 156 to 352 million hectares (2.26-fold) by the end of the century.

TABLE 2.45 Production, consumption, imports, and exports of 17 oils and fats (million tonnes) by country/region for the calendar years 2000 to 2004 by country/region

	Production					Consumption				
	2000	**2001**	**2002**	**2003**	**2004**	**2000**	**2001**	**2002**	**2003**	**2004**
World	114.8	117.6	120.6	125.4	131.1	113.3	117.9	121.5	125.7	130.9
EU-25	17.1	17.0	16.9	16.8	16.9	19.7	20.5	20.8	21.4	21.9
CIS	3.9	3.7	3.8	4.5	4.9	4.1	4.5	4.7	4.9	5.1
US	15.6	15.4	15.8	15.2	14.7	14.2	14.4	14.3	14.3	14.5
Canada	2.2	2.0	1.8	2.0	2.3	1.4	1.4	1.4	1.4	1.4
Argentina	5.7	5.1	5.6	6.3	6.1	0.9	0.8	0.8	0.9	0.9
Brazil	5.3	5.8	6.4	6.9	7.2	4.5	4.4	4.4	4.6	4.6
China	14.9	16.1	16.1	16.5	17.2	17.5	19.1	20.4	22.3	24.4
India	6.7	6.7	7.1	6.5	8.3	11.7	12.1	12.2	12.0	12.5
Malaysia	12.4	13.5	13.5	15.1	15.8	2.5	2.6	2.7	2.8	3.0
Indonesia	8.6	9.6	11.1	12.4	14.1	3.3	3.5	3.6	3.8	3.9
	Imports					**Exports**				
World	35.62	38.22	40.94	44.12	46.55	35.98	38.19	40.72	44.25	46.02
EU-25	5.03	5.68	6.21	6.40	6.90	2.54	2.13	2.31	1.95	1.88
CIS	1.05	1.47	1.58	1.46	1.38	0.83	0.72	0.75	1.16	1.23
US	1.75	1.80	1.83	1.67	2.18	2.75	2.74	3.50	2.96	2.44
Canada	0.37	0.39	0.40	0.39	0.40	1.14	1.00	0.88	0.93	1.33
Argentina	0.03	0.03	0.02	0.04	0.03	4.75	4.52	4.67	5.41	5.57
Brazil	0.34	0.22	0.27	0.16	0.14	1.15	1.78	2.14	2.47	2.68
China	2.94	3.07	4.35	6.20	7.41	0.15	0.14	0.10	0.07	0.07
India	5.33	5.41	5.17	5.46	4.79	0.26	0.27	0.20	0.23	0.34
Malaysia	0.42	0.65	0.81	0.91	1.22	9.92	11.62	11.81	13.37	13.64
Indonesia	0.08	0.09	0.07	0.07	0.06	5.45	5.93	7.78	8.56	10.28

Note: These figures cover 17 oils and fats including four of animal origin. CIS = Commonwealth of Independent States (former Soviet Union).
Source: Adapted from *Oil World Annual 2005*, ISTA Mielke GmbH, Hamburg, 2005.

TABLE 2.46a Range of fatty acid composition for some major oils taken from the Codex Alimentarius

Acid	Coconut	Corn	Cottonseed	Groundnut	Palm	Palm Olein	Palm Stearin
6:0	ND–0.7	ND	ND	ND	ND	ND	ND
8:0	4.6–10.0	ND	ND	ND	ND	ND	ND
10:0	5.0–8.0	ND	ND	ND	ND	ND	ND
12:0	45.1–53.2	ND–0.3	ND-0.2	ND–0.1	ND–0.5	0.1–0.5	0.1–0.5
14:0	16.8–21.0	ND–0.3	0.6–1.0	ND–0.1	0.5–2.0	0.5–1.5	1.0–2.0
16:0	7.5–10.2	8.0–16.5	21.4–26.4	8.0–14.0	39.3–47.5	38.0–43.5	48.0–74.0
16:1	ND	ND–0.5	ND–1.2	ND–0.2	ND–0.6	ND–0.6	ND–0.2
17:0	ND	ND–0.1	ND–0.1	ND–0.1	ND–0.2	ND–0.2	ND–0.2
17:1	ND	ND–0.1	ND–0.1	ND–0.1	ND	ND–0.1	ND–0.1
18:0	2.0–4.0	ND–3.3	2.1–3.3	1.0–4.5	3.5–6.0	3.5–5.0	3.9–6.0
18:1	5.0–10.0	20.0–42.2	14.7–21.7	35.0–69.0	36.0–44.0	39.8–46.0	15.5–36.0
18:2	1.0–2.5	34.0–65.6	46.7–58.2	12.0–43.0	9.0–12.0	10.0–13.5	3.0–10.0
18:3	ND–0.2	ND–2.0	ND–0.4	ND–0.3	ND–0.5	ND–0.6	ND–0.5
20:0	ND–0.2	0.3–1.0	0.2–0.5	1.0–2.0	ND–1.0	ND–0.6	ND–1.0
20:1	ND–0.2	0.2–0.6	ND–0.1	0.7–1.7	ND–0.4	ND–0.4	ND–0.4
20:2	ND	ND–0.1	ND–0.1	ND	ND	ND	ND
22:0	ND	ND–0.5	ND–0.6	1.5–4.5	ND–0.2	ND–0.2	ND–0.2
22:1	ND	ND–0.3	ND–0.3	ND–0.3	ND	ND	ND
22:2	ND	ND	ND–0.1	ND	ND	ND	ND
24:0	ND	ND–0.5	ND–0.1	0.5–2.5	ND	ND	ND
24:1	ND	ND	ND	ND–0.3	ND	ND	ND

Note: ND = not detected
Source: Food and Agriculture Organization (FAO).

TABLE 2.46b Range of fatty acid composition for some major oils taken from the Codex Alimentarius

Acid	Palm Kernel	LEAR	Rapeseed	Sesame	Soybean	Sunflower	Sunflower HO
6:0	ND–0.8	ND	ND	ND	ND	ND	ND
8:0	2.4–6.2	ND	ND	ND	ND	ND	ND
10:0	2.6–5.0	ND	ND	ND	ND	ND	ND
12:0	45.0–55.0	ND	ND	ND	ND-0.1	ND–0.1	ND
14:0	14.0–18.0	ND–0.2	ND–0.2	ND–0.1	ND–0.2	ND–0.2	ND–0.1
16:0	6.5–10.0	2.5–7.0	1.5–6.0	7.9–12.0	8.0–13.5	5.0–7.6	2.6–5.0
16:1	ND-0.2	ND–0.6	ND–3.0	0.1–0.2	ND–0.2	ND–0.3	ND–0.1
17:0	ND	ND–0.3	ND–0.1	ND–0.2	ND–0.2	ND–0.2	ND–0.1
17:1	ND	ND–0.3	ND–0.1	ND–0.1	ND–0.1	ND–0.1	ND–0.1
18:0	1.0–3.0	0.8–3.0	0.5–3.1	4.8–6.1	2.0–5.4	2.7–6.5	2.9–6.2
18:1	12.0–19.0	51.0–70.0	8.0–60.0	35.9–42.3	17.0–30.0	14.0–39.4	75.0–90.7
18:2	1.0–3.5	15.0–30.0	11.0–23.0	41.5–47.9	48.0–59.0	48.3–74.0	2.1–17.0
18:3	ND–0.2	5.0–14.0	5.0–13.0	0.3–0.4	4.5–11.0	ND–0.3	ND–0.3
20:0	ND–0.2	0.2–1.2	ND–3.0	0.3–0.6	0.1–0.6	0.1–0.5	0.2–0.5
20:1	ND–0.2	0.1–4.3	3.0–15.0	ND–0.3	ND–0.5	ND–0.3	0.1–0.5
20:2	ND	ND–0.1	ND–1.0	ND	ND–0.1	ND	ND
22:0	ND–0.2	ND–0.6	ND–2.0	ND–0.3	ND–0.7	0.3–0.5	0.5–1.6
22:1	ND	ND–2.0	>2.0–60.0	ND	ND–0.3	ND–0.3	ND–0.3
22:2	ND	ND–0.1	ND–2.0	ND	ND	ND–0.3	ND
24:0	ND	ND–0.3	ND–2.0	ND–0.3	ND–0.5	ND–0.5	ND–0.5
24:1	ND	ND–0.4	ND–3.0	ND	ND	ND	ND

Note: ND = not detected
LEAR = low-erucic rapeseed oil (canola)
HO = high-oleic
Source: Food and Agriculture Organization (FAO).

TABLE 2.47 Sterols (mg/100 g oil) in a range of crude vegetable oils

	Camp	Stigma	Sito	Δ-5-Ave	Total	Esterified	Free
Coconut	7.8	12.5	48.6	–	68.9	26.3	41.5
Corn	200.5	67.7	645.7	10.4	924.3	423.3	485.5
Cotton (a)	33.3	5.0	401.8	19.4	459.5	87.1	402.0
Olive (b)	1.7	–	130.3	44.3	176.3	30.6	151.4
Palm	13.9	9.5	42.6	3.3	69.3	16.3	49.2
Palm olein	19.5	10.5	51.0	–	81.0	23.0	55.8
Peanut (c)	37.8	21.9	169.0	–	228.7	73.2	133.4
Rapeseed	293.0	–	419.8	110.9	823.8	475.4	336.2
Soybean	57.1	57.7	173.4	13.5	301.7	78.7	239.4
Sunflower (d)	41.0	33.7	265.3	43.2	383.2	114.0	285.5

Note: Major sterols are campesterol, stigmasterol, sitosterol, and Δ-5-avenasterol. (a) degummed, (b) cold pressed, (c) refined, (d) bleached.
Source: Adapted from Verleyen, T. et al., *J. Am. Oil Chem. Soc.,* 79, 117–122, 2002. The original paper contains further information on processed oils and details individual sterols that are free and esterified.

TABLE 2.48 Content of eight tocols in selected vegetable oils, animal fats, and nuts and berries

	Tocopherols (mg/100g)					Tocotienols (mg/100g)				
	α-	β-	γ-	Δ-	total	α	β	γ	Δ	total
Soybean	10		59	26	96					0
Palm	26		32	7	65	14	3	29	7	53
Rapeseed	17		35	1	53					0
Sunflower	49		5	1	55					0
Groundnut	13		22	2	37					0
Coconut	0.5		0.6		1	0.5		2	0.6	3
Olive	20	1	1		22					0
Corn	11	5	60	2	78					0
Safflower	39		17	24	80					0
Sesame	1	1	24	3	290					0
Hemp			150		150					0

(Continued)

TABLE 2.48 Continued

	Tocopherols (mg/100g)					Tocotienols (mg/100g)				
Wheatgerm	121	65	24	25	235	2	17			19
Rice	12	4	5		21	18	2	57		77
Barley	35	5	5		45	67	12	12		91
Oats	18	2	5	5	30	18		3		21
Rye	74	17		104	192					0
Butter	2				2					0
Lard	1				1	1				1
Margarine	7		51	3	62					0
Eggs	2		0.6	0.4	3					0
Almonds	27		1		28	1				1
Walnuts	1		17	2	20					0
Peanuts	11		8		19					0
Pistachios	3		30	1	34	1		4		5
Pecans	1		2		21					0
Cashew			4		4					0
Avocados	3				3					0
Green peas			6	1	7					0
Sea buckthorn (a)	200	10	15	10	235	3	5	3	1	12
Sea buckthorn (b)	100	20	100	10	230	5	5	5	2	17
Gooseberry	19		55	5	79					0
Jostaberry	59		73	4	136					0
Blackcurrant	60		103	7	170					0
Evening primrose	16		42	7	65					0
Redcurrant	26	3	87	29	145					0
Cloudberry	60		140		200					0
Raspberry	50		240	40	330					0
Cranberry	20		10		30			150		150
Lingonberry	20		10		30			120		120
Bilberry	20		10		30			70		70

Note: Blank values indicate nothing was detected. Other sources with tocols below 1 are cited in the original reports.
(a) pulp, (b) seed
The values cited in this table do not agree entirely with those cited in the text.
Source: Adapted Stone W. L. and Papas A., in Gunstone F.D. (Ed.) *Lipids for Functional Foods and Nutraceuticals*, The Oily Press, Bridgwater, England, 2003; Papas A.M., *The Vitamin E Factor*, Harper Collins, New York, 1999; and Baoru Yang, *Lipid Technol.*, 15, 125–130, 2003.

TABLE 2.49 Some physical and chemical properties of major vegetable oils

	Specific gravity	Temp °C	Refractive index 40°C	Iodine value	Sapon. value	Nonsap (%)
Canola	0.914–0.920	20/20	1.465–1.467	110–126	182–193	<2.1
Castor	0.956–0.970	15.5/15.5	1.466–1.473	81–91	176–187	
Cocoa butter	0.973–0.980	25/25	1.456–1.458	32–40	192–200	0.2–1.0
Coconut	0.908–0.921	15.5/15.5	1.448–1.450	6–11	248–265	<1.5
Corn	0.917–0.925	15.5/15.5	1.465–1.468	107–128	187–195	1–3
Cottonseed	0.918–0.926	20/20	1.458–1.466	100–115	189–198	<2
Groundnut	0.914–0.917	20/20	1.460–1.465	86–107	187–196	<1.1
Linseed	0.924–0.930	25/25	1.472–1.475	170–203	188–196	0.1–2.0
Olive	0.910–0.916	20/20	1.468–1.470(20)	75–94	184–196	1.5
Palm	0.891–0.899	50/20	1.449–1.455(50)	50–55	190–209	<1.4
Palm kernel	0.899–0.914	40/20	1.452–1.488	14–21	230–254	<1.1
Palm olein	0.899–0.920	40/20	1.458–1.459	>55	194–202	<1.4
Palm stearin	0.881–0.891	60/20	1.447–1.451	<49	193–205	<1.0
Rice bran oil	0.916–0.921	25/25	1.465–1.468	99–108	181–189	3–5
Sesame	0.915–0.923	20/20	1.465–1.469	104–120	187–195	<2.1
Soybean	0.919–0.925	20/20	1.466–1.470	124–139	189–195	<1.6
Sunflower	0.918–0.923	20/20	1.467–1.469	118–145	188–194	<1.6

Source: Adapted from Firestone, D. (Ed.) *Official Methods and Recommended Practices of the American Oil Chemists' Society: Physical and Chemical Characteristics of Oils, Fats and Waxes*, AOCS Press, Champaign, IL, 1997, reprinted as a book and disc, 1999.

References

Baoru Yang, Natural vitamin E: activities and sources, *Lipid Technol.*, **15**, 125–130, 2003.

Fry, J., The world's oil and fat needs in the 20th century — lessons from the 21st century, Lecture in Hull cited by Gunstone, F.D., Production and trade of vegetable oils in Gunstone, F.D., Ed., *Vegetable Oils in Food Technology — Composition, Properties and Uses,* Blackwell Publishing, Oxford, 2002.

Papas, A.M., *The Vitamin E Factor*, Harper Collins, New York, 1999.

Reaney, M.J.T., Vegetable oils as biodiesel, in *Bailey's Industrial Oil and Fat Products,* 6th ed., vol. 6, Shahidi, F., Ed., Wiley Interscience, New York, 2005, p. 252.

Robbelen, G., Downey, R.K., and Ashri, A., Eds., *Oil Crops of the World,* McGraw-Hill, New York, 1989.

Stone W. L. and Papas A., Tocopherols, tocotrienols, and vitamin E, in Gunstone F.D., Ed., *Lipids for Functional Foods and Nutraceuticals*, The Oily Press, Bridgwater, England, 2003.

Verleyen, T. et al., Analysis of free and esterified sterols in vegetable oils. *J. Am. Oil Chem. Soc.,* **79**, 117–122, 2002.

2.3 Minor oils from plant sources

2.3.1 Introduction

There is no accepted definition of "minor oil," so it is necessary to indicate how this term will be interpreted here. Fatty oils produced in such large amounts that they are recognised as commodity oils have been discussed in Section 2.2 (Major Oils from Plant Sources). But, in addition, there is a wide range of oils produced, sold, and used in still lower quantities. The list is almost endless and the author has made his own selection based on the frequency with which they are reported in the literature and their appearance in lists of specialist oil suppliers. These are presented below in alphabetical order after some general points have been made.

These minor oils are generally of interest because they contain a fatty acid or other component that gives the oil interesting dietary or technical properties. Such oils are usually available in only limited quantities and, if they are to be marketed, it is essential to ensure that the sources located will provide a reliable and adequate supply of good quality material. If the oils are to be used as dietary supplements, as health foods, as gourmet oils, or in the cosmetics industry, it is important that the seeds be handled, transported, and stored under conditions that maintain quality. It may also be necessary to consider growing the crops in such a way as to minimise the level of pesticides.

Many fruits are now processed at centralised facilities. This means that larger quantities of "waste products" are available at one centre and can be more easily treated to recover oil and other valuable by-products. This is particularly relevant in the fruit industry where pips, stones, and kernels are available in large quantities.

Extraction can be carried out by cold pressing at temperatures not exceeding 45°C, pressing at higher temperatures, and/or solvent extraction. Solvent extraction is not favoured for high-quality gourmet oils. Supercritical fluid extraction with carbon dioxide is an acceptable possibility, but only limited use is made of this method. A further possibility is to use enzymes to break down cell walls followed by extraction under the mildest possible conditions. Some specialty oils, such as walnut, virgin olive, hazelnut, and pistachio, can be used as expressed, merely after filtering, but for others some refining is generally necessary. If the oil has a characteristic flavour of its own, it may be desirable to retain this, and high-temperature deodorisation must then be excluded or operated at the lowest possible temperature. Once obtained in its final form, the oil must be protected from deterioration — particularly by oxidation. This necessitates the use of stainless steel equipment, blanketing with nitrogen, and avoiding unnecessary exposure to heat and light. At the request of the customer, natural and/or a synthetic antioxidant can be added to provide further protection.

Useful information related to this topic can be found in the following: Eckey (1954), Robbelen et al. (1989), Pritchard (1991), Kamel et al. (1994), Padley (1994), Ucciani (1995a), Firestone (1996), Abbott et al. (1997), Mazza (1998), Gunstone (1999, 2001), CTFA (2002), Bhattacharrya (2002), Aitzetmuller et al. (2003), Connock (website), Statfold oils (website), and Ienica (website). Other web sites also furnish information on many of the individual oils. For all the oils, a fatty acid composition has been reported and information about the minor components (tocopherols, sterols, carotenes, etc.) is also sometimes available. Based on this, claims are frequently made for the superior properties of these oils. These may be valid, but there are few, if any, where tests have been carried out to support the claims.

Most vegetable oils contain only three acids at levels exceeding 10% [palmitic (16:0), oleic (18:1), and linoleic (18:2)] and these three frequently having a combined level of 90% or more. This means that other acids, such as Δ9-hexadecenoic, stearic, or linolenic acid are generally present at low levels, if at all. The many oils of this type can be subdivided into those in which oleic acid dominates, those in which linoleic dominates, and those in which these two acids are present at similar high levels. Palmitic acid, though always present, is seldom the dominant component. Beyond these, however, are some oils with less-common acids, sometimes at quite high concentration.

2.3.1.1 Short and medium chain acids

While most oils contain virtually only C_{16} and C_{18} fatty acids, a small number are characterised by a dominance of acids of shorter chain lengths. Two commodity oils (coconut and palm kernel) are known collectively as lauric oils because they contain around 50% of lauric acid (12:0) accompanied by 8:0, 10:0, and 14:0 at lower levels (Gunstone, 2002). Among the minor oils, there are some with a similar lauric-rich fatty acid composition (e.g.,

babassu) and some in which the shorter chain acids dominate as in the cuphea oils (Table 2.51 and Table 2.55).

2.3.1.2 Stearic acid

Stearic acid is more significant in fats from domesticated land animals (especially sheep) than in vegetable oils. Nevertheless, there are some oils in which stearic acid accompanies palmitic and oleic acids as a major component. This holds for cocoa butter (palmitic acid ~26%, stearic acid ~34%, and oleic acid ~35%) and for a range of tropical fats with similar chemical composition and similar physical properties (Table 2.54).

2.3.1.3 Hexadecenoic and erucic acids

Oleic acid (18:1) is the most common monounsaturated acid and also the most common acid produced in nature. There are, however, some other monounsaturated acids that become significant in certain vegetable fats. These may be isomers of oleic acid with the unsaturated centre different from the common Δ9 (such as petroselinic and *cis*-vaccenic) or they may be acids of different chain length of which the most common are hexadecenoic (16:1), present in macadamia oil and sea buckthorn oil as well as erucic acid (22:1) in some forms of rapeseed oil and in crambe oil.

2.3.1.4 Petroselinic acid

Petroselinic acid (Δ6*c*–18:1) is an uncommon isomer of oleic acid that is present at high levels in a restricted range of seed oils — especially those from plants of the Umbelliferae family. Oleic acid is usually present also at lower levels. With unsaturation starting on an even carbon atom, the Δ6 acid has a higher melting point than isomers in which unsaturation starts on an odd carbon atom, such as oleic. Petroselinic acid melts at 29ºC compared with 11ºC for oleic acid. It is formed in seeds by an unusual biosynthetic pathway. The unsaturated centre is introduced at the C_{16} stage by a Δ4-desaturase and desaturation is followed by chain elongation.

$$16{:}0 \rightarrow \Delta 4\text{-}16{:}1 \rightarrow \Delta 6\text{-}18{:}1$$

2.3.1.5 γ-Linolenic acid (GLA)

The most common polyunsaturated fatty acids occurring in seed oils are linoleic acid (Δ9,12–18:2) and α-linolenic acid (Δ9,12,15–18:3), but in a few species the α-linolenic acid is accompanied or replaced by γ-linolenic acid (Δ6,9,12–18:3, GLA) that is now recognised as a fatty acid with beneficial health properties. It is a biological intermediate in the conversion of freely available linoleic acid to the important, but less readily available, arachidonic acid. This change is a three-step process involving Δ6-desaturation, elongation, and Δ5-desaturation of which the first step is considered to be rate-determining.

$$\underset{\text{linoleic}}{9,12\text{-}18{:}2} \rightarrow 6,9,12\text{-}18{:}3 \rightarrow 8,11,14\text{-}20{:}3\text{-} \rightarrow \underset{\text{arachidonic}}{5,8,11,14\text{-}20{:}4}$$

A similar sequence of changes converts α-linolenic acid first to stearidonic acid (Δ6,9,12,15–18:4) and then to eicosapentaenoic acid (20:5) and docosahexaenoic acid (22:6). Echium oil serves as a source of stearidonic acid and GLA is present in a number of seed oils of which three are commercially available (blackcurrant, borage, and evening primrose). A case has been made for incorporating this acid into our dietary intake and companies have been growing the required seeds and producing these oils (see Borage).

2.3.1.6 Acids with conjugated unsaturation

As indicated above, the most common polyunsaturated fatty acids in vegetable oils have methylene-interrupted patterns of unsaturation. However, acids with conjugated unsaturation are present at high levels in a small number of seed oils. These are mainly 18:3 acids with unsaturation at Δ9,11,13 or Δ8,10,12, and are all derived metabolically from linoleic acid. There are also some tetraene acids (Δ9,11,13,15–18:4) derived from α-linolenic acid. Conjugated diene acids occur only very rarely in seed oils. The intensive study of the animal-derived conjugated linoleic acids (18:2) has led to consideration of the potential value of the plant-derived conjugated trienes and tetraenes.

2.3.1.7 Cocoa butter alternatives

Cocoa butter is an important commodity that carries a premium price. Cheaper alternatives with similar physical properties, such as materials derived from lauric oils, can be used, but products containing these fats cannot be called chocolate and are generally described as confectionery fats. However, in a number of countries, including those of the EU, products designated chocolate can contain up to 5% of fats other than cocoa butter taken from a prescribed list. These include palm mid-fraction and five tropical fats listed in Table 2.54.

A brief account of over 100 minor oils follows. These are presented in alphabetical order and fatty acid composition data are collected together in Table 2.50 and Table 2.51. By perusal of these tables, it is easy to compare fatty acid composition. Those who have access to the second edition of this work will find details of earlier work on some minor oils in Padley's extensive survey of this topic (1994).

2.3.2 Aceituno (*Simarouba glauca*)

Aceituno oil comes from trees grown in Central and South America. The nuts furnish an oil (30%) rich in oleic acid (Table 2.50). The major triacylglycerols are SOO (42%), SOS (29%), and OOO (15%), where S and O represent saturated acids and oleic acid respectively (Ucciani, 1995a).

2.3.3 Acorn (*Quercus* spp)

Acorns, containing about 5% oil, are important components of the diets of free-range pigs. A recent study of three

TABLE 2.50 Fatty acid composition of minor oils rich in C_{16} and C_{18} acids

Oil	16:0	18:0	18:1	18:2	18:3	Other
Aceituno	12	28	58			
Acorn	14	1–3	57–63	17–22	1–3	
Alfalfa				34	25	
Allanblackia		>50	>40			
Almond			65–70			
Amaranthus	19	3	22	45		
Ambrette	26		33	37		
Apricot	5	1	66	29		
Argan			42–47	31–37		
Argemone	12–15		28–29	~55		
Arnebia	7		14	23	45	GLA 3, SA 4
Avocado	10–20		60–70	10-15		
Babassu						See Table 2.51
Baobab	25–46		21–39	12–29		
Basil	6–11		9–13	18–31	44–65	
Blackcurrant						See Table 2.53
Borage						See Table 2.53
Borneo tallow	18	46	35			
Buffalo gourd	9	2	25	62		
Calendula				28–34		Cal 53-62
Camelina			10–20	16–24	30–40	See text
Camelina			14–20	19–24	27–35	See text
Candlenut	6–8	2–3	17–25	38–45	25–30	
Candlenut (kukui)	6–8	2–5	24–29	33–39	21–30	
Caraway						Pet 35–43
Carrot						Pet 66–73
Cashew	9–14	6–12	57–65	16–18		
Chaulmoogra						See text
Cherry			30–40	40–50		Elst 6–12
Chestnut	15		54	25		
Chia	9–11		7–8	20–21	52–63	
Chinese vegetable tallow	75		20–25			See text
Coffee robusta	32		13	41		
Coffee arabica	34		8	44		
Cohune						See Table 2.51
Coriander						Pet 31–75
Crambe						Er 50–55
Cranberry	5		25	38	28	
Cuphea						See Table 2.55
Cupuacu date seed	6–12	22–35	39–47	3–9		Ar 10–12
Date						See Table 2.51
Dimorphotheca						See text
Echium						See Table 2.53
Euphorbia lathyris			80–85			
Euphorbia lagascae	4		19	9		Ver 64
Evening primrose						See Table 2.53
Grapeseed	6–8	3–6	12–25	60–76		
Gevuina						See text
Hazelnut			74–80	6–8		See text
Hemp	4–9	2–4	8–15	53–60	15–25	GLA 0–5, SA 0–3
Honesty						Er 41, Ner 22
Hyptis				22–31	51–64	See text
Illipe						See Borneo tallow
Jatropha curcas	10–17	5-10	36–64	18–45		
Jojoba						See text
Kapok	22		21	37		CP acids 13
Karanja	4–8	2–9	44–71	2–18		Ar 2–5, Eic 9–12
Kiwi			13	16	63	Sat 8
Kokum	35	52–56	39–42	0–2		
Kusum	5–9	2–6	40–67	2–7		Ar 20–31
Lesquerella	1	2	15	7		See text
Lupin	9	8	45	25	11	20:0, 22:0. 20:1, 22:1; see text

(Continued)

TABLE 2.50 Continued

Oil	16:0	18:0	18:1	18:2	18:3	Other
Macadamia			55–65			Hex 16–23, Eic 1–3
Mahua	22–37	19–24	37–46			
Mango	3–18	24–57	34–56	1–13		
Mango	10	35	49	5		
Manketti			18–24	39–47		See text
Marula	11	7	75	5		
Meadowfoam						See text
Melon	11	7	10	71		See text
Melon	12	11	14	63		See text
Moringa	12	4	77	2	2	Eic 2
Mowrah	27	9	39	24		See text
Mowrah	15–32	16–26	32–45	14–18		See text
Murumuru						See Table 2.51
Mustard			23	9	10	Eic 8 Er 43
Mustard (low erucic)	3	2	64	17	10	See text
Neem	16–19	15–18	46–57	9–14		Ar 1–3
Ngali						See Table 2.58
Nigella	10		35	45		See text
Niger	6–11	6–11	6–11	71–79		
Nutmeg						See Table 2.51
Oats	13–28		19–53	24–53	1–5	
Oiticica						See text
Parsley						Pet 80
Passionfruit	8–12	2–3	13–20	65–75		
Peach	9		67	21		
Pecan	5–11		49–69	19–40		
Perilla			13–15	14–18	57–64	
Phulwara butter	61	3	31	5		
Pistachio	10	3	69	17		
Plum			71	16		
Poppy	10		11	72	5	
Pumpkin	4–14	5–6	21–47	35–59		
Pumpkin (white)	10.7	8.1	30.0	48.5		
Pumpkin (black)	5.7	6.2	34.9	52.1		
Purslane	15	4	18	33	26	See text
Raspberry	3		9	55	33	See text
Ricebran	16		42	37		
Rose hip			8	54	32	Sat 5
Rubber seed	9–11	8–12	17–30	35–41	14–26	
Sacha inchi			6–9	34–39	47–51	Sat 5–7
Sal	2–8	35–48	35–42	2–3		Ar 6–11
Salicornia bigelovii	8		12	74		
Sea buckthorn						See Table 2.59
Shea	4–8	23–58	33–68	4–8		
Shikoin						See text
Sisymbrium irio	6	2	12	17	35	See text
Stillingia			13	23	47	See Chinese vegetable tallow
Tamanu	12	13	34	38		
Teaseed	5–17		58–87	7–17		
Tobacco				>70		
Tomato	12–16		16–25	50–60	~2	
Tung						Elst ~69
Ucuhuba						See Table 2.51
Vernonia						See text
Walnut				50-60	13–15	
Watermelon	9–11		13–19	62–71		
Wheatgerm				~60	5	

Notes: Headings refer to palmitic, stearic, oleic, linoleic, and α-linolenic acid.
Ar = arachidic, Cal = calendic, CP = cyclopropene acids, Eic = eicosenoic acid, Elst = eleaostearic acid, Er = erucic acid, GLA = γ-linolenic acid, Hex = hexadecenoic acid, Ner = nervonic acid; Pet = petroselinic acid, SA = stearidonic acid, Ver = vernolic acid, Sat = saturated acids
Useful information on many of these oils and on many others is be found in the SOFA database described by Aitzetmüller et al., 2003.

TABLE 2.51 Seed oils containing short and medium chain fatty acids (see also cuphea oils in Table 2.55)

	8:0	10:0	12:0	14:0	16:0	18:0	18:1	18:2	Other
Babassu	6	4	45	17	9	3	13	3	
Cohune	7–9	6–8	44–48	16–17	7–10	3–4	8–10	1	
Date (deglit nour)			18	10	11		41	12	
Date (allig)			6	3	15		48	21	
Murumuru			42	37					See text
Nutmeg			3–6	76–83	4–10		5–11	0–2	
Pycnanthus kombo			5	62	3		6		14:1 24
Ucuhuba			13	69	7		+	+	

species (*Querces ilex, Q. suber, Q. faginea*) details their fatty acids (Table 2.50), triacylglycerols (rich in OOO, POO, PLO, and PPL), and unsaponifiable components. These oils are particularly rich in sterols (8.6–11.4g/kg, 80% β-sitosterol) and may be a useful source of phytochemicals (Leon-Camacho et al., 2004). The oils extracted by hexane and by supercritical carbon dioxide have been compared in respect of fatty acid and triacylglycerols, sterols, tocopherols, and phospholipids by Lopes and Bernardo-Gil (2005).

2.3.4 Alfalfa (*Medicago sativa* and *M. falcate*)

Alfalfa seeds (*M. sativa*) contain only 7.8% of a highly unsaturated oil (Table 2.50) rich in carotenes and in lutein. It has been claimed that the seeds lower LDL cholesterol in patients with hyperlipoproteinemia and that the oil reduces erythema caused by sunburn (Firestone, 1996; Connock, website).

2.3.5 Allanblackia (*Allanblackia floribunda* and other species)

Allanblackia nuts are unusual in containing a stearic-rich oil (Table 2.50). They are being grown in Ghana to produce a useful crop providing useful local employment (Anon., 2005b).

2.3.6 Almond (*Prunus dulcis, P. amygdalis, Amygdalis communis*).

Almond oil is an oleic-rich oil (65 to 70%) also containing linoleic, palmitic, and minor acids (Table 2.50) though its fatty acid composition can vary widely. Its major triacylglycerols (Prats-Moya et al., 1999) are OOO (38%), OOL (24%), and OOP (11%). Cherif et al. (2004) have reported fatty acid and triacylglycerol composition for three almond cultivars during maturation. Like other low-saturated, high-monounsaturated oils, almond oil shows high oxidative and cold-weather stability (slow to deposit crystals). The oil is commonly used in skin-care and massage products because of its nongreasy nature, good skin feel, reasonable price, and consumer appeal. Almond nuts are reported to lower cholesterol levels and the FDA permits the following claim for a limited range of nuts, including almonds: "Scientific evidence suggests, but does not prove, that eating 1.5 ounces per day of most nuts as part of a diet low in saturated fat and cholesterol may reduce the risk of heart disease." (Ucciani, 1995a, Firestone, 1996, Bernardo-Gil, 2002, Watkins, 2005).

2.3.7 Amaranthus (*Amaranthus cruentus*)

Amaranthus is a grain with only low levels (6 to 9%) of oil. A study of 21 accessions gave the following results: oil content 5 to 8% (mean 6.5), palmitic 8 to 22%, stearic 1 to 4%, oleic 16 to 25%, linoleic 41 to 61% (mean values are cited in Table 2.50), tocopherols 2.8 to 7.8 mg/100g of seed. The average content of tocopherols is 4.94 mg/100g of seed with the major components being α- and β-tocopherols at 2.17 and 1.66 mg/100g seed, respectively (Budin et al., 1996). The high level of β-tocopherol is unusual and in contrast to the results of an earlier study (Lehmann et al., 1994). Another study of five accessions shows palmitic (21 to 24%), oleic (23 to 31%), and linoleic acid (39 to 48%) as the major components and gives details of the triacylglycerol composition (Jahaniaval et al., 2000). Amaranthus oil is unusual among vegetable oils in its relatively high level (6 to 8%) of squalene and this concentration can be raised 10-fold by short-path, high-vacuum distillation. There is no other convenient vegetable source of this C_{30} hydrocarbon other than olive oil (see Section 2.2), which has a squalene level of 0.3 to 0.7% rising to 10 to 30% in its deodoriser distillate (Sun et al., 1997).

2.3.8 Ambrette (*Abelmoschus moschatus*)

Ambrette is cultivated in Asia, Africa, and South America for its seeds with their characteristic musky odour. The seeds also contain ~16% of oil and it is used in India as a minor edible oil comparable to groundnut oil. Its fatty acid composition is cited in Table 2.50 (Rao et al., 1982).

2.3.9 Apricot (*Prunus armeniaca*)

Apricot seed oil is used in cosmetics, particularly as a skin-conditioning agent, and is also available as a gourmet oil for food use. It generally contains oleic (58 to 74%) and

linoleic acids (20 to 34%) with the results for one study being given in Table 2.50. With its low content of saturated acids, it shows excellent cold weather stability. The fatty acid composition of the phospholipids has been reported and tocopherol levels are given as 570 to 900 mg/kg. (Kamel et al., 1992; Anon., 1993; Ucciani, 1995°; Zlatanov et al., 1998).

2.3.10 Argan (*Argania spinosa*)

The argan tree grows mainly in Morocco and also in Israel. Its seeds contain about 50% of an oil rich in oleic and linoleic acids (Table 2.50). Sterols, phenols, tocopherols, and carotenoids are present in the unsaponifiable portion of the oil (~1.0%) and give the oil high oxidative stability. It is used locally to protect and soften skin. The pressed oil has a nutty flavour and is used in food preparation (Ucciani, 1995a; Yaghmur et al., 1999; Hilali et al., 2005; Watkins, 2005).

2.3.11 Argemone (*Argemone mexicana*)

Argemone seeds contain about 39% of oil with palmitic, oleic, and linoleic acids as major component acids (Table 2.50) (Ucciani, 1995a). Recent Indian work (Rauf et al., 2004) gives very different results for *A. mexicana* seed oil (Table 2.52). The regiospecific results are slightly surprising in that palmitic acid is slightly enriched in the *sn*-2 position, but the PUFA are not. The dominant oleic acid appears to be randomly distributed on the basis of the limited information provided.

2.3.12 Arnebia (*Arnebia griffithii*)

This highly unsaturated oil contains α-linolenic acid, γ-linolenic acid, and stearidonic acid, in addition to palmitic, oleic, and linoleic acids (Table 2.50) (Ucciani, 1995a).

2.3.13 Avocado (*Persea americana* and *P. gratissima*)

The avocado grows in tropical and subtropical countries between 40°N and 40°S and particularly in California, Florida, Israel, New Zealand, and South Africa. As with the palm and the olive, lipid is concentrated in the fruit pulp (4 to 25%) from which it can be pressed. There is very little oil in the seed (2%). The oil is used widely in cosmetic products. Avocado oil is easily absorbed by the skin and its unsaponifiable material is reported to provide some protection from the sun. It has been claimed that mixtures of avocado and soybean oil may help osteoarthritis. It is also available as a high-oleic specialty oil for food use and is produced and marketed in New Zealand as an alternative to olive oil. Avocado oil is rich in chlorophyll, making it green before processing. It contains palmitic, oleic, and linoleic acids as its major fatty acids (Table 2.50). Its unsaponifiable matter (~1%), total sterol (mainly β-sitosterol), and tocopherol levels (130 to 200 mg/kg, mainly γ-tocopherol) have been reported (Eckey, 1954; Werman et al., 1987; Swisher, 1993; Lozano et al., 1993; Ucciani, 1995a; Firestone, 1996; Eyres et al., 2001; Birkbeck, 2002; Bruin, 2003).

TABLE 2.52 Fatty acid composition of *Argemone mexicana* seed oil

sn-	16:0	18:0	18:1	18:2	18:3
1,2,3	13.3	4.0	70.8	7.2	4.7
2	15.5	3.8	70.9	6.6	3.2
1,3	12.1	4.2	70.7	7.5	5.5

Source: Rauf, A., et al., *J. Oleo Sci.,* **53**, 531–536, 2004.

2.3.14 Babassu (*Orbignya martiana* and *O. oleifera*)

This palm, grown in South and Central America, contains a lauric oil in its kernel (Table 2.51). Annual production is small and uncertain (100 to 300 kt), but Codex values have been established. In line with other lauric oils, it is rich in lauric and myristic acids. It is used as a skin cosmetic and is being considered in Brazil as a biofuel, as the oil or as its methyl esters, alone or mixed with mineral diesel (Ucciani, 1995a; Firestone, 1996).

2.3.15 Baobab (*Adansonia digitata*)

Baobab is an African tree whose seeds are eaten raw or roasted by the local population. It provides an oil of long shelf life that is used in cosmetics and also reported to be edible. The seed oil is reported to contain palmitic, oleic, and linoleic acids along with minor amounts of stearic and cyclopropene acids (Table 2.50). If the oil does contain this last type of acid, then it is probably unwise to use it for food and cosmetic purposes. However, one supplier of the oil (Statfold, website) gives a specification that does not include cyclopropene acids, but has palmitic (22%), oleic (34%), and linoleic (30%) as the major acids (Ucciani, 1995a; Firestone, 1996; PhytoTrade, website).

2.3.16 Basil (*Ocimum* spp)

Basil seed oil is obtained in a yield of 300 to 400kg/hectare. The seeds contain 18 to 36% of a highly unsaturated oil, which is rich in linolenic acid and also contains palmitic, oleic, and linoleic acids (Table 2.50) (Ucciani, 1995a; Angers et al., 1996).

2.3.17 Blackcurrant (*Ribes niger*)

Blackcurrant seed oil is of interest and of value because it contains γ-linolenic acid (n-6 18:3) and stearidonic acid (n-3 18:4). These are important metabolites of linoleic and

TABLE 2.53 Component acids of oils containing γ-linolenic acid (γ-18:3) and stearidonic acid (18:4) (typical results, % wt)

	16:0	18:0	18:1	18:2	γ-18:3	18:4	Other
Evening primrose	6	2	9	72	10	Tr	1
Borage	10	4	16	38	23	Tr	9 (a)
Blackcurrant	7	2	11	47	17	3	13 (b)
Echium	6	3	14	13	12	17	35 (c)
Canola-GM (d)	5	3	23	27	36	–	6

(a) Including 20:1 (4.5), 22:1 (2.5), and 24:1 (1.5)
(b) Including α-18:3 (13)
(c) Including α-18:3 (33)
(d) Genetically modified canola oil

linolenic acid, respectively (Table 2.53). Blackcurrant seed oil is also a rich source of tocopherols (1700 mg/kg) (Yang, 2003a). More general information about oils containing GLA (gamma-linolenic acid) is included in the entry for Borage oil. The oils are used in cosmetics and also as dietary supplements. Blackcurrant oil is extracted from the seeds, themselves a by-product of the production of juice from the berries (Ucciani, 1995a; Firestone, 1996).

Kallio et al. (2005) examined regioisomeric triacylglycerols in other *Ribes* species (northern red currant, *R. spicatum* and alpine currant *R. alpinum*) by tandem mass spectrometry.

2.3.18 Borage (*Borago officinalis*, Starflower)

γ-Linolenic acid (Δ6,9,12-18:3, GLA) is an interesting material with beneficial health properties. Claims have been made for its use in the treatment of multiple sclerosis, arthritis, eczema, premenstrual syndrome, and other diseases (Horrobin, 1992). As already reported in the Section 2.3.1 (Introduction), GLA is a biological intermediate in the conversion of freely available linoleic acid to the important but less readily available arachidonic acid.

GLA is present in a number of seed oils of which blackcurrant, borage, and evening primrose are commercially available (Table 2.53). The production and uses of these oils have been reviewed by Clough (2001a, 2001b). Borage oil, with just below 25%, is the richest source of γ-linolenic acid and there are several reports on ways to isolate the pure acid or to enhance its level in the oil by enzymic and other methods (Hayes et al., 2000; Chen et al., 2001; Ju et al., 2002; Tuter et al., 2003). There are many other plant sources of γ-linolenic acid, including hop (*Humulus lupulus*, 3 to 4%), hemp (C*annabis sativa*, 3 to 6%), red currant (*Ribes rubrum*, 4 to 6%), and gooseberry seeds (*Ribes uva crispa*, 10 to 12%) (Traitler et al., 1984) and others reported by Ucciani (1995b). The value of these GLA-containing oils is such that a genetically modified canola oil rich in γ-linolenic acid (43%) has been developed (Liu et al., 2001; Wainwright et al., 2003) and work is proceeding to develop GLA in a safflower oil (Anon., 2004a; Flider, 2005). Wretensjo et al. (2002, 2003) have described the nature of the sterols and alkaloids in borage oil. Pyrrolizidine alkaloids are present in crude borage only at levels below 100 ppb and these are reduced by a factor of 30,000 by the refining process. General reviews on γ-linolenic acid have been prepared by Gunstone (1992, 2003a), Huang et al. (1996, 2001, and Eskin (2002).

2.3.19 Borneo tallow (*Shorea stenoptera*)

This solid fat, also known as illipe butter, contains palmitic, stearic, and oleic acid (Table 2.50). It is one of a group of tropical fats that generally resemble cocoa butter in the proportions of these three acids. The fats, therefore, have similar triacylglycerol composition and display similar melting behaviour. Its major triacylglycerols are POP (7%), POSt (34%), and StOSt (47%). Along with palm oil, kokum butter, sal fat, shea butter, and mango kernel fat, it is one of six permitted tropical fats that can partially replace cocoa butter in chocolate (Table 2.54). An interesting account of the commercial development of illipe, shea, and sal fats has been provided by Campbell (2002) and further information is available in articles and books devoted to cocoa butter and to chocolate (Stewart et al., 2002 Timms, 2003).

2.3.20 Buffalo gourd (*Cucurbita foetidissima*)

The buffalo gourd is a vine-like plant growing in semi-arid regions of the U.S., Mexico, Lebanon, and India. The seed contains good quality oil (32 to 39%) and protein. The oil is very variable in fatty acid composition, thus lending itself to seed breeding. A typical fatty acid composition is detailed in Table 2.50 (Vasconcellos et al., 1981; Ucciani, 1995a; Firestone, 1996; Badifu et al., 1991).

2.3.21 Calendula (*Calendula officinalis*, marigold)

Interest in this seed oil is based on the fact that it contains significant levels of calendic acid (53 to 62%) along with linoleic acid (see Table 2.50). Calendic acid (Δ8*t*,10*t*,12*c*-18:3) is a conjugated trienoic acid and this makes the oil an effective drying agent. Its alkyl esters can be used as a reactive diluent in alkyd paints to replace volatile organic compounds. Calendula oil is also a rich source of γ-tocopherol (1820 ppm in the crude oil). The crop is being studied, particularly in Europe, to improve its agronomy. It has been reported that soybeans have been genetically modified to contain 15% of calendic acid. (Ucciani, 1995a; Janssens et al., 2000a, 2000b). A recent report (Ozgul-Yucel, 2005) reports on this and other oils containing acids with conjugated unsaturation grown in Turkey.

2.3.22 Camelina (*Camelina sativa*, also called false flax and gold of pleasure)

In addition to its interesting fatty acid composition, this plant attracts attention because it grows well with lower

inputs of fertilisers and pesticides than traditional crops. The plant can be grown on poorer soils and is reported to show better gross margins than either rapeseed or linseed. The seed yield is in the range 1.5 to 3.0 tonnes per hectare and the oil content between 36 and 47%. Attempts are being made to optimise the agronomy. The oil has an unusual fatty acid composition and two sets of data are given in Table 2.50. It contains significant levels of oleic, linoleic, linolenic, and of C_{20} and C_{22} acids, especially eicosenoic (20:1). It contains a range of tocols (5 to 22, mean 17 mg/100g). Detailed tocopherol analysis shows that this is mainly γ-tocopherol and/or β-tocotrienol (>80%). (Budin et al., 1995). Despite its high level of unsaturation, the oil shows reasonable oxidative stability. Its use in paints, varnishes, inks, cosmetics, and as a food oil is being examined and developed. It provides a useful dietary source of linolenic acid as an alternative to linseed (flax) oil. Camelina oil is reported to contain cholesterol at a level of 188 ppm, which is unusually high for a vegetable source (Bonjean et al., 1995; Ucciani, 1995a; Zubr et al., 1997; Hebard, 1998; Leonard, 1998; Steinke et al., 2000a,2000b; Shukla et al., 2002).

2.3.23 Candlenut (*Aleurites moluccana* lumbang, kemiri, kukui)

This is a tropical tree with nuts containing a highly unsaturated oil rich in linoleic acid and linolenic acid (Table 2.50). Its iodine value, however, is not as high as that of linseed oil. It is used for cosmetic purposes and has been recommended for the treatment of burns (Ucciani, 1995a; Firestone, 1996).

2.3.24 Caraway (*Carum carvii*)

This is one of a group of plants whose seed oils contain petroselinic acid (Δ6–18:1). This reaches levels of 35 to 43% in caraway, 66 to 73% in carrot, 31 to 75% in coriander, and ~80% in parsley (Ucciani, 1995a). This isomer of oleic acid is a potential source of lauric and adipic acids as products of oxidative cleavage. The C_6 dibasic acid is an important component of many polyamides (nylons) and is usually made from cyclohexane by a reaction reported to be environmentally unfriendly. The use of petroselinic acid in food and in skin-care products has been described in two patents (Anon., 2003a).

2.3.25 Carrot (*Daucus carota*)

See Caraway.

2.3.26 Cashew (*Anacardium occidentale*)

Toschi et al. (1993) have given details of the fatty acids, triacylglycerols, sterols, and tocopherols in cashew nut oil. The fatty acid composition of this oleic-rich oil is given in Table 2.50 and the major triacylglycerols are OOO, POO, OOSt, OOL, and POL. The oil contains α- (2-6mg/100mg of oil), γ- (45-83), and δ- (3-8) tocopherols and is used in cosmetic preparations. (Ucciani, 1995a; Firestone, 1996; Watkins, 2005).

2.3.27 Chaulmoogra (*Hydnocarpus kurzii*, *Taraktogenus kurzii*)

Seed oils of the Flacourtiaceae are unusual because they contain high levels of cyclopentenyl fatty acids [C_5H_7(X)COOH] in which X is a saturated or unsaturated alkyl chain. The most common are hydnocarpic (16:1), chaulmoogric (18:1), and gorlic (18:2) acids. Chaulmoogra oil has been used in folk medicine for the treatment of leprosy, but there is no scientific evidence to support this claim (Ucciani, 1995a). Christie et al. (1989) have reported that *T. kurzii* oil contains these three acids at levels of 33.9, 27.5, and 21.2%, respectively.

2.3.28 Cherry (*Prunus cerasus*)

Obtained by cold pressing and filtering, this oil is reported to be used in the unrefined state as a specialty oil for salad dressings, baking, and for shallow frying and also in the production of skin-care products. However, its fatty acid composition is unusual in that, in addition to oleic and linoleic acids, it contains α-eleostearic acid (6 to 12%, Δ9*c*11*t*13*t*-18:3), (Kamel et al., 1992; Comes et al., 1992; Anon., 1993; Ucciani, 1995a) (Table 2.50). Some of these potential uses are surprising for an oil containing a conjugated triene acid and it should not be considered as an edible oil. The fatty acid composition of the phospholipids has been reported (Zlatanov et al., 1998).

2.3.29 Chestnut (*Castanea mollisma*)

Chestnut oil contains the usual palmitic, oleic, and linoleic acids (Padley, 1994) (Table 2.50).

2.3.30 Chia (*Salvia hispanica*)

Chia seeds contain 32 to 38% of a highly unsaturated oil. The fatty acid composition for five samples from Argentina is shown in Table 2.50 (Ayerza, 1995; Ucciani, 1995; Firestone, 1996).

2.3.31 Chinese vegetable tallow and stillingia oil (*Sapium sebiferum*, *Stillingia sebifera*)

This seed is unusual in that it yields lipids of differing composition from its outer seed coating (Chinese vegetable tallow, 20 to 30%) and from its kernel (stillingia oil, 10 to 17%) (Jeffrey et al., 1991; Ucciani, 1995a). The former oil, rich in palmitic and oleic acid (Table 2.50), is mainly a mixture of PPP (20 to 25%) and POP (~70%) tri-acylglycerols and, therefore, is a

potential confectionery fat. However, it is difficult to obtain the fat free from stillingia oil (the kernel oil). The latter is considered to be nutritionally unacceptable and, therefore, Chinese vegetable tallow is not used for food purposes. Stillingia oil is quite different in composition. In addition to oleic, linoleic, and linolenic acids, it contains novel C_8 hydroxy allenic and C_{10} conjugated dienoic acids combined as a C_{18} estolide attached to glycerol at the *sn*-3 position, thus:

$$\text{Glyc-OCOCH=C=CH(CH}_2)_4\text{OCOCH=CHCH=CH(CH}_2)_4\text{CH}_3$$

2.3.32 Coffee (*Coffea arabica* and *C. robusta*)

Coffee seed oil consists mainly of palmitic, oleic acid, and linoleic acid. Figures for the *robusta* and *arabica* oils are given in Table 2.50 (Rui Alves et al., 2003). Segall et al. (2005) identified 13 triacylglycerols from coffee beans. The major components were PLL (25.9%), PLP (13.7%), LLL (11.8%), POL (8.8%), PLSt (8.7%), StLL (8.3%), and OLL (7.8%).

2.3.33 Cohune (*Attalea cohune*)

Cohune seeds contain a typical lauric oil (Table 2.51) (Ucciani, 1995a; Firestone, 1996).

2.3.34 Coriander (*Coriandrum sativum*)

(See Caraway.) Attempts are being made to develop coriander with its high level of petroselinic acid as an agricultural crop. Transfer of the necessary desaturase to rape would provide an alternative source of petroselinic if successful (Ucciani, 1995a; Firestone, 1996; Foglia et al., 2000). A paper on the oxidative stability of a range of oils provides details of fatty acids, polar lipids, sterols, and tocols of coriander (Ramadan et al., 2004).

2.3.35 Crambe (*Crambe abyssinica, C. hispanica*)

Present interest in this oil, particularly in North Dakota and in Holland, depends on the fact that it is a potential source of erucic acid (50 to 55%), an acid with several industrial uses. This was once the major acid in rapeseed oil, but modern varieties of this seed produce low-erucic oils (such as canola) suitable for food use. (See Rapeseed oil, Section 2.2.12) (Lessman et al., 1981; Leonard, 1994; Ucciani, 1995a; Firestone, 1996; Steinke et al., 2000a, 2000b; Hebard. 2002; Temple-Heald, 2004; Anon., 2004e).

2.3.36 Cranberry (*Vaccinium macrocarpon*)

This seed oil is sold on the basis of its balance of omega-6 and omega-3 acids (Table 2.50) and of its very high content of tocotrienols (Table 2.48).

2.3.37 Cuphea

Cuphea plants furnish seeds with oils rich in one or more of C_8, C_{10}, C_{12}, or C_{14} saturated acids. They generally contain >30% of oil and are expected to produce a commercial crop in the period 2005 to 2010. Problems of seed dormancy and seed shattering have already been solved. Since markets for lauric oils already exist, there should be no difficulty in substituting cuphea oils. Pandey et al. (2000) have described the oil (17 to 29%) from *Cuphea procumbens* containing 88 to 95% of decanoic acid. (Ucciani, 1995a; Firestone, 1996; Isbell, 2002).

2.3.38 Cupuacu butter (*Theobroma grandiflora*, also called Cupu Assu kernel oil)

This is a solid fat containing palmitic, stearic, arachidic, oleic, and linoleic acid. With the fatty acid composition shown in Table 2.50, it will be rich in SOS triacylglycerols and have melting properties similar to cocoa butter (Firestone, 1996).

2.3.39 Date seed (*Phoenix dactylifera* L)

Two date cultivars (deglit nour and allig) contain oil (10.2 and 12.7%) rich in oleic acid along with a range of C_8 to C_{18} acids. These include lauric, myristic, palmitic, linoleic, and other minor acids. Details are given in Table 2.51. Despite the presence of medium chain acids this oil with its high levels of C_{18} acids is not a typical lauruc acid (Ucciani, 1995a; Besbes et al., 2004).

2.3.40 Dimorphotheca (*Dimorphotheca pluvialis*)

The seed of *Dimorphotheca pluvialis* is not very rich in oil (13 to 28%, typically about 20%), but it contains an unusual C_{18} hydroxy fatty acid (~60%) with the hydroxyl group adjacent (allylic) to a conjugated diene system (Firestone, 1996). Because of this structural feature, this acid is chemically unstable and easily dehydrates to a mixture of conjugated 18:3 acids (Δ8,10,12 and Δ9,11,13). Dimorphecolic acid provides a convenient source of 9-hydroxy- and 9-oxostearate and of hydroxy epoxy esters.

$$\text{CH}_3(\text{CH}_2)_4\text{CH=CHCH=CHCH(OH)(CH}_2)_7\text{COOH}$$

Dimorphecolic acid (9-OH 10*t*12*c*-18:2)

2.3.41 Echium (*Echium plantagineum*)

Only a few seeds contain stearidonic acid (Δ-6,9,12,15-18:4) and attempts are being made to grow *Echium plantagineum* as a source of this acid. Most of the oils from these seeds contain linoleic and γ-linolenic acid as well as α-linolenic and stearidonic acid (Ucciani, 1995a; Chen et al., 2001; Anon., 2003c; Kallio et al., 2003). A typical fatty acid analysis of refined echium oil is given in Table 2.53. With

almost 60% of the acids having three or four double bonds, the oil is highly unsaturated. Another convenient source of stearidonic acid is the readily available blackcurrant seed oil even though it only contains 2.5 to 3.0% of this acid. Stearidonic acid is the first metabolite in the conversion of α-linolenic to EPA and DHA and arguments for dietary supplements containing γ-linolenic acid can also be applied to stearidonic acid.

2.3.42 *Euphorbia lathyris* (caper spurge)

Attempts are being made to develop this plant as a source of an oil rich in oleic acid (80 to 85%). It is a Mediterranean annual with about 50% oil in its seed, but problems associated with seed shattering and the presence of a carcinogenic milky sap have still to be overcome through plant breeding (Ucciani, 1995a).

2.3.43 *Euphorbia lagascae*

This euphorbia species is one of a limited number of plants that contain significant proportions of epoxy acids in their seed oils (see Vernonia oil). With ~64% of vernolic acid (12,13-epoxyoleic) and minor proportions of palmitic, oleic, and linoleic acid this oil is rich in triacylglycerols containing two or three vernolic chains. (Table 2.50) However, not all reports include vernolic acid and there may be some confusion between the species examined (Ucciani, 1995a; Firestone, 1996).

2.3.44 Evening primrose (*Oenothera biennis*, *O. lamarckiana*, and *O. parviflora*)

This GLA-containing oil is discussed in Section 2.3.18 (Borage oil) (Table 2.53).

2.3.45 Flax (*Linum usitatissimum*)

Flax oil was discussed as linseed oil in Section 2.2.8 on major vegetable oils.

2.3.46 *Gevuina avellana* (Chilean hazelnut)

The so-called Chilean hazelnuts differ from traditional hazelnuts (*Corylus avellana,* also called filberts, see Section 2.3.48, Hazelnut). *Gevuina avellana* is native to Argentina and Chile and attempts are being made to produce commercial crops in Chile and in New Zealand. The fatty acid composition is unusual in that the unsaturated centres occupy unconventional positions and in a range of chain lengths (C_{16} to C_{24}). The oil content of the kernels is 40 to 48% and the oil contains a significant quantity of α-tocotrienol (130 mg/kg). The oil is rich in monounsaturated acids and is often compared with macadamia oil, but *Gevuina* seeds do not have the hard shell of macadamia nuts. The fatty acid composition is given below. The double bond positions are unusual and unrelated to each other except that three are n-5 olefininc groups. These unexpected results (Ucciani, 1995a; Bertoli et al., 1998) have been extended by Aitzetmüller (2004) who has reported that in addition to oleic (29.3%) and linoleic acid (8.8%), the oil contains several n-5 acids (~51%) including 16:1 (24.2%), 18:1 (8.4%), 20:1 (8.5%), and 22:1 (9.8%). The plant is being developed in New Zealand as a cool climate alternative to macadamia oil (Anon., 2003d). However, these two oils differ in their fatty acid composition.

2.3.47 Gold of Pleasure

See Camelina.

2.3.48 Grapeseed (*Vitis vinifera*)

Grapeseeds produce variable levels of an oil (6 to 20%) that is now available as a gourmet oil and for which Codex values have been reported (Ucciani, 1995a; Firestone, 1996; Beveridge et al., 2005). The oil is linoleic-rich and also contains palmitic, stearic, and oleic acids (Table 2.50). In common with other oils rich in linoleic acid, it is reported to have a beneficial effect on the skin. Some samples of grapeseed oil have higher PAH (polycyclic aromatic hydrocarbons) levels than is desired and Moret et al. (2000) described the effect of processing on the PAH content of the oil.

2.3.49 Hazelnut (*Corylus avellana*, filberts)

Hazelnut oil is rich in oleic acid and also contains linoleic acid (Table 2.50). The level of saturated acids is low. Hazelnuts grown mainly in Turkey and also in New Zealand produce 55 to 63% of oil. The fatty acid composition is very similar to that of olive oil, and hazelnut oil is sometimes added as an adulterant to the more costly olive oil. There have been several reports on methods of detecting this adulteration, many of them related to the presence of filbertone (structure given below) in hazelnut oil (see discussion on olive oil, Section 2.2.9). Hazelnuts appear in a short list of nuts for which a health claim may be made (Anon., 2003b). Other details are found in reports by Bertoli (1998), Savage et al. (1997), Gordon et al. (2001), Ruiz del Castillo et al. (2001, 2003), Bernardo-Gil (2002), Bewadt et al. (2003), Christopoulou et al. (2004), Zabaras et al. (2004), Crews et al. (2005a), and Watkins (2005).

Filbertone is (*E*)-5- methylhept-2-en-4-one
$CH_3CH_2CH(CH_3)COCH{=}CHCH_3$

2.3.50 Hemp (*Cannabis sativa*, marijuana)

Hemp seed oil (25 to 34% of whole seed, 42 to 47% of dehulled seed) has an interesting fatty acid composition (Table 2.50). In addition to the usual range of C_{16} and C_{18}

acids, the oil contains low levels of γ-linolenic (0 to 5%) and stearidonic acid (0 to 3%). The oil is a rich source of tocopherols — virtually entirely the γ-compound — at 1500 mg/kg and is used in cosmetic formulations. Evidence from a study in Finland indicates that dietary consumption of hemp seed oil leads to increased levels of γ-linolenic acid in blood serum. The growing of hemp is banned in the U.S. (though permitted in Canada) and, therefore, supplies of hemp seed oil, if required in the U.S., must be imported. Further details are available in references Ucciani (1995a), Firestone (1996), Anon. (2001), Przybylski (2002,) Yang et al. (2003), Illingworth (2004), Przybylski (2006), and hemptrade (Website).

2.3.51 Honesty (*Lunaria annua*)

This seed oil contains significant levels of erucic (22:1) and nervonic acids (24:1) and is being studied as a new crop because it is a good source of nervonic acid, which may be useful in the treatment of demyelinating disease (Sargent et al., 1994; Ucciani, 1995a).

2.3.52 Hyptis (*Hyptis* spp)

Hagemann et al. (1967) have reported the fatty acid composition of oils from six different *hyptis* species. Five contain high levels of linolenic and of linoleic acid (Table 2.50) but the oil from *Hyptis suaveolens* contains less than 1% of linolenic acid with 77 to 80% of linoleic acid and palmitic (8 to 15%) and oleic acids (6 to 8%) (Ucciani, 1995a).

2.3.53 Illipe

See Borneo tallow

2.3.54 Kidney beans (*Phaseolus vulgaris*)

Kidney beans contain only low levels of lipid (~1.8%), but the beans are widely consumed and in some communities act as a significant source of lipid. Yoshida et al. (2005) have examined five cultivars grown in Japan. The lipids are mainly triacylglycerols (34 to 46% containing 16:0 8 to 13%, 18:1 8 to 14%, 18:2 17 to 26%, and 18:3 49 to 58%) and phospholipids (50 to 59%, with the same major acids, but in different proportions). The low omega-6/omega-3 ratio adds to the dietary value of this foodstuff.

2.3.55 *Jatropha curcas*

Jatropha oil is present in the kernel of *Jatropha curcas,* also known as the physic nut, which grows in India, Indonesia, Nicaragua, and other tropical regions. The oil content is around 50%. This is mainly triacylglycerols with 0.4% of unsaponifiable material. The oil is rich in palmitic (16%), oleic (51%), and linoleic acid (23%). These numbers are typical and other levels have been reported (Table 2.50). New interest in this oil is based on its potential as a source of biodiesel and ambitious plans to grow *Jatropha curcas* in India have been announced. (Anon., 2005a; SOFA database and websites devoted to *jatropha* oil).

2.3.56 Jojoba (*Simmondsia chinensis*)

Jojoba oil is a valuable source of C_{20} and C_{22} compounds. The oil has already been developed as a marketable product, but is available in only limited supply. It is produced by a plant resistant to drought and to desert heat. It takes 5 to 7 years to first harvest, 10 to 17 years to full yield, and has a life span of about 100 years. Jojoba plants are grown in the Southwestern U.S., Mexico, Latin America, Israel, South Africa, and Australia. Yields are reported to be about 2.5 tonnes of oil/hectare.

Jojaba oil contains only traces of triacylglycerols and is predominantly a mixture of wax esters based mainly on C_{20} and C_{22} monounsaturated acids and alcohols. The oil contains esters with 40, 42, and 44 carbon atoms with two isolated double bonds, one in the acyl chain and one in the alkyl chain. Tobaras et al. (2003) have compared the physical properties and the chemical composition (Table 2.56) of cold-pressed, expelled, and bleached (expelled) jojoba oil. The oil serves as a replacement for sperm whale oil proscribed in many countries because the sperm whale is an endangered species. As a high-priced commodity, jojoba oil is used in cosmetics. As it gets cheaper through increasing supplies, it will be used as a superior lubricant. It is also considered as a possible biofuel after methanolysis — presumably as a mixture of methyl esters and long-chain alcohols. The oil is fairly pure as extracted, has a light colour, and is resistant to oxidation because its two double bonds are well separated. The oil can be chemically modified by reaction of the double bonds, such as hydrogenation, stereomutation, epoxidation and sulfochlorination (Wisniak, 1987; Ucciani, 1995a; Firestone, 1996).

2.3.57 Kapok (*Bombax malabaricum*, *Ceiba pentandra*)

This name is applied to a number of tropical trees of the bombax family. The oil is a by-product of kapok fibre production. In addition to palmitic, oleic, and linoleic acids (Table 2.50), it also contains about 13% of cyclopropene acids (malvalic and sterculic) that make it unsuitable for food use (Ucciani, 1995a; Firestone, 1996).

2.3.58 Karanja (*Pongamia glabra*)

Karanja seed oil from India is rich in C_{18} and C_{20} monounsaturated acids (Table 2.50) (Ucciani, 1995a; Bhattacharyya, 2002). It is being investigated as a local source of biodiesel.

2.3.59 Kiwi (*Actinidia chinensis*, *A. deliciosa*)

The seed of this fruit furnishes a linolenic-rich oil with lower levels of linoleic, oleic, and saturated acids (Table 2.50) (Ucciani, 1995a).

2.3.60 Kokum (*Garcinia indica*)

Both kokum and mahua fats are rich in saturated and oleic acids and contain high levels of SOS triacylglycerols (Table 2.57). These fats can be used directly or be fractioned separately or as blends to produce stearins used as cocoa butter extenders. Kokum butter is one of the six permitted fats (palm oil, illipe butter, kokum butter, sal fat, shea butter, and mango kernel fat) that can partially replace cocoa butter in chocolate in EU countries. Kokum butter is a stearic acid-rich fat and Bhattacharyya (2002) has reported the figures cited in Table 2.50 (Ucciani, 1995; Firestone, 1996; Jeyarani et al., 1999; Maheshwari et al., 2005).

2.3.61 Kusum (*Schleichera trijuga*)

Kusum seed oil is an unusual oleic-rich oil (40 to 67%) also containing significant quantities of arachidic acid (20:0) and lower levels of palmitic, stearic, and linoleic acids (Table 2.50) (Ucciani, 1995a; Firestone, 1996; Bhattacharyya, 2002).

2.3.62 Lesquerella (*Lesquerella fendleri)*

The only oil of commercial significance with a hydroxy acid is castor oil, but among the new crops being seriously developed there are two containing hydroxy acids. Lesquerella oils have some resemblance to castor oil, but *Dimorphotheca pluvialis* seed oil contains a different kind of hydroxy acid. Plants of the *Lesquerella* species are characterised by the presence of the C_{20} bis-homologue of ricinoleic acid — lesquerolic acid — sometimes accompanied by other acids of the same type at lower levels:

ricinoleic acid	12-OH Δ 9-18:1
densipolic acid	12-OH Δ 9,15-18:2
lesquerolic acid	14-OH Δ 11-20:1
auricolic acid	14-OH Δ 11,17-20:2

A typical analysis of *L. fendleri* seed oil showed the presence of palmitic, stearic, oleic, linoleic, and linolenic along with lesquerolic (54%), and auricolic (4%) acids (Table 2.50). Because lesquerolic acid is the C_{20} homologue of ricinoleic with the same β-hydroxy alkene unit, it undergoes similar chemical reactions, but produces (some) different products (See Section 2.2.2, Castor oil). For example, pyrolysis should give heptanal and 13-tridecenoic acid (in place of 11-undecenoic acid from castor oil). This could be converted to 13-aminotridecanoic acid, the monomer required to make nylon-13. Similarly, alkali fusion will give 2-octanol and dodecanedioic acid in place of decanedioic (sebacic) acid from castor oil. This C_{12} dibasic acid is already available from petrochemical products and has a number of applications. The free hydroxyl group in castor and lesquerella oils can be esterified with fatty acids, such as oleic acid, to give an estolide. Partial acylation of lesquerella oil with cinnamic acid or 4-methoxycinnamic acid produce products, which are active as sunscreens (Compton et al., 2004; Compton, 2005).

Abbott (1997) has reviewed the status and potential of lesquerella as an industrial crop. The protein from this seed has been characterised by Wu et al. (2005). Lesquerella plants can be grown in saline soils (Ucciani, 1995a; Firestone, 1996; Isbell et al., 2002). Attempts are being made to develop a variety that also will grow in salty water.

2.3.63 Lupin (*Lupinus albus*)

White lupin is a legume crop sometimes grown as a green manure because of the nitrogen fixation that takes place. It has potential as a component of foods and animal feed. Two papers (Bhardwaj et al. and Hamama et al., 2005) report on the seed and its oil as produced in the U.S. The oil (7 to 8%) contains saturated (~12%), monounsaturated (50 to 53%, C_{16} to C_{22} mainly 18:1) and polyunsaturated acids (35 to 37%, linoleic and linolenic acids in a ratio of about 2:1). Details are also given for phospholipids, triterpene alcohols, and phytosterols.

2.3.64 Macadamia (*Macadamia integrifolia, M. tetraphylla*)

Macadamia nuts are used as a snack food and it has been claimed that their consumption reduces total and LDL serum cholesterol (Colquhoun et al., 1996). They are rich in oil (60 to 70%) used in cosmetics and available as a gourmet oil. It is characterised by its high level of C_{16} to C_{20} monoene acids (~80%) (Table 2.50), which makes it good for skin care, though low levels of tocopherols limit its oxidative stability. Gevuina oil is sometimes used in place of macadamia oil; however, their detailed fatty acid compositions differ more than some have recognised. Certain health benefits have been claimed for hexadecenoic acid with the most convenient sources being sea buckthorn oil and macadamia oil (Macfarlane et al., 1981; Croy, 1994; Yang et al., 2003; Anon., 2003f; Watkins, 2005).

2.3.65 Mahua (*Madhuca latifolia*)

See Kokum fat and Mango kernel fat. Bhattacharyya (2002) gives the fatty acid composition of mahua fat indicated in Table 2.50 (Ucciani, 1995a).

2.3.66 Mango (*Mangifera indica*)

The fruit of the mango is consumed in large quantities. The kernel contains 7 to 12% of stearic acid-rich lipid

TABLE 2.54 Tropical fats that may partially replace cocoa butter in some countries

Common name	Botanical name	Major triacylglycerols (%)		
		POP	POSt	StOSt
Cocoa butter	*Theobroma cacao*	16	38	23
Palm mid fraction	*Elaies guinensis*	57	11	2
Borneo tallow (illipe)	*Shorea stenoptera*	6	37	49
Kokum butter	*Garcinia indica*	1	5	76
Mango kernel stearin	*Mangifera indica*	2	13	55
Sal stearin	*Shorea robusta*	1	10	57
Shea stearin	*Butyrospermum parkii*	1	7	71

Note: Major SOS triacylglycerols are shown as typical values (P = palmitic, O = oleic, St = stearic, S = saturated). See Timms 2003, Chapter 5.

with palmitic, oleic, and linoleic acids also. Ranges and typical values are given in Table 2.50. The fat is fractionated to give a lower melting olein with excellent emollient properties. The accompanying stearin (Table 2.54) can serve as a cocoa butter equivalent and as a component of a *trans*-free bakery shortening, along with fractioned mahua fat. It is one of six permitted fats (palm oil, illipe butter, kokum butter, sal fat, shea butter, and mango kernel fat) that can partially replace cocoa butter in chocolate in EU countries. (Osman et al., 1981; Ucciani, 1995a; Firestone, 1996; Yella Reddy, 2001; Bhattacharyya, 2002; Gunjikar, 2005).

2.3.67 Manketti (*Ricinodendron rauttanenni*)

This oil, used widely in Namibia as an emollient, contains eleostearic (9c11t13t-18:3) and other conjugated octadecatrienoates (total 6 to 28%) as well as oleic, linoleic, and a range of minor acids (Ucciani, 1995a).

2.3.68 Marigold (*Calendula officinalis*)

See Calendula.

2.3.69 Marula (*Sclerocarya birrea*)

Marula oil is an oleic-rich oil also containing palmitic, stearic, and linoleic acids (Table 2.50) (Ucciani, 1995a).

2.3.70 Meadowfoam (*Limnanthes alba*)

This oil is unusual in that over 95% of its component acids are C_{20} or C_{22} compounds and include Δ5-20:1 (63 to 67%), Δ5-22:1 (2 to 4%), Δ13-22:1 (16 to 18%), and Δ5,13-22:2 (5 to 9%). It is grown in the U.S. and its potential uses are being thoroughly examined. Winter cultivars now being developed are expected to improve the suitability of the crop to conditions in Northern Europe (Joliff, 1981; Ucciani, 1995a; Firestone, 1996; Isbell, 1997). Potential uses of this oil include cosmetic applications, production of dimer acid, as a lubricant, and via a wide range of novel derivatives based on reaction at the Δ5 double bond (Isbell, 1998; Isbell et al., 2001).

2.3.71 Melon (*Citrullus colocythis* and *C. vulgaris*)

Akoh and Nwosu (1992) report the major fatty acids in the total lipids from two samples (Table 2.50). In a later paper, three cutivars (Hy-mark, Honey Dew, and Orange Flesh) are described in terms of lipid content (25.7 to 28.6%) and fatty acid composition (Ucciani, 1995a; Bora et al., 2000).

2.3.72 Moringa (*Moringa oleifera*, *M. stenopetala*)

Dried moringa seeds contain about 35% of oil rich in oleic acid, also having palmitic, stearic, linoleic, linolenic, and eicosenoic acids (Table 2.50). The oil has high oxidative stability resulting from its low levels of polyunsaturated fatty acids and from the presence of the flavone myricetin that is a powerful antioxidant. In the more recent study, cold-pressed oil (36%) is compared with that extracted by chloroform/methanol (45%) and the composition of the fatty acids and sterols is given. The oil is reported to contain 20:0, 20:1, 22:0, 22:1, and 26:0 at around 11% (total) (Bhattacharyya, 2002; Lalas et al., 2002, 2003). One specification (Statfold, website) indicates the presence of palmitoleic acid (8%) in this oil. A study of the oil produced in Pakistan (Anwar et al., 2005) includes details of sterols and tocopherols. See also Moringa (website).

2.3.73 Mowrah (*Madhuca latifolia*, *M. longifolia*, *M. indica*)

This comes mainly from India where the fat is used for edible and industrial purposes. The nuts contain 46% oil with variable levels of palmitic, stearic, oleic, and linoleic acid (Table 2.50). The mowrah fat examined by De et al. (2002) differs somewhat, particularly in the levels of stearic and linoleic acid (Ucciani, 1995a; Firestone, 1996; Bhattacharyya, 2002). A more recent article (Ramadan and Moersel, 2006) draws attention to the radical scavenging activity of this fat.

2.3.74 Murumuru butter (*Astrocaryum murumuru*)

This solid fat is rich in saturated acids (89%, mainly lauric and myristic) and has 11% of unsaturated acids that are almost entirely oleic (Padley, 1994) (Table 2.51).

TABLE 2.55 Fatty acid composition (% of total) of oils from selected *Cuphea* species

	8:0	10:0	12:0	14:0	16:0	18:1	18:2
C. pulcherrina	94.4	3.3	0.0	0.0	0.6	0.7	1.0
C. koehneana	0.6	91.6	1.5	0.6	1.3	1.1	3.1
C. calophylla	0.1	5.0	85.0	6.8	1.1	0.5	1.3
C. salvadorensis	25.3	0.9	2.8	64.5	5.2	0.5	0.5
C. denticula	0.0	0.0	0.0	0.0	33.0	9.8	53.2

Source: Adapted from Pandey, V., et al., *Eur. J. Lipid Sci and Technol.*, **102**, 463–466, 2000.

TABLE 2.56 Alcohol and acid composition of jojoba oil

	Long chain acids			Long chain alcohols		
	Cold-pressed	Expelled	Expelled and Bleached	Cold-pressed	Expelled	Expelled and Bleached
16:0	1.1	0.9	0.8	Trace	Trace	Trace
18:1 (Δ 9)	9.3	8.9	8.4	0.7	0.7	0.8
20:1 (Δ 11)	75.0	75.7	76.6	41.8	41.6	41.3
22:1 (Δ 13)	13.4	13.4	13.0	48.4	48.3	48.5
24:1 (Δ 15)	1.2	1.0	1.1	9.0	9.4	9.1

2.3.75 Mustard (*Brassica alba*, *B. hirta*, *B. nigra*, *B. juncea*, *B. carinata*)

Mustard seeds contain 24 to 40% of an erucic acid-rich oil (Table 2.50). The plant is grown extensively in India. Canadian investigators have bred *Brassica juncea* (oriental mustard) from an Australian line with low erucic acid and low glucosinolate, so that it has a fatty acid composition similar to that of canola oil from *B. napus* and *B. rapa.* This makes it possible to expand the canola growing area of Western Canada (Ucciani, 1995a; Firestone, 1996; Gunstone, 2000).

2.3.76 Neem (*Azadirachta indica*)

This interesting seed oil, sourced mainly from India, contains chemicals used to control 200 species of insects. For example, the oil prevents larval insects from maturing. Bhattacharyya (2002) has reported the fatty acid composition given in Table 2.50 (Ucciani, 1995a; Firestone, 1996; Anon., 1994; Melton, 2003).

2.3.77 Ngali nut (*Canarium* spp)

Analyses of five different *Canarium* spp have been reported (Ucciani, 1995a) (Table 2.58). They contain the same major component acids, but at differing levels.

2.3.78 Nigella (*Nigella sativa*, black cumin)

Typically nigella oil contains palmitic, oleic, and linoleic acid (Table 2.50). Related species (*N. arvensis* and *N. damascena)* give similar oils with less oleic and more linoleic acid. The presence of low levels of 20:1 (Δ11*c*, 0.5–1.0%) and higher levels of 20:2 (Δ11*c*14*c*, 3.6–4.7%) in all of these oils may be of taxonomic significance. In one analysis the oil contained the following major triacylglycerols: LLL 25%, LLO 20%, LLP 17%, LOP 13%, and LOO 10% reflecting the high level of linoleic acid. The seeds appear to contain an active lipase and the oil quickly develops high levels of free acid. The oil is reported to be a good source of thymoquinone and to assist in the treatment of prostate problems (Ustun et al., 1990; Ucciani, 1995a; Zeitoun et al., 1995; Aitzetmüller et al., 1997; Takuri et al., 1998; Atta, 2003). A paper on the oxidative stability of black cumin oil provides details of fatty acids, polar lipids, sterols, and tocols (Ramadan et al., 2004).

2.3.79 Niger (*Guizotia abyssinica*)

This oil comes mainly from Ethiopia. The seeds contain 29 to 39% of oil rich in linoleic acid (71 to 79%) along with palmitic, stearic, and oleic acids (Table 2.50). The major triacylglycerols and sterols (particularly sitosterol,

TABLE 2.57 Fatty acids and triacylglycerols of kokum and mahua fats (see also Table 2.54)

	Fatty acids				Triacylglycerols		
Oil source	16:0	18:0	18:1	18:2	StOSt	POSt	POP
Kokum	2.0	49.0	49.0	0	72.3	7.4	0.5
Mahua	23.5	20.0	39.0	16.7	10.6	22.2	18.9
Stearin[a]	15.7	37.8	35.5	11.1	46.2	15.0	9.7

[a] Obtained by dry fractionation of a 1:1 mixture of the two oils.

TABLE 2.58 Fatty acid composition of the seed oils from five *Canarium* species

	Palmitic	Stearic	Oleic	Linoleic
C. commune	30	10	40	19
C. ovatum	33–38	2–9	44–60	0–10
C. patentinervium	33	10	27	28
C. schweinfurthii	1	-	84	15
C. vulgare	29	12	49	10

campesterol, stigmasterol, and Δ5-avenasterol) have been identified. The oil is rich in α-tocopherol (94 to 96% of total values ranging from 657 to 853 mg/kg) and is a good source of vitamin E. It is used for both edible and industrial purposes (Dutta et al., 1994; Ucciani, 1995a; Firestone, 1996; Marini et al., 2003). A paper on the oxidative stability of niger oil provides details of fatty acids, polar lipids, sterols, and tocols (Ramadan et al., 2004).

2.3.80 Nutmeg (*Myristica malabarica* and other *M.* species)

Not surprisingly, considering its botanical name, seeds of the *Myristica* species (~40% oil, though this may contain essential oil and resins) are rich in myristic acid (~40%). Higher levels were quoted in earlier work and one report is cited in Table 2.50 and 2.51 (Eckey, 1954; Hilditch et al., 1964).

2.3.81 Oats (*Avena sativa*)

This grain seed contains 4 to 8% of lipid, though somewhat more in certain strains. The major component acids are palmitic, oleic, linoleic, and linolenic acid (Table 2.50). The oil contains triacylglycerols (51%), di- and mono-acylglycerols (7%), free acids (7%), sterols and sterol esters (each 3%), glycolipids (8%), and phospholipids (20%). The special features of this oil are utilised in various ways. It is reported to show cholesterolemic and antithrombotic activity, and is used as an appetite suppressant in "Olibra", used in cosmetics by virtue of its glycolipids, and can be used in baking, increasing loaf volume at levels as low as 0.5%. Oat lipids are discussed in several recent reviews (Firestone, 1996; Anon., 1998; Zhou et al., 1999; Herslof, 2000; Holmback et al., 2001; Peterson, 2002). Moreau et al. (2003) have examined the pressurized liquid extraction of polar and nonpolar lipids in corn and oats with hexane, methylene chloride, isopropanol, and ethanol. The phytosterols have been detailed by Jiang and Wang (2005).

2.3.82 Oiticica (*Licania rigida*)

The kernel oil, obtained from this Brazilian tree, is characterised by its high level (~78%) of licanic acid (4-oxo-9*c*11*t*13*t*-octadecatrienoic acid) — a keto derivative of the more familiar eleostearic acid. The oil shows drying properties, but does not dry as quickly as tung oil (Ucciani, 1995a; Firestone, 1996).

2.3.83 Parsley (*Petroselinium sativum*)

See Caraway.

2.3.84 Passionfruit (*Passiflora edulis*)

This popular fruit contains about 20% of oil in its seed and is available as a gourmet oil for use in specialty foods and salad dressings. It is linoleic-rich with palmitic and oleic acids (Table 2.50). Its high level of linoleic acid is believed to make the oil good for skin care (Anon., 1991; Ucciani, 1995a; Firestone, 1996). A recent paper provides a comparative study of fatty acid profiles of Ugandan passiflora seed oils (Nyanzi et al., 2005).

2.3.85 Peach (*Prunus persica*)

Peach kernels contain 44% of an oleic-rich oil, which also contains palmitic and linoleic acid (Table 2.50) (Ucciani, 1995a; Firestone, 1996; Zlatanov, 1998).

2.3.86 Pecan (*Carya pecan*, *C. illinoensis*)

Pecan oil, containing palmitic, oleic, and linoleic acids (Table 2.50), is reported to lower blood cholesterol and the FDA allows such a claim to be made in respect of pecan nuts (Firestone, 1996; Bewadt, 2003; Watkins, 2005).

2.3.87 Perilla (*Perilla frutescens*)

Perilla is a linolenic-rich oil (57 to 64%) used as a drying oil. It also contains oleic and linoleic acids (Table 2.50) and comes mainly from Korea and India. Recent descriptions of this oil come from these two countries (Longvah et al., 1991; Shin et al., 1994; Ucciani, 1995a; Firestone, 1996; Kim et al., 2002).

2.3.88 Phulwara butter (*Madhuca butyracea* or *Bassia butyracea*)

This solid fat is exceptionally rich in palmitic acid and would be expected to contain high levels of POP among its triacylglycerols. Bhattycharyya (2002) reports the values cited in Table 2.50 (Ucciani, 1995a).

2.3.89 Pine nuts (*Pinus* spp)

There are many species of pine-producing nuts with similar but not identical seed oils. Interesting information is available in publications by Wolff and his colleagues (1995, 1997, 1998). The oil content for 18 species ranges from 13 to 35%. The oils are of interst in that they

contain one or more Δ5 acids (up to a totals of 27%) in addition to oleic (14 to 36%) and linoleic (43 to 56%) acids. The 5 acids include Δ5*c*9*c*12*c*-18:3 (pinoleic) and Δ5*c*11*c*14*c*-20:3 (sciadonic). It has been claimed that pinolenic acid is the active component in these oils acting as an appetite suppressant. They are believed to stimulate the hormone cholcystokinin, which reduces the desire to eat (Watkins, 2005).

2.3.90 Pistachio (*Pistachio vera*)

Pistachio nuts, produced mainly in Iran, are widely consumed as shelled nuts. They contain about 60% of an oil used for cooking and frying. Mean fatty acid levels for five varieties are given in Table 2.50. The regiospecific distribution of fatty acids and the triacylglycerol composition of Algerian *Pistacia atlantica* have been reported by Yousfi et al. (2005). Triacylglycerol composition has been suggested as a method of determining the country of origin of pistachio nuts. The FDA allows the following claim in respect of pistachio nuts: "Scientific evidence suggest, but does not prove, that eating 1.5 ounces a day of most nuts as part of a diet low in saturated fat and cholesterol may reduce the risk of heart disease." (See Dyszel et al., 1990; Firestone, 1996; Maskan et al., 1998; Yildiz et al., 1998; Bernardo-Gil et al., 2002; Watkins, 2005.)

2.3.91 Plum (*Prunus domestica*)

The kernels contain oil (41%) that is rich in oleic acid with significant levels of linoleic acid as well (Table 2.50) (Padley, 1994; Zlatanov, 1998).

2.3.92 Poppy (*Papaver somniferium*)

Opium is obtained from unripe capsules and from the straw of the poppy plant, but the narcotic is not present in the seed, which is frequently used as birdseed. It contains 40 to 70% of a semidrying oil used by artists and also as an edible oil. Rich in linoleic acid, it also contains palmitic, oleic, and linolenic acids (Ucciani, 1995a; Firestone, 1996; Singh et al., 1998). Oxidative deterioration of poppy seeds and of the derived oil is related to the damage that the seeds may suffer during harvesting (Wagner et al., 2003). Krist et al. (2005) have measured the volatile compounds in poppy seed oil (mainly pentanol, hexanal, hexanol, 2-pentylfuran, and caproic acid) and the triacylglycerols (mainly PLL, PLO, LLL, OLL and OOL).

2.3.93 Pumpkin (*Cucurbita pepo*)

Pumpkin seed oil is a linoleic-rich oil also containing palmitic, stearic, oleic, and a wide range of minor component acids. It has attracted attention because of its reported potential to cure prostate disease. The fatty acid composition differs between black and white pumkin seeds (Table 2.50) and information is available on its triacylglycerols, sterols, and tocopherols; on the effect of microwave treatment; and on oils extracted with supercritical carbon dioxide. (Ucciani, 1995a; Firestone, 1996; Fruhwirth et al., 2003; Murkovic et al., 2004; Siegmund et al., 2004; Yoshida et al., 2004; Yu Wenli et al., 2004).

2.3.94 Purslane (*Portulaca oleracea*)

The plant (leaves, stem, and whole plant) is reported to be the richest vegetable source of n-3 acids with low levels of the 20:5, 22:5, and 22:6 members. This is a surprising and unlikely result and needs to be confirmed (Omara-Olwaea et al., 1991). These acids have not been identified in the seed oil that contains palmitic, stearic, oleic, linoleic, and linolenic acids (Table 2.50).

2.3.95 Raspberry (*Rubus idaeus*)

Raspberry seed oil is highly unsaturated with palmitic, oleic, linoleic, and linolenic acids (Table 2.50). It is reported to be a rich source of tocopherols (3300 mg/l of oil) divided between the α- 500, γ- 2400, and δ-compounds 400 (Table 2.48) (Yang, 2003a). The raspberry is one of a group of fruits, along with boysenberry, Marion blackberry and an evergreen blackberry, that grow on leafy canes and are describes as caneberries. Their fatty acid composition and tocopherol levels are reported by Bushman et al., 2004.

2.3.96 Rose hip (*Rosa canina*, hipberry)

Rose hips are best known for the high level of vitamin C in their fleshy parts, but the seeds contain a highly unsaturated oil that is used in cosmetics. The oil contains linoleic and linolenic acids in addition to oleic and saturated acids (Table 2.50). The yield of pressed oil is increased by enzymatic pretreatment (Concha et al., 2004).

2.3.97 Rubber seed oil (*Hevea brasiliensis*)

Rubber seed oil is highly unsaturated oil containing both linoleic and linolenic acids. It is becoming important as a binder in surface coatings and as a plasticise/stabiliser for PVC and natural rubber. Several properties that assist in the authentication of this oil are reported by Aigboudion and Bakare, 2005.

2.3.98 Sacha inchi (*Pilkenetia volubilis,* Inca peanut)

This highly unsaturated oil from plants in the tropical jungles of Central and South America is rich in linoleic and linolenic acids with only low levels of oleic and saturated

acids (Table 2.50). This makes it comparable to, but not identical with, linseed oil (Guillen et al., 2003).

2.3.99 Sal (*Shorea robusta*)

This Northern India tree is felled for its timber. Its seed oil is rich in stearic acid and can be used as a cocoa butter equivalent (CBE). The major acids are palmitic, stearic, oleic, linoleic, and arachidic acid (20:0) (Table 2.50 and Table 2.54) and its major triacylglycerols are of the SUS type required of a cocoa butter equivalent. Sal olein is an excellent emollient and sal stearin, with POP 1%, POSt 13% and StOSt 60%, and is a superior cocoa butter equivalent. It is one of the six permitted fats (palm oil, illipe butter, kokum butter, sal fat, shea butter and mango kernel fat) that can partially replace cocoa butter in chocolate (Ucciani, 1995a; Shukla, 1995; Firestone, 1996; Lipp et al., 1998; Birkett, 1999; Talbot, 1999; Stewart, 2002). Commercial sal fat may contain varying levels of epoxy and hydroxy acids depending on how the fat has been stored.

2.3.100 *Salicornia bigelovii*

This annual dicotyledon is of interest because it is a halophyte, growing in areas that support only limited vegetation. When growing, it can be irrigated with salt water (Flider, 2004). It produces seeds at a level of 1.7 to 2.3 t/h that furnishes oil (26 to 33%) and meal with 40% protein. The oil is rich in linoleic acid and also contains oleic, palmitic, and lower levels of stearic and α-linolenic acids (Table 2.50). Its tocopherols (720 ppm) are mainly the α- and γ-compounds and its sterol esters (4%) are mainly stigmasterol, β-sitosterol, and spinasterol (Lu et al., 2000). Imai et al. (2004) have characterised the complex lipids in a related species — *Salicornia europaea.*

2.3.101 Sea buckthorn (*Hippophae rhamnoides*)

This is a hardy bush growing wild in several parts of Asia and Europe and now cultivated in Europe, North America, and Japan. It is resistant to cold, drought, salt, and alkali. Two different oils are available in the seeds and in the pulp/peel, but these are not always kept separate. In the combined oil, the seed furnishes 16 to 25% of the total oil and the balance comes from the pulp/peel. The seed oil is rich in oleic, linoleic, and linolenic acids, but the berry oil contains significant levels of palmitoleic acid (see Table 2.59). Several health benefits are claimed for this oil now available in encapsulated form and being incorporated into functional foods. The oil is rich in sterols, carotenoids (especially β-carotene), tocopherols (2470 mg/l of oil) and phenolic acids (Zadernoeski et al., 2005). Sea buckthorn pulp is rich in α-tocopherol (2000), while the seed oil is rich in α- (1000) and γ-tocopherols (1000) (Li et al., 1998; Yang et al., 2001a, 2001b, 2003b; Kallio et al., 2002; Yang et al., 2003; Zadernowski et al., 2003). The crop grown in Canada is sufficient to meet the demand in North America (10 tonnes of oil from 1500 tonnes of fruit) and to supply oil to Europe as well (Anon., 2003e).

2.3.102 Shea (*Butyrospermum parkii*, shea butter, karate butter)

This fat comes from trees grown mainly in West Africa and contains an unusually high level of unsaponifiable material (~11%), including polyisoprene hydrocarbons. It is rich in stearic acid, but its fatty acid composition varies with its geographical source. It contains palmitic, stearic, oleic, and linoleic acid (Table 2.50). Shea fat can be fractionated to give a stearin (typically POP 1%, POSt 8%, and StOSt 68%) that can be used as a cocoa butter equivalent. It is one of the six permitted fats (palm oil, illipe butter, kokum butter, sal fat, shea butter and mango kernel fat) that may partially replace cocoa butter in chocolate (Ucciani, 1995a; Firestone, 1996; Shukla, 1996; Lipp et al., 1998; Birkett, 1999; Stewart et al., 2002; Lovett, 2005). Its tocopherol content is affected by the climate under which it grows (Maranz et al., 2004). The fat may be added to animal feed to prevent diarrhea (Anon., 2004b).

Alendar (2004) reports that shea butter contains an interesting and useful unsaponifable fraction (2 to 5%) rich in triterpene alcohols, such as α- and β-amyrins (27 and 10% of total triterpene alcohols), lupeol (22%), and butyrospermol (25%). These alcohols occur mainly as esters with fatty acids or cinnamic acid and are bioactive compounds used in cosmetics (see "Personal care products", Section 9.4).

2.3.103 Shikonoin seed (*Lithospermum* spp)

Several plants of the *Lithospermum* genus have been examined. They belong to the Boraginaceae family and many of them contain γ-linolenic (18:3) and stearidonic acid (18:4) (see Borage) (Ucciani, 1995a; Miller et al., 1968).

TABLE 2.59 Fatty acid composition (% wt. mean of 21 samples) of seed oil and berry oil from sea buckthorn

	16:0	16:1-Δ 9	18:0	18:1-Δ 9	18:1-Δ 11	18:2	18:3
Seed oil	7.7	–	2.5	18.5	2.3	39.7	29.3
Berry oil	23.1	23.0	1.4	17.8	7.0	17.4	10.4

Source: Adapted from Yang, B. and Kallio., H. *J. Agric. Food Chem.*, **49**, 1939–1947, 2001.

2.3.104 *Sisymbrium irio*

Sisymbrium irio is one of several *sisymbrium* oils reported by Ucciani (1995a). These come from brassica species and, therefore, it is not surprising that many contain long chain monoene acids. *Sisymbrium irio* seed oil, for example, is reported to contain 20:1 (9.2%) and 22:1 (erucic acid, 9.1%) in addition to palmitic, oleic, linoleic, and linolenic acid (Table 2.50). Aubert-Mammou et al. (1996) have identified 19 acids in this seed oil and have reported a regiospecific analysis. All acids are present in the *sn*-1/3 positions, but the 2-position is occupied almost solely by unsaturated C_{18} acids.

2.3.105 Tamanu (*Calophyllum tacanahaca*)

Tamanu oil is obtained from nuts that grow on the ati tree and is important in Polynesian culture. It has four major component acids (palmitic, stearic, oleic, and linoleic) and a typical analysis is reported in Table 2.50. It is claimed to be helpful in the treatment of many skin ailments (tamanu, website).

2.3.106 Teaseed (*Thea sinensis, T. sasangua*)

These seeds contain 56–70% oil. The oleic-rich oil also contains palmitic and linoleic acid as major fatty acids (Table 2.50). Myristic, stearic, eicosenoic, and docosenoic acids may also be present (Firestone, 1996).

2.3.107 Tobacco (*Nicotiana tobacum*)

Tobacco seeds contain an oil rich in linoleic acid, but with virtually no linolenic acid (Table 2.50). After refining, it can be used for edible purposes or as a nonyellowing drying oil. In one sample of the oil, the major triacylglycerols were LLL (38%), LLO (24%), and LLS (20%) (Pritchard, 1991; Firestone, 1996; Ucciani, 1995a).

2.3.108 Tomato seed (*Lycopersicum esculentum*)

Tomato seed oil is a linoleic-rich vegetable oil with an unusually high level of cholesterol. The fatty acid composition is reported in Table 2.50 (Ucciani, 1995a; Firestone, 1996). Tomatoes themselves are rich in carotenoids and attempts are being made to recover these from tomato waste (Anon., 2004c).

2.3.109 Tung (*Aleurites fordii*)

Tung oil comes mainly from China, thereby explaining its alternative name of China wood oil. It is characterised by the presence of a conjugated triene acid (α-eleostearic, 9*c*11*t*13*t*-18:3, ~69%). The oil dries more quickly than linseed with its nonconjugated triene acid, but oxidised tung oil contains less oxygen (5%) than does oxidised linseed oil (12%). Put another way, tung oil hardens at a lower level of oxygen uptake than linseed oil. This oil is exported mainly from China (30 to 40,000 tonnes) and imported mainly by Japan, South Korea, Taiwan, and the U.S. (Ucciani, 1995a; Firestone, 1996).

2.3.110 Ucuhuba (*Virola surinamensis*)

This tree grows in South America. Its seeds provide one of the few oils rich in myristic acid along with lauric, palmitic, oleic, and linoleic (Table 2.51). These values are reflected in the triacylglycerol composition: MMM 43%, MML 31%, and LMP 10% where L, M, and P represent lauric, myristic, and palmitic acid chains (Ucciani, 1995a; Firestone, 1996).

2.3.111 Vernonia oils

A small number of seed oils contain epoxy acids and sometimes these unusual acids attain high levels. Such oils show a wide range of chemical reactions producing compounds of unusual structure with properties of potential value. The most common acid of this type is vernolic (12,13-epoxyoleic) acid — a monoepoxide of linoleic acid. First identified in *Vernonia anthelmintica* with 72% of vernolic acid, the acid has also been recognised in the seed oils of *V.galamensis* (73 to 78%), *Cephalocroton cordofanus* (62%), *Stokes aster* (65 to 79%), *Euphorbia lagescae* (57 to 62%), *Erlanga tomentosa* (52%), *Crepis aureus* (52 to 54%), and *C. biennis* (68%). With these high levels of vernolic acid, the triacylglycerols in these seed oils are rich in esters with two or three vernolic acid groups. *V. galamensis* seed oil, for example, is reported to contain 50 to 60% of trivernolin and 21 to 28% of glycerol esters of the type V_2X, where V and X represent vernolic and other acyl groups, respectively. Attempts are being made to domesticate V*ernonia galamensis* and *Euphorbia lagescae* (Ucciani, 1995a; Firestone, 1996; Sherringham et al., 2003).

2.3.112 Walnut (*Juglans regia*)

Walnut oil is an unsaturated oil containing both linoleic and linolenic acids (Table 2.50) and is rich in tocopherols (~1500 mg/kg of oil). It is used as a gourmet oil in Japan, France, and other countries. Recent papers report the detailed composition (fatty acids, triacylglycerols sterols, and tocopherols) of oil extracted with hexane and with supercritical carbon dioxide. The two products contain ~300 and 400 ppm of tocopherols, respectively, of which 82% is the β/γ compounds (Ucciani, 1995a; Firestone, 1996; Oliveira et al., 2002; Crowe et al., 2003; Watkins, 2005; Crews et al., 2005b). The FDA has agreed to a qualified health claim on walnuts (Anon., 2004e). The triacylglycerol composition of several walnut cultivars collected between 2001 and 2003 has been reported. Nine groups of triacylglycerol were recognized, of which four generally exceed 10%: LLL (35 to 41%), OLL (14 to

28%), LLLn (12 to 23%), and PLL (7 to 11%). The triacylglycerol composition depends on genetic and environmental factors (Amaral et al., 2004).

2.3.113 Watermelon (*Citrullus vulgaris*)

Watermelon seeds yield an oil rich in linoleic acid (Table 2.50) and in lycopene (Ucciani, 1995a; Firestone, 1996; Anon., 2002).

2.3.114 Wheatgerm (*Triticum aestivum*)

This oil is highly unsaturated with a high level of linoleic and some linolenic acid (Table 2.50). It is valued for its high tocopherol levels (~2500 mg/l of oil) and is reported to lower total and LDL cholesterol levels. α-, β-, γ-, and δ-Tocopherols are present at levels of 1210, 65, 24, and 25 mg/l of oil, in addition to small amounts of tocotrienols (Barnes et al., 1980; Barnes, 1982; Yang et al., 2003a).

References

Abbott, T.P., et al., On-line chemical database for new crop seeds, *J. Am. Oil Chem. Soc.,* **74**, 723–726, 1997: www.ncaur.usda.gov/nc/ncdb/search.html-ssi

Abbott, T.P., Status of lesquerella as an industrial crop, Inform, **8**, 1169–1175, 1997.

Aigbodion, A.I. and Bakare, I.O., Rubber seed oil quality assessment and authentication, *J. Am. Oil Chem. Soc.,* **82**, 465–469, 2005.

Aitzetmüller, A., et al., Seed oils of *Nigella* species and of closely related genera, *Oleagineux Corps gras Lipides,* **4**, 385–388, 1997.

Aitzetmüller, K., et al., A new database for seed oil fatty acids — the database SOFA. *Eur. J. Lipid Sci. Technol.,* **105**, 92–103, 2003: www.bagkf.de/sofa

Aitzetmüller, K., Chilean hazelnut (*Gevuina avellana*) seed oil, *J. Am. Oil Chem. Soc.,* **81**, 721–723, 2004.

Akoh, C.C. and Nwosu, C.V. Fatty acid composition of melon seed oil lipids and phospholipids, *J. Am. Oil Chem. Soc*., **69**, 314–316, 1992.

Alendar, J., Shea butter – a multifunctional ingredient for food and cosmetics, *Lipid Technol.*, **16**, 202–205, 2004.

Amaral, J.S., et al., Triacylglycerol composition of walnut (*Juglans regia* L.) cultivars: characterisation by HPLC-ELSD and chemometrics, *J. Agri. Food Chem.,* **52**,7964–7969, 2004.

Angers, P., et al., Fatty acid variation in seed oil among O*cimum* species, *J. Am. Oil Chem. Soc*., **73**, 393–395, 1996.

Anon., Advisory Committee in Novel Foods and Procedures (UK), Annual Report, pp. 7 and 30–31, 1991.

Anon., Cherry and apricot oils are safe for food use*, Lipid Technol.,* **5***,* 53, 1993.

Anon., Neem: source of insecticide, fungicide, *Inform,* **5**, 713, 1994.

Anon., Olibra confirmed as appetite suppressant, *Lipid Technol. Newsl.,* **4**, 105–106, 1998.

Anon., Hemp oil study finds higher GLA levels, *Inform*, **12**, 1213, 2001.

Anon., Watermelon offers as much lycopene as tomatoes, *Lipid Technol. Newsl.,* **8**, 56–57, 2002.

Anon., Unilever US 6,365,175 and Elizabeth Arden US 6,455,057 and EP 1200058 cited in *Lipid Technol.,* **15**, 118 and 119, 2003a.

Anon., New health claim guidelines published, *Inform*, **14**, 476, 2003b.

Anon., Echium oil, *Lipid Technol. Newsl.,* **9**, 56, 2003c.

Anon., Gevuina provides alternative to macadamia, *Lipid Technol. Newsl.,* **9**, 96, 2003d.

Anon., Good prospects for sea buckthorn in North America*, Lipid Technol.*, **15**, 99–100, 2003e.

Anon., Macadamia nuts positively affect serum cholesterol, *Inform*, **14**, 353–354, 2003f.

Anon., GLA-enriched safflower oil to be produced, *Inform*, **15**, 526, 2004a.

Anon., Shea oil formulations to prevent diarrhoea, *Lipid Technol.*, **16**, 190, 2004b.

Anon., Carotenoids and other food additives made from tomato waste, *Lipid Technol.* **16**, 77, 2004c.

Anon., FDA partially supports health claims for walnuts, *Inform*, **15**, 354, 2004d.

Anon., More Crambe grown in the UK for industrial markets, *Lipid Technol.*, **16**, 75, 2004e.

Anon., Jatropha oil, *Lipid Technol*., **17**, 147, 2005a.

Anon., Allanblackia nut oil, *Inform*, **16**, 618, 2005b.

Anwar, F., et al., Interprovenance variation in the composition of *Moringa oleifera* oilseeds from Pakistan, *J. Am. Oil Chem. Soc.* **82**, 45–51, 2005.

Atta, M.B., Some characteristics of nigella (*Nigella sativa* L.) seeds cultivated in Egypt and its lipid profile, *Food Chem.,* **83**, 63–68, 2003.

Aubert-Mammou, C., et al., Les triacylglycerols de l'huile graines de sisymbre (*Sisymbrium* sp. L., Brassicaceae), *Oleagineaux Corps gras Lipides*, **3**, 65–69, 1996.

Ayerza, R., Oil content and fatty acid composition of chia (*Salva hispanica* L) from five Northwestern locations in Argentina, *J. Am. Oil Chem. Soc.,* **72**, 1079–1081, 1995.

Badifu, G.I.O., Chemical and physical analyses of oils from four species of Cucurbitaceae, *J. Am. Oil Chem. Soc.,* **68***,* 428–432, 1991.

Barnes, P.J., Lipid composition of wheat germ and wheat germ oil, *Fat Sci. Technol.,* **84**, 256–269, 1982.

Barnes, P.J. and Taylor, P.W., The composition of acyl lipids and tocopherols in wheat germ oils from various sources. *J. Sci. Food Chem.,* **31**, 997–1006, 1980.

Bernardo-Gil, M.G., et al., Supercritical fluid extraction and characterisation of oil from hazelnut, *Eur. J. Lipid Sci. Technol.,* **104***,* 402–409, 2002.

Bertoli, C., et al., Characterization of Chilean hazelnut (*Gevuina avellana* Mol) seed oil, *J. Am. Oil Chem. Soc*., **75**, 1037–1040. 1998.

Besbes, S., et al., Date seeds: chemical composition and characteristic profiles of the lipid fraction, *Food Chem.*, **84**, 577–584, 2004.

Bewadt, S. and Aparicio, R., The detection of the adulteration of olive oil with hazelnut oil: a challenge for the chemist, *Inform*, **14***,* 342–343, 2003.

Beveridge, T.H.J., et al., Yield and composition of grape seed oils extracted by supercritical carbon dioxide and pertroleum ether: varietal effects, *J. Agric. Food Chem.,* **53**, 1799–1804, 2005.

Bhardwaj, H.L., et al., Fatty acids and oil content in white lupin seed as affected by production processes, *J. Am. Oil Chem. Soc.,* **81**, 1035–1038, 2004.

Bhattacharyya, D.K., Lesser-known Indian plant sources for fats and oils, *Inform*, **13**, 151–157, 2002.

Birkbeck, J., Health benefits of avocado oil, *Food New Zealand*, April/May, pp. 40–42, 2002.

Birkett, J., Manufacture and use of fats in chocolate, in *Oils and Fats Handbook,* vol. 1*, Vegetable Oils and Fats*, Rossell. B., Ed., Leatherhead Food RA, Leatherhead, U.K., Ch. 1–22, 1999.

Bonjean, A., et al., Que penser de la cameline en 1995? O*leagineux Corps gras Lipides*, **2**, 97–100, 1995.

Bora, P.S., et al., Characterization of the seed oils of some commercial cultivars of melon, *Eur. J. Lipid Sci. Technol.,* **102**, 266–269, 2000.

Bruin, FR2822068 and EP 1213975, cited in *Lipid Technol.,* **15**, 71, 2003.

Budin, J.T., et al., Some compositional properties of camelina, *Camelina sativa* L. Crantz) seed and oils, *J. Am. Oil Chem. Soc.,* **72**, 309–315, 1995.

Budin, J.T., et al., Some compositional properties of seeds and oils of eight *Ameraranthus* species, *J. Am. Oil Chem. Soc.,* **73***,* 475–481, 1996.

Bushman, B.S., et al., Chemical composition of caneberry (*Rubus* spp) seeds and oils and their antioxidant potential, *J. Agric. Food Chem.,* **52**, 7982–7987, 2004.

Campbell, I., An exotic tale, Part I: Illipe — the uncertain crop: www.britanniafood.com/invite, 2002.

Chen, T.C. and Ju, Y-H., Polyunsaturated fatty acid concentrates from borage and linseed oil fatty acids, *J. Am. Oil Chem. Soc.,* **78,** 485–488, 2001.

Cherif, A., et al., Kernel fatty acid and triacylglycerol composition for three almond cultivars during maturation, *J. Am. Oil Chem. Soc.,* **81**, 901–905, 2004.

Christie, W.W., et al., Analysis of seed oils containing cyclopentenyl fatty acids by combined chromatographic procedures, *Lipids*, **24**, 116–120, 1989.

Christopoulou, E., et al., Effectiveness of determination of fatty acids and triglycerides for the detection of adulteration of olive oils with vegetable oils, *Food Chem.*, **84***,* 463–474, 2004.

Clough, P.M., Specialty vegetable oils containing γ-linolenic acid and stearidonic acid in Gunstone, F.D., Ed., *Structured and Modified Lipids,* Marcel Dekker, New York, pp. 75–117, 2001a.

Clough, P., Sources and production of speciality oils containing GLA and stearidonic acid, *Lipid Technol.,* **13***,* 9–12, 2001b.

Colquhoun, D.M., et al., Effects of a macadamia nut enriched diet on serum lipids and lipoproteins compared with a low fat diet, *Food Austral.,* **48**, 531–537, 1996.

Comes, F., et al., Fatty acids and triacylglycerols of cherry seed oil, *J. Am. Oil Chem. Soc.,* **69**, 1224–1227, 1992.

Compton, D.L., et al., Cinamoyl esters of lesquerella and castor oil: novel sunscreen active ingredients, *J. Am. Oil Chem. Soc.,* **81**, 945–951, 2004.

Compton, D.L., Sunscreens based on vegetable oil, *Lipid Technol.,* **17**, 276–279, 2005.

Concha. J., et al., Enzymatic pre-treatment on rose-hip oil extraction: hydrolysis and pressing conditions*. J. Am. Oil Chem. Soc.,* **81**, 549–552, 2004.

Connock, www.connock.co.uk/vegetable_oils.htm and www.creative-compounds.co.uk

Crews, C., et al, Study of the main constituernts of some authentic hazelnut oils, *J. Agric. Food Chem.,* **53**, 4843–4852, 2005a.

Crews, C., et al, Study of the main constituents of some authentic walnut oils, *J. Agric. Food Chem.,* **53**, 4853–4860, 2005b.

Crowe, T.D. and White, P.J., Oxidative stability of walnut oils extracted with supercritical carbon dioxide, *J. Am. Oil Chem. Soc.,* **80***,* 575–578, 2003.

Croy, C., Macadamia nut oil, *Inform*, **5**, 970–971, 1994.

CTFA, Cosmetic, Toiletries, and Fragrances Association, *CTFA International Cosmetic Ingredient Dictionary and Handbook*, 9th ed., Micelle Press, Dorset, England, 2002; also: www.ctfa.org

De, B.K., et al., Bio- and auto-catalytic esterification of high acid mowrah fat and palm kernel oil, *Eur. J. Lipid Sci. Technol.,* **104**, 167–173, 2002.

Dutta, P.C., et al., Variation in lipid composition of niger seed (*Guizotia abyssinica*) samples collected from different regions in Ethiopia, *J. Am. Oil Chem. Soc.*, **71**, 839–845, 1994.

Dyszel, S.M. and Pettit, B.C., Determination of the country of origin of pistachio nuts by DSC and HPLC, *J. Am. Oil Chem. Soc.,* **67**, 947–951, 1990.

Eckey, E.W., *Vegetable Fats and Oils.* Reinhold Publishing Corporation, New York, 1954.

Eskin, N.A.M., Authentication of evening primrose, borage, and fish oils, in Jee. M., Ed., *Oils and Fats Authentication*, Blackwell Publishing, Oxford, 95–114, 2002.

Eyres, L., Sherpa, N., and Hendriks, G., Avocado oil: a new edible oil from Australasia, *Lipid Technol.*, **23***,* 84–88, 2001.

Firestone, D., Ed., *Physical and Chemical Characteristics of Oils, Fats, and Waxes,* AOCS Press, Champaign IL, 1997; republished as a book and a disc, 1999.

Flider, F.J., Salicornia: an oilseed for saltwater agriculture, *Inform*, **15**, 78–79, 2004.

Flider, F.J., GLA: uses and new sources, *Inform*, **16**, 279–282, 2005.

Foglia, T.A. et al. Selectivity of lipases: isolation of fatty acids from castor, coriander, meadowfoam oils, *Eur. J. Lipid Sci. Technol.,* **102***,* 612–617, 2000.

Fruhwirth, G.O., Wennzl, T., El-Toukhy, R., Wagner, F.S., and Hermetter, A., Flourescence screening of antioxidant capacity in pumpkin seed oil and other natural oils, *Eur. J. Lipid Sci. Technol.*, **105**, 266–274, 2003.

Gordon, M.H., Covell, C., and Kirsch, N., Detection of pressed hazelnut oil in admixtures with virgin olive oil by analysis of polar components, *J. Am. Oil Chem. Soc.,* **78**, 621–624, 2001.

Guillen, M.D., Ruiz A., Cabo. N, Chirinos, R., and Pascual, G., Characterisation of sachi inchi (*Plukenetia volubilis* L) oil by FTIR spectroscopy and ^{1}H NMR. Comparison with linseed oil, *J. Am. Oil Chem. Soc.,* **80**, 755–762, 2003.

Gunjiker, J., Mango kernel fat — an exotic fat, *Inform*, **16**, 232–234, 2005.

Gunstone, F.D., Gamma-linolenic acid — occurrence and physical and chemical properties, *Prog. Lipid Res.,* **31**, 145–161, 1992.

Gunstone, F.D., What else beside commodity oils and fats? *Fett/Lipid,* **101**, 124–131, 1999.

Gunstone, F.D., Oilseed crops with modified fatty acid composition, *J. Oleo Sci.*, **50**, 269–279, 2000.

Gunstone, F.D., Minor oils, specialty oils, and super-refined oils, in Gunstone, F.D., Ed., *Structured and Modified Lipids,* Marcel Dekker, New York, 185–208, 2001.

Gunstone, F.D., Ed., *Vegetable oils in Food Technology: Composition, Properties, and Uses*, Blackwell Publishing, Oxford, England, 2002.

Gunstone, F.D., Ed., *Lipids for Functional Foods and Nutraceuticals*, The Oily Press, Bridgwater, England, 2003a.

Gunstone, F.D., γ-Linolenic acid (GLA), in Gunstone, F.D., Ed., *Lipids for Functional Foods and Nutraceuticals,* The Oily Press, Bridgwater, England, 272–279, 2003b.

Hagemann, J.M., et al., Seed oils of Labiatae, *Lipids*, **2**, 371–380, 1967.

Hammama, A.A., et al., Phytosterols, triterpene alcohols, and phospholids in seed oil from white lupin, *J. Am. Oil Chem. Soc.,* **81**, 1039–1044, 2004.

Hayes, D.G., et al., Urea-based fractionation of seed oil samples containing fatty acids and triacylglycerols of polyunsaturated and hydroxy fatty acids, *J. Am. Oil Chem. Soc.,* **77**, 207–213, 2000.

Hebard, A.B., Growing high-erucic rapeseed oil as an example of identity preservation, *Lipid Technol.,* **14***,* 53–55, 2002.

Hebard, A., *Camelina sativa* — a pleasurable experience or another false hope, *Lipid Technol.*, **10**, 81–83, 1998.

hemptrade: www.hemptrade.ca

Herslof, B.G., Glycolipids herald a new era for food and drug products, *Lipid Technol.* **12**, 125–128, 2000.

Hilali, M., et al, Influence of origin and extractive method on argan oil physico-chemical characteristics and composition, *J. Agric. Food Chem.,* **53**, 2081–2087, 2005.

Hilditch, T.P. and Williams, P.N., *The Chemical Constitution of Natural Fats,* 4th ed., Chapman and Hall, London, 336, 1964.

Holmback, J.M., et al., Characterisation of *N*-acylphosphatidylethanolamine and acylphosphatidylglycerol in oats, *Lipids*, **36**, 153–165, 2001.

Horrobin, D.F., Nutritional and medical importance of gamma-linolenic acid, *Prog. Lipid Res.,* **31**, 163–194, 1992.

Huang, Y-S. and Ziboh, V A., *γ-Linolenic Acid: Recent Advances in Biotechnology and Clinical Applications,* AOCS Press, Champaign, IL, 2001.

Huang. Y-S. and Mills, D.E., *γ-Linolenic Acid,* AOCS Press, Champaign, IL, 1996.

Ienica: www.ienica.net

Illingworth, C., Sowing seeds for better hemp, *Inform,* **15**, 339–340, 2004.

Imai, H., et al., Chemical characterisation of glycerolipids and cerebrosides in a halophytic plant, *Salicornia europaea L.*, *J. Oleo Sci.*, **53**, 337–341, 2004.

Isbell, T.A., Development of meadowfoam as an industrial crop through novel fatty acid derivatives, *Lipid Technol.,* **9**, 140–144, 1997.

Isbell, T.A., Novel chemistry of Δ 5 fatty acids, in *Lipid Synthesis and Manufacture,* Gunstone, F.D., Ed., Sheffield Academic Press, Sheffield, U.K., 401–421, 1998.

Isbell, T.A. and Cermak, C.S., Synthesis of δ-eicosanolactone and δ-docosanolactone from meadowfoam oil, *J. Am. Oil Chem. Soc.,* **78***,* 527–531, 2001.

Isbell, T.A. and Cermak, S C., Synthesis of triglyceride estolides from lesquerella and castor oils, *J. Am. Oil Chem. Soc.*, **79**, 1227–1233, 2002.

Isbell, T.A., Current progress in the development of *Cuphea, Lipid Technol.*, **14***,* 77–80, 2002.

Jahaniaval, F., et al., Fatty acid and triacylglycerol compositions of seed oils of five *amararanthus* accessions and their comparison to other oils*, J. Am. Oil Chem. Soc.*, **77**, 847–852, 2000.

Janssens, R.J., *Calendula* oil: seed classification, oil processing and quality aspects, *Lipid Technol.*, **12**, 53–58, 2000a.

Janssens, R.J. and Vernooij, W.P., *Calendula officinalis*: a new source for pharmaceutical, oleochemical, and functional compounds, *Inform,* **12**, 468–477, 2000b.

Jeffrey, B.S.J. and Padley, B.F., Chinese vegetable tallow — characterization and contamination by stillingia oil, *J. Am. Oil Chem. Soc.,* **68**, 123–127, 1991.

Jeyarani, Y. and Yella Reddy, S., Heat-resistant cocoa butter extenders from mahua (*Madhuca latifolia*) and kokum (*Garcinia indica*) fats, *J. Am. Oil Chem. Soc.*, **76***,* 1431–1436, 1999.

Jolliff, G.D., Development and production of meadowfoam (*Limnanthes alba*), in Pryde, E.H., et al., Eds., *New Sources of Fats and Oils*, AOCS Press, Champaign, IL, 269–285, 1981.

Ju, Y-H. and Chen, T-C., High purity γ-linolenic acid from borage oil fatty acids, *J. Am. Oil Chem. Soc.,* **79**, 29–32, 2002.

Kallio, H., Stearidonic acid, in Gunstone, F.D., Ed., *Lipids for Functional Foods and Nutraceuticals*, The Oily Press, Bridgwater, England, 280–284, 2003.

Kallio, H., et al., Triacylglycerols, glycerophospholipids, tocopherols and tocotrienols in berries and seeds of two subspecies (ssp *sinensis* and ssp. *mongolica)* of sea buckthorn (*Hippophae rhamnoides*), *J. Agric. Food Chem.*, **50**, 3004–3009, 2002.

Kallio, H.L., et al., Analysis of regioisomers of triacylglycerols of northern currant seed oil by tandem mass spectrometry, *Eur. J. Lipid Sci. Technol.,* **107**, 101–106, 2005.

Kamel, B.S. and Kakuda, Y., Characterization of the seed oil and meal from apricot, cherry, nectarine, peach and plum, *J. Am. Oil Chem. Soc.*, **69**, 492–494, 1992.

Kamel, B.S. and Kakuda, Y., Eds., *Technological Advances in Improved and Alternative Sources of Lipids,* Blackie Academic and Professional, London, 1994.

Kim, I-H., et al., Lipase catalysed acidolysis of perilla oil with caprylic acid to produce structured lipids, *J. Am. Oil Chem. Soc.,* **79**, 363–367, 2002.

Krist, S., et al, Analysis of volatile compounds and triglycerides of seed oils extracted from different poppy varieties (*Papaver somniferum* L), *J. Agric. Food Chem.*, **53**, 8310–8316, 2005.

Lalas, S. and Tsaknis, J., Extraction and identification of natural antioxidant from the seeds of the *Moringa oleifera* tree variety of Malawi, *J. Am. Oil Chem. Soc.,* **79***,* 677–683, 2002.

Lalas, S., et al., Characterisation of *Moringa stenopetala* seed oil variety "Marigat" from island Kokwa, *Eur. J. Lipid Sci. Technol.,* **105***,* 23–31, 2003.

Lehmann, J.W., et al., Vitamin E isomers in grain ameraranthus *Amaranthus* spp., *Lipids,* **29***¸* 177–181, 1994.

Leon-Camacho, et al., Acorn (*Quercus* spp) fruit lipids: saponifiable and unsaponifiable fractions: a detailed study, *J. Am. Oil Chem. Soc.,* **81**. 447–453, 2004.

Leonard, C., Sources and commercial applications of high-erucic vegetable oils, *Lipid Technol.*, **6**, 79–83, 1994.

Leonard, E.C., Camelina oil: α-linolenic source, *Inform*, **9**, 830–838, 1998.

Lessman, K.J. and Anderson, W.P., Crambe, in Pryde, E.H., et al., Eds., *New Sources of Fats and Oils,* AOCS Press, Champaign, IL, 223–246, 1981.

Li, T.S.C. and Wang, L.C.H., Physiological components and health effects of ginseng, *Echinacea* and sea buckthorn, in Mazza, G., Ed., *Functional Foods — Biochemical and Processing Aspects,* Technomic, Lancaster, PA, 329–356, 1998.

Lipp, M. and Anklam, E., Review of cocoa butter and alternative fats for use in chocolate: Part A. Compositional data, Part B. Analytical approaches for identification and determination, *Food Chem.,* **62**, 73–97 and 99–108, 1998.

Liu, J-W., et al., Characterization of oil exhibiting high γ-linolenic acid from a genetically transformed canola strain, *J. Am. Oil Chem. Soc.,* **78**, 489–493, 2001.

Longvah, T. and Deosthale, Y.G., Chemical and nutritional studies on hanshi (*Perilla frutescens*), a traditional oilseed from northeast India, *J. Am. Oil Chem. Soc.,* **68**, 781–784, 1991.

Lopes, I.M.G. and Bernardo-Gil, M.G., Characterisation of acorn oils by hexane and by supercritical carbon dioxide, *Eur. J. Lipid Sci. Technol.,* **107**, 12–19, 2005.

Lovett, P.N., Shea butter industry expanding in West Africa, *Inform,* **16**, 273–275, 2005.

Lozano, Y.F., et al., Unsaponifiable matter, total sterol and tocopherol contents of avocado oil varieties, *J. Am. Oil Chem. Soc.,* **70**, 561–565, 1993.

Lu, Z., et al., *Salicornia bigelovii*: an overview, *Inform,* **11**, 418–423, 2000.

Macfarlane. N. and Harris, R.V., Macadamia nuts as an edible oil source, in Pryde, E.H., et al., Eds., *New Sources of Fats and Oils*, AOCS Press, Champaign, IL, 103–108, 1981.

Maheshwari, B. and Reddy, S.Y., Application of kokum (*Garcinia indica*) fat as cocoa butter improver on chocolate, *J. Sci. Food Agric*., **85**, 135–140, 2005.

Maranz, S. and Wiseman, Z., Influence of climate on the tocopherol content of shea butter, *J. Agric. Food Chem.,* **52**, 2934–2937, 2004.

Marini, F., et al., Characterization of the lipid fraction of niger seeds (*Guizotia abyssinica* Cass) from different regions of Ethiopia and India and chemometric authentication of their geographical origin, *Eur. J. Lipid Sci. Technol.*, **105**, 697–704, 2003.

Maskan, M. and Karatas, S., Fatty acid oxidation of pistachio nuts stored under various atmospheric conditions and different temperatures, *J. Sci. Food Agric.,* **77**, 334–340, 1998.

Mazza, G., Ed., *Functional Foods — Biochemical and Processing Aspects,* Technomic Publishing Company, Lancaster, PA, 1998.

Melton, L., Slimming pills for insects, *Chem. Brit*., **39**, 24–26, 2003 (Dec).

Miller, R.W., et al., Search for new seed oils XV, oils of boraginaceae, *Lipids*, **3**, 43–45, 1968.

Moreau, R.A., et al., Pressurised liquid extraction of polar and nonpolar lipids in corn and oats with hexane, methylene chloride, isopropanol, and ethanol, *J. Am. Oil Chem. Soc.,* **80**, 1063–1067, 2003.

Moret, S., et al., Processing effects on the polyaromatic hydrocarbon content of grapeseed oil, *J. Am. Oil Chem. Soc.,* **77**, 1289–1292, 2000.

Moringa:www.moringaexports.com

Murkovic, M., et al., Changes in chemical composition of pumpkin seeds during the roasting process for production of pumpkin seed oil (Part 1: nonvolatile compounds), *Food Chem.,* **84**, 359–365, 2004.

Nyanzi, S.A., et al., A comparative study of fatty acid profiles of *Passiflora* seed oils from Uganda, *J. Am. Oil Chem. Soc*., **82**, 41–44, 2005.

Oliveira, R., et al., Characterisation and supercritical carbon dioxide extraction of walnut oil, *J. Am. Oil Chem. Soc.,* **79**, 225–230, 2002.

Omara-Alwala, T.R., et al., Omega-three fatty acids in purslane (*Portulaca oleracea*), *J. Am. Oil Chem. Soc.,* **68**, 198–199, 1991.

Osman, S.M. Mango fat, in *New Sources of Fats and Oils*, Pryde, E.H., et al., Eds., AOCS Press, Champaign, IL, 129-140, 1981.

Ozgul-Yucel, S., Determination of conjugated linolenic acid content of selected oils grown in Turkey, *J. Am. Oil Chem. Soc.,* **82**, 893–897, 2005.

Padley, F.B., Major vegetable fats in Gunstone F.D., et al., Eds., *The Lipid Handbook,* 2nd ed., Chapman and Hall, London, pp. 53–146, 1994.

Pandey, V., et al., *Cuphea* a rich source of medium chain triglycerides: Fatty acid composition and oil diversity, in *Cuphea procumbens. Eur. J. Lipid Sci. Technol*., **102**, 463–466, 2000.

Peterson, D.M., Oat lipids: composition, separation, and applications, *Lipid Technol.*, **14**, 56–59, 2002.

PhytoTrade Africa: www.sanprota.com/products/baobob

Prats-Moya, M.S., et al., A chemometric study of genotypic variation in triacylglycerol composition among selected almond cultivars, *J. Am. Oil Chem. Soc*., **76**, 267–272, 1999.

Pritchard, J.L.R., Analysis and properties of oilseeds, in *Analysis of Oilseeds, Fats, and Fatty Foods*, Rossell, J.B., Pritchard, J.L.R., Eds., Elsevier Applied Science, London, 39–102, 1991.

Przybylski, R. and Fitzpatrick, K.C., Does hempseed need to be a forbidden crop? *Inform*, **13**, 489–491, 2002.

Przybylski, R., Hempseed — revival of a forgotten oilseed crop, *Lipid Technol.,* **18**, 58–62, 2006.

Ramadan, M.F., et al., Radical scavenging activity of black cumin (*Nigella sativa* L.), coriander (*Coriandrum sativum* L.), and niger (*Guizotia abyssinica* Cass) crude seed oils and fractions, *J. Agric. Food Chem.,* **51**, 6961–6969, 2003.

Ramadan, M.F. and Moersel, J-T., Mowrah butter: nature's novel fat, *Inform*, **17**, 124–126, 2006.

Rao, Y.R., et al., Safety evaluation of ambrette (*Abelmoschus moschatus* Linn) seed oil, *J. Am. Oil Chem. Soc.,* **82**, 749–752, 2005.

Rauf, A., et al., Triacylglycerols — analysis of three medicinal seed oils using pancreatic lipase, *J. Oleo Sci.,* **53**, 531–536, 2004.

Robbelen, G., et al., Eds., *Oil Crops of the World — Their Breeding and Utilization,* McGraw-Hill, New York, 1989.

Rui Alves, M., et al., Contribution of FA profile obtained by high-resolution GC/chemometric techniques to the authenticity of green and roasted coffee varieties, *J. Am. Oil Chem. Soc*., **80**, 511–517, 2003.

Ruiz del Castillo, M.L. and Herraiz, M. Ultrasonically assisted solid-phase extraction and GC analysis of filbertone in hazelnut oil, *J. Am. Oil Chem. Soc.,* **80**, 307–310, 2003.

Ruiz del Castillo, M.L., et al., Off-line coupling of high-performance liquid chromatography and ^{1}H nuclear magnetic resonance for the identification of filbertone in hazelnut oil, *J. Am. Oil Chem. Soc.,* **78**, 1261–1265, 2001.

Sargent, J. and Coupland, K., Applications of specialised oils in the nutritional therapy of demyelinating disease, *Lipid Technol.*, **6**, 10–14, 1994.

Savage, G.P., et al., Lipid composition and oxidative stability of oils in hazelnuts (*Corylus avellana* L.) grown in New Zealand, *J. Am. Oil Chem. Soc.,* **74**, 755–759, 1997.

Sherringham, J.A., et al., New chemical feedstocks from unsaturated oils, *Lipid Technol.,* **12**, 129–132, 2003.

Shin, H-S. and Kim S-W., Lipid composition of perilla seed, *J. Am. Oil Chem. Soc*., **71**, 619–622, 1994.

Shukla, V.K.S. Confectionery fats, in *Developments in Oils and Fats*, Hamilton, R.J., Ed., London: Blackie, 66–94, 1995.

Shukla, V.K.S., et al., Camelina oil and its unusual cholesterol content, *J. Am. Oil Chem. Soc*., **79**, 965–969, 2002.

Siegmund, B. and Murkovic, M., Changes in chemical composition of pumpkin seeds during the roasting process for production of pumpkin seed oil (Part 2: volatile compounds), *Food Chem.,* **84**, 367–374. 2004.

Singh, P.S., et al., Variation of major fatty acids in F_8 generation of opium poppy (*Papaver somniferum* × *Papaver setigerum*) genotypes, *J. Sci. Food Agric.,* **76**, 168–172, 1998.

Smith, J.R., *Safflower*. AOCS Press, Champaign, IL, 1996.

Statfold oils: www.statfold-oils.co.uk/specifications

Steinke, G., et al., Lipase catalysed alcoholysis of crambe oil and camelina oil for the preparation of long-chain esters, *J. Am. Oil Chem. Soc.,* **77***,* 361–366, 2000a.

Steinke, G., et al., Alkali-catalysed alcoholysis of crambe oil and camelina oil for the preparation of long-chain esters, *J. Am. Oil Chem. Soc.,* **77***,* 367–371, 2000b.

Stewart, I.M. and Timms, R.E., Fats for chocolate and sugar confectionery, in Rajah, K.K., Ed., *Fats in Food Technology,* Sheffield Academic Press, Sheffield, U.K., 159–191, 2002.

Sun, H., et al., Fractionation of squalene from Ameraranth seed oil, *J. Am. Oil Chem. Soc.,* **74**, 413–418, 1997.

Swisher, H.O., Avocado oil — from food use to skin care, *J. Am. Oil Chem. Soc.,* **65***,* 1704–1706, 1993.

Takruri, H.R.H. and Dameh, M.A.F., Study of the nutritional value of black cumin seeds (*Nigella sativa* L), *J. Sci. Food Agric.,* **76,** 404–410, 1998.

Talbot, G., Manufacture and use of fats in non-chocolate confectionery. In *Oils and Fats Handbook, Vol. 1, Vegetable Oils and Fats*, Rossell. B., Ed., Leatherhead Food RA, Leatherhead, U.K., 1999, Co 1–12, 1999.

Tamanu**:** http://tamanu.biz http://tamanu.biz

Temple-Heald, C., High-erucic oil: its production and uses, in Gunstone, F.D., Ed., *Rapeseed and Canola Oil,* Blackwell Publishing, Oxford, 111–130, 2004.

Timms, R E., *Confectionery Fats Handbook — Properties, Production and Application,* The Oily Press, Bridgwater, England, 2003.

Tobaras, L., et al., Effect of the extraction and bleaching processes on jojoba (*Simmondsia chinensis*) wax quality, *Eur. J. Lipid Sci. Technol.* **105**, 749–753, 2003.

Toschi, T.G., et al., A study on cashew nut oil composition, *J. Am. Oil Chem. Soc*., **70**, 1017–1020, 1993.

Traitler, H., et al., Characterisation of γ-linolenic acid in *Ribes* seed, *Lipids*, **19**, 923–928, 1984.

Tuter, M., et al., Partial purification of *Nigella sativa* L. seed lipase and its application in hydrolytic reactions. Enrichment of γ-linolenic acid from borage oil, *J. Am. Oil Chem. Soc.,* **80**, 237–241, 2003.

Ucciani, E., *Nouveau Dictionnaire des Huiles Vegetales*, Tec and Doc, New York, 1995a.

Ucciani, E. Sources potentielles d'acide gamma-linolenique; un review. *Oleagineaux Corps gras Lipides*, **2**, 319–322, 1995b.

Ustun, G., et al., Investigation of the technological properties of *Nigella sativa* (black cumin) seed oil, *J. Am. Oil Chem. Soc.,* **67**, 958–960, 1990.

Vasconcellos, J.A., et al., The buffalo gourd, *Cucurbita foetidissima* HBK, as a source of edible oil, in Pryde, E.H., et al., Eds., *New Sources of Fats and Oils,* AOCS Press, Champaign, IL, 55–68, 1981.

Wagner, K-H., et al., Effects of seed damage on the oxidative stability of poppy seed oil, *Eur. J. Lipid Sci. Technol.,* **105**, 219–224, 2003.

Wainwright, O.E., et al., Effects of high γ-linolenic acid canola oil compared with borage oil on reproduction, growth, and brain and behavioural development in mice, *Lipids*, **38**, 171–178, 2003.

Watkins, C., The world of nuts, *Inform,* **16**, 200–203, 2005.

Werman, M.J. and Neeman, I., Avocado oil production and chemical characteristics, *J. Am. Oil Chem. Soc.,* **64**, 229–232, 1987.

Wisniak. J., Ed., *The Chemistry and Technology of Jojoba Oil*, AOCS Press, Champaign, IL, 1987.

Wolff, R.L. and Bayard, C.C., Fatty acid composition of some pine seed oils, *J. Am. Oil Chem. Soc.,* **72**, 1043–1046, 1995.

Wolff, R.L., New tools to explore lipid metabolism, *Inform*, **8**, 116–119, 1997.

Wolff, R.L., et al., Fatty acids of the seeds from pine species of the *Ponderosa-Banksiana* and *Halepensis* sections. The peculiar taxonomic position of *Pinus pinaster*, *J. Am. Oil Chem. Soc.,* **75**, 45–50, 1998.

Wretensjo, I. and Karlberg, B., Characterization of sterols in refined borage oil by GC-MS. *J. Am. Oil Chem. Soc.***, 79***,* 1069–1074, 2002.

Wretensjo, I. and Karlberg, B., Pyrrolizidine alkaloid content in crude and processed borage oil from different processing stages. *J. Am. Oil Chem. Soc.,* **80**, 963–970, 2003.

Wu, V.W. and Hojilla-Evangelista, M.P., *Lesquerella fendleri* protein fractionation and characterisation, *J. Am. Oil Chem. Soc.,* **82**, 53–56, 2005.

Yaghmur, et al., Argon oil-in-water emulsions; preparation and stabilization, *J. Am. Oil Chem. Soc.,* **76**, 15–18, 1999.

Yang, B., Natural vitamin E: activities and sources, *Lipid Technol.,* **15***,* 125–130, 2003a.

Yang, B., Palmitoleic acid, in *Lipids for Functional Foods and Nutraceuticals,* Gunstone, F.D., Ed., The Oily Press Bridgwater, England, 266–272, 2003b.

Yang, B., et al., Oils containing oleic, palmitoleic, γ-linolenic and stearidonic acids, in Gunstone, F.D., Ed., *Lipids for Functional Foods and Nutraceuticals,* Oily Press, Bridgwater, U.K., 263–290, 2003.

Yang, B. and Kallio., H. Fatty acid composition of lipids in sea buckthorn (*Hippophae* rhamnoides L.) berries of different origin, *J. Agric. Food Chem*., **49**, 1939–1947, 2001.

Yang, B., et al., Phytosterols in sea buckthorn (*Hippophae* rhamnoides L.) berries: identification and effects of different origins and harvesting times, *J. Agric. Food Chem*., **49***,* 5620–5629, 2001.

Yella Reddy, S. and Jeyarani, T., Trans-free bakery shortenings from mango kernel and mahua fats by fractionation and blending, *J. Am. Oil Chem. Soc.,* **78**, 635–640, 2001.

Yildiz, M., et al., Oil composition of pistachio nuts (*Pistachio vera* L) from Turkey, *Fett/Lipid,* **100**, 84–86, 1998.

Yoshida, H., et al., Lipid classes, fatty acid composition and triacylglycerol molecular species in the kernels of pumpkin (*Cucurbita* spp) seeds, *J. Sci. Food Agric.,* **84**, 158–163, 2004.

Yoshida, H., et al., Lipid classes, fatty acid composition and triacylglycerol molecular species in the kernels of kidney beans (*Phaseolus vulgaris*, L.), *Eur. J. Lipid Sci. Technol*., **107**, 307–315, 2005.

Yousfi, M., et al., Triacylglycerol composition of oil from *Pistacia atlantica* fruit growing in Algeria, *J. Am. Oil Chem. Soc.*. **82**, 93–96, 2005.

Yu Wenli, et al., Comparison of two kinds of pumpkin seed oils obtained by supercritical CO_2 extraction, *Eur. J. Lipid Sci. Technol.* **106**, 355–358, 2004.

Zabaras, D. and Gordon, M.H., Detection of pressed hazelnut oil in virgin olive oil by analysis of polar components: improvement and validation of the method. *Food Chem.*, **84**, 475–483, 2004.

Zadernowski, R. et al., Tocopherols in sea buckthorn (*Hippophae rhamnoides* L) berry oil, *J. Am. Oil Chem. Soc.,* **80***,* 55–58, 2003.

Zadernowski, R. et al., Composition of phenolic acids in sea buckthorn (*Hippophae rhamnoides* L) berries, *J. Am. Oil Chem. Soc.,* **82***,* 175–179, 2005.

Zeitoun, M.A.M. and Neff, W.E., Fatty acid, triacylglycerol, tocopherol, sterol phospholipid composition and oxidative stability of Egyptian *Nigella sativa* seed oil, *Oleagineux Corps gras Lipides,* **2**, 245–248, 1995.

Zhou, M., et al., Oat lipids — a review, *J. Am. Oil Chem. Soc.,* **76**, 159–169, 1999.

Zlatanov. M. and Janakieva, I., Phospholipid composition of some fruit-stone oils of Rosaceae species, *Fett/Lipid,* **100**, 312–315, 1998.

Zubr, J., Oil-seed crop: *Camelina sativa. Ind. Crops Prod*., **6***,* 113–119, 1997.

2.4 Milk fats, animal depot fats, and fish oils

This section of Chapter 2 is devoted to animal fats of commercial and nutritional importance as well as covering butter (milk fat), lard from pigs, tallow (mainly from cattle), chicken fat, and fish oils. Apart from some comments on less common milk fats, there is no discussion of fats from animals in general. Aspects of this topic have been reviewed by Haas (2005), Ackman (2005), Bimbo (2005), Hettinga (2005), Hammond (2006), Scheeder (2006a and b), Hjaltason and Haraldsson (2006a and b), and Haraldsson and Hjaltasan (2006). Milk lipids are also discussed in Section 2.7.

2.4.1 Milk fats and butter

2.4.1.1 Introduction

General information on milk fat is available in books authored or edited by Jensen (1995 and 2000) and by Rossell (2003) and in a chapter by Hettinga (2005). More detailed sources are cited throughout this section.

Cow milk is a complex biological fluid secreted in the udder. Its main constituent is water, but it also contains fat, sugar, salt, enzymes, and vitamins. Milk yield is usually 10 to 20 litres/day, though higher volumes have been recorded under favourable conditions. The quantity and quality of the fat depend on many factors including, among others, health and age of the animal, time period since previous milking, stage of lactation, and diet. Typically, cow milk contains water (88%), fat (3.8%), protein (3.3%, mainly casein and whey proteins), and carbohydrate (4.7%, mainly lactose).

Bovine milk lipids are chiefly triacylglycerols (95 to 98%) along with diacylglycerols (1.3 to 1.6%), monoacylglycerols (trace), free acids (0.1 to 0.4%), phospholipids (0.8 to 1.0%), sterols (0.2 to 0.4%), and other minor components.

Dietary fat ingested by ruminants is subject to extensive biohydrogenation by microorganisms present in the rumen. As a consequence, most of the dietary PUFA — whether from grass or from concentrates — is converted to saturated or monounsaturated acids and the latter are predominantly 18:1 acids with *trans* unsaturation. This has a consequence for the nutritional value of milk and butter, and reference will be made later to attempts to overcome this loss of PUFA.

Cow milk fat is consumed mainly as milk, butter, cheese, or other dairy products and is an important source of dietary lipids. Milk fat has a complex fatty acid composition with high levels of short and medium chain acids and with many uncommon fatty acids present at low levels, including those with *trans* unsaturation. Attempts to extend the usefulness of butter fat by fractionation and to find alternative uses for the fat or its fractions are hampered by the high cost of the starting material compared

with that of vegetable oils and by legislation that strictly defines the term butter.

2.4.1.2 Major fatty acids

Cow milk fat has been studied extensively, and over 400 different fatty acids have been identified (Jensen, 1995 and 2000). However, fatty acid composition is discussed here in terms of only 20 or fewer components, though some information on the chemical nature of the remainder will be given. These acids have 4 to 26 carbon atoms and this wide range of chain length and the significant quantities of short-chain acids have the consequence that results expressed as % mol differ considerably from those cited as % wt. This is illustrated in the figures in Table 2.60 showing that the $C_4 - C_{10}$ acids together are 10.4% by weight, but 20.5% on a molar basis. The level of butyric acid at around 4% by weight may be considered to be small, but it should be recognised that this is equivalent to about 8.5% on a molar basis and since this acid is likely to occur only once in any triacylglycerol molecule (at the *sn*-3 position), a quarter of all the triacylglycerol molecules in butter contain butyric acid.

Some typical data for milk and butter cited by Jensen (1995) are presented in adapted form in Table 2.61. The major acids (86.6% wt) are the C_4–C_{12} short and medium chain acids (13.8%), myristic acid (10.6%), palmitic acid (28.2%), stearic acid (12.6%), and oleic acid (21.4%). This same report indicates the presence of straight-chain saturated acids (65.8%), branched-chain saturated acids (2.6%), monounsaturated acids (27.4%, of which about 3% are *trans* acids), and polyunsaturated acids (4.1%). Precht (1990) has provided a more detailed analysis of 83 different milk samples covering 45 constituent fatty acids. This complexity of fatty acid composition has been a challenge to lipid analysts, and over the years improved methods have been developed giving more detailed and correct results. As a consequence, many of the earlier results are incomplete and erroneous.

2.4.1.3 Minor fatty acids

In contrast to most other fats, milk fats contain saturated acids of chain length between C_4 and C_{26} with significant levels of those between 4:0 and 18:0. In addition, there are acids with an odd number of carbon atoms, such as 15, 17, and 19 including straight and branched-chain members (iso and anteiso acids).

TABLE 2.60 Thirteen major fatty acids in butterfat presented as % wt and % mol

Acid	% wt	% mol	Acid	% wt	% mol
4:0	3.7	9.6	16:0	27.4	24.8
6:0	2.4	4.8	16:1	1.6	1.5
8:0	1.4	2.2	18:0	13.9	11.4
10:0	2.9	3.9	18:1	28.0	23.0
12:0	3.2	3.7	18:2	2.0	1.7
14:0	11.2	11.4	18:3	1.0	0.8
14:1	1.0	1.1			

Source: Adapted from Rossell, B., (Ed.) *Oils and Fats Volume 3 Dairy Fats*, Leatherhead Food International, Leatherhead, England, 2003.

TABLE 2.61 Eighteen major fatty acids in bovine milk fat and in butter (%wt)[a]

Acid	Milk	Butter	Acid	Milk	Butter
4:0	4.5	5.3	14:1	0.9	0.9
6:0	2.3	2.8	16:1 n-7	1.8	1.4
8:0	1.3	1.6	18:1 c	21.4	20.8
10:0	2.7	3.1	18:1 t	1.7	–
12:0	3.0	3.4	20:1	0.6	0.3
14:0	10.6	10.8	18:2 n-6	2.9	2.0
15:0i	0.7	0.3			
15:0	1.0	1.0	Saturated	69.1	69.0
16:0	28.2	28.1	Monounsaturated	25.5	22.5
17:0i	0.7	0.5	Polyunsaturated	2.9	2.0
17:0	0.6	0.6	Not included	2.5	6.5
18:0	12.6	10.6			

[a] See also the section on minor acids. These are average values and individual samples show considerable variation depending on diet (see Table 2.62) and on other factors.
i = iso, c = *cis*, t = *trans*

Source: Adapted from Jensen, R.G., in *Fatty Acids in Foods and Their Health* Implication, 2nd ed., Ching Kuang Chow (Ed.) Marcel Dekker, New York, 2000, 109–123.

The monounsaturated acids are mainly, but not entirely, C_{18} compounds. These are complex mixtures of *cis* and *trans* acids with unsaturated centres in many different positions. The major *cis* isomer is oleic (Δ9*c*), *trans* isomers range from Δ6 to Δ16 with the Δ11 isomer (vaccenic) predominating and result from biohydrogenation of PUFA in the rumen. The total content of 18:1 acids is usually 19 to 23% with the level of *trans* acids being around 2.5 to 4%.

According to Jensen (1995 and 2000) the total content of eleven PUFA is about 4%. These are mainly linoleic (~2.4%) and linolenic acid (~1.1%). ARA (0.14%), EPA (0.09%), and DHA (0.01%) are each present at the very low levels indicated (work by Iverson and Shepherd, 1986; cited by Jensen, 2000).

Hydroxy and keto acids are also present in milk fat. The former are precursors of γ- and δ-lactones formed through intramolecular esterification of appropriate hydroxy acids. Methyl ketones also present result from decarboxylation of 3-keto acids. The lactones and ketones are not themselves acids and, therefore, are not present as glycerol esters. Nevertheless, they are derived from fatty acids, they are components of milk fat, and they add significantly to the characteristic flavour of milk. Jensen (1995) reports the existence of 85 hydroxy acids of which 9 are C_8-C_{14} 4- and 5- hydroxy acids furnishing γ- and δ-lactones, respectively. Some 60 to 70 keto acids have also been identified. These have 10 to 24 carbon atoms and may be saturated or unsaturated. The keto group may occupy several positions in the alkyl chain, but only those

with a keto group at position 3 are readily decarboxylated. Milk fat also contains trace amounts of 11-cyclohexylundecanoic acid and of acids containing a furan unit.

An exciting development in lipid science in recent years has been the recognition of the importance of certain octadecadienoic acids (18:2) with conjugated unsaturation (conjugated linoleic acid, CLA), which are produced by ruminants and appear in low but significant levels in the milk and meat of these animals. The term "conjugated linoleic acid (CLA)" is used to describe any 18:2 acid with conjugated diene unsaturation. Such acids are minor components of the human diet. Ruminant fats contain a range of CLA isomers among which the 9*c*11*t* acid (rumenic) is dominant. The 7*t*9*c* and 10*t*12*c* dienes are also present at lower levels along with many other isomers. There is evidence that individual isomers within this group inhibit the growth of cancer cells and that they promote the formation of protein at the expense of fat (Yurawecz et al., 1999; Sebedio et al., 2003). The first of these properties is important in the management of cancer and the second in animal husbandry (Jahreis et al., 2002). The best CLA available for purchase is a mixture of the 9*c*11*t* and 10*t*12*c* isomers resulting from carefully controlled alkali isomerisation of a linoleic acid-rich oil such as safflower.

CLA has been identified at low levels in milk fat (3 to 6 mg/g of total fat), butter fat (12 to 14 mg/g), and cheeses (2 to 20 mg/g). Extensive studies have been made of factors influencing the level of CLA in milk fats and of ways of enhancing these (Fernie, 2003; Lock and Bauman, 2004).

2.4.1.4 Changes in fatty acid composition either through dietary changes or through fractionation

Milk fat composition is generally different in the summer and winter as a consequence of dietary changes. In the summer, the animals feed on fresh green pasture; in the winter they are kept indoors and are fed on forage and concentrates (Table 2.62). The level of short- and medium-chain acids is lower and the level of C_{18} acids is higher in the summer than during the winter.

Saturated C_4–C_{14} acids and about one-half of the C_{16} acid are produced by *de novo* synthesis in the mammary gland. The rest of the C_{16} acid and the saturated and unsaturated C_{18} acids are derived from dietary sources or by mobilisation of body fat reserves during early lactation. It follows that only part of the milk fatty acids can be modified through a change of dietary intake. Further, since in ruminants unsaturated acids (free) are subject to biohydrogenation in the rumen, it is necessary to protect such acids during their passage through the rumen if they are to be incorporated unchanged in the milk fat. This was first achieved through coating the unsaturated oil (soybean, linseed, rape/canola), but two other methods are now more commonly employed. In the first, calcium salts are used as lipid source. These remain as (unreactive) salts in the rumen, but are converted to acids in the more acidic conditions of the abomasum and enter the duodenum as fatty acids available for digestion. Alternatively, the lipid is hardened to the point where it remains solid in the rumen, but melts in the abomasum. The resulting changes in the milk fat may seem small in terms of fatty acid composition, but they are slightly greater in their effect on triacylglycerol composition and may be enough to allow the butter to spread directly from the refrigerator. It is important that the dietary supplement contain appropriate proportions of omega-9, omega-6, and omega-3 unsaturated acids and that it is over 75% protected from metabolism in the rumen. Lock and Bauman (2004) have reviewed attempts to modify the fat composition of milk fats.

TABLE 2.62 Fatty acid composition (% wt) of French butters (12 samples) collected in May and June and in October and November

Acid	May–June			Oct–Nov		
	mean	min	max	mean	min	max
4:0	3.8	3.6	4.0	4.3	3.8	4.6
6:0	2.4	2.3	2.5	2.5	2.3	2.8
8:0	1.4	1.4	2.0	1.5	1.4	1.7
10:0	3.1	2.9	3.5	3.3	3.0	3.6
12:0	3.6	3.2	4.1	3.7	3.4	4.4
14:0	11.0	10.4	11.6	11.3	10.7	11.7
15:0	1.1	1.1	1.2	1.1	1.0	1.3
16:0	27.0	24.9	29.2	29.3	24.1	31.5
16:1 (*c*/*t*)	2.0			1.9		
18:0	11.0	10.0	12.5	9.6	9.1	10.8
18:1 (*c*/*t*)	24.0	22.4	25.7	22.6	20.8	26.6
18:2 n-6	1.2	1.0	1.5	1.3	1.1	1.6
Other	8.4			8.6		

Source: Adapted from Wolff, R.L., *J. Amer. Oil Chem. Soc.,* **71**, 277–283, 1994. Figures for other times of the year are given in a later paper by Wolff et al., 1995.

In times of over supply, there is an interest in extending the range of applications of milk fat by fractionation. However, the triacylglycerol composition of milk fat is so complex (no individual triacylglycerol exceeds 5%; see Table 2.64) that differences between crystallised fractions are not so marked as with simpler vegetable oils such as palm oil. Nevertheless, useful separations have been achieved producing fractions that are harder and fractions that are softer than the original milk fat. The lower melting (softer) fractions are employed to make spreadable butter and the harder fractions find pastry applications (Deffense, 1995; De Greyt et al., 1995 and 2001; Kaylegian, 1999; van Aken et al., 1999; Gibon, 2002 and 2006; Deffense et al., 2003; Timms, 2005).

In Europe, butters are designated as "butter" only when they contain 80% fat, "three-quarter fat butter" has 60 to 62% fat, "half fat butter" has 39 to 41% fat, and "dairy fat spreads" have other fat levels. In the U.S., "light butter" must contain less than half of the normal level of fat and "reduced butter" less than one-quarter of the normal level.

Blends of butter and vegetable oil — generally soybean oil — are now available in many countries. These cannot be called butter, but are given an appropriate name that the consumer comes to think of as "spreadable butter." Such products are made in New Zealand by fractionation of butter, followed by recombination of appropriate fractions.

In India, milk fat is consumed partly as butter, but also as ghee. This is a concentrate of butter fat with over 99% milk fat and less than 0.2% moisture. Its lower water content gives it a shelf life of 6 to 8 months even at ambient tropical temperatures. Butter or cream is converted into ghee by controlled heating to reduce the water content to below 0.2%. In other procedures, the aqueous fraction is allowed to separate and some of it is run off before residual moisture is removed by heating (Achaya, 1997; Rajah, 1999).

2.4.1.5 Stereospecific distribution of fatty acids in milk fat and triacylglycerol composition by carbon number and by molecular species

Studies by Christie and Clapperton (1982) and others have shown that in milk fat triacylglycerols short-chain acids (C_4–C_{10}) are concentrated at the *sn*-3 position and medium- and long-chain saturated acids are enriched at the *sn*-1 and 2 positions (Table 2.63).

The number of potential triacylglycerols in milk fat with over 10 significant acids (leading to a potential of 10^3 triacylglycerols) and over 400 acids in total is exceedingly large. In qualitative terms, they are unusual in the location of all the short chain acids (C_4–C_8) in the *sn*-3 position with 12:0, 14:0, and 16:0 concentrated in the *sn*-2 position. Table 2.64 lists 22 triacylglycerol groups present at levels of 1.0% or above. This selection falls into two groups: those with one short chain acid and carbon number between 34 and 40, and those without a short-chain acid having carbon numbers between 44 and 54. Even these 22 groups of triacylglycerols account for only 42.6% of the total.

TABLE 2.63 Stereospecific analysis of triacylglycerols from bovine milk (% mol)

Acid	TAG	*sn*-1	*sn*-2	*sn*-3
4:0	11.8	–	–	35.4
6:0	4.6	–	0.9	12.9
8:0	1.9	1.4	0.7	3.6
10:0	3.7	1.9	3.0	6.2
12:0	3.9	4.9	6.2	0.6
14:0	11.2	9.7	17.5	0.4
15:0	2.1	2.0	2.9	1.4
16:0	23.9	34.0	32.3	5.4
16:1	2.6	2.8	3.6	1.4
17:0	0.8	1.3	1.0	0.1
18:0	7.0	10.3	9.5	1.2
18:1	24.0	30.0	18.9	23.1
18:2	2.5	1.7	3.5	2.3

Source: Adapted from Christie, W.W. and Clapperton, J.L., *J. Soc. Dairy Technol.,* **35**, 22–24, 1982.

TABLE 2.64 Major triacylglycerols in bovine milk lipids listed by increasing carbon number

Carbon number	Fatty acids	% (mol)	Carbon number	Fatty acids	% (mol)
34:0	BMP	3.0	40:2	BOO	1.5
36:0	CMP	1.4	44:1	DPO	1.6
36:0	BMSt	1.3	46:1	LPO	1.2
36:0	BPP	3.2	48:1	MPO	2.8
36:1	BMO	1.8	50:1	MOSt	1.4
38:0	CPP	1.5	50:1	PPO	2.3
38:0	BPSt	2.5	50:2	MOO	1.3
38:1	BPO	4.2	52:1	POSt	2.2
40:0	CPSt	1.1	52:2	POO	2.5
40:1	CPO	2.0	54:2	OOSt	1.2
40:1	BOSt	1.6	54:3	OOO	1.0
Total					42.6

Notes: The carbon number indicates the total number of carbon atoms in the three acyl chains.
Fatty acids: B = butyric, C = caproic, D = capric (decanoic), L = lauric, M = myristic, P = palmitic, St = stearic, O = oleic.
Symbols, such as BMP, stand for all triacylglycerols containing these three acyl chains. Even allowing for all these isomeric species together, they represent less than one-half of the milk triacylglycerols. It is known from other evidence that short-chain acids (C_4-C_{10}) are concentrated at the *sn*-3 position and medium- and long-chain saturated acids are enriched at the *sn*-1 and 2 positions (see Table 2.63).
Source: Adapted from Jensen, R. G. (Ed.) *Handbook of Milk Fat Composition,* Academic Press, San Diego, 1995.

The figures in Table 2.65 report the triacylglycerols in milk fat by carbon number (the sum of the carbon numbers in the three acyl chains). Each carbon number group will contain several different triacylglycerols. The distribution pattern is bimodal with peaks at 38 and 50. The former will be triacylglycerols with one short chain and two long chains, such as all the isomeric triacylglycerols containing C_4, C_{16}, and C_{18} fatty acids with the C_{18} acids being saturated or unsaturated. Triacylglycerols with carbon number 50 will be mainly 16/16/18 or 14/18/18 compounds and include all the stereoisomers of these. Again, the C_{18} chains may be saturated or unsaturated.

2.4.1.6 Phospholipids and other minor components

The phospholipids present in milk fat (0.8 to 1.0%) are mainly phosphatidylcholines (PC), phosphatidylethanolamines (PE), and sphingomyelins (SM) at the levels given in

TABLE 2.65 Triacylglycerol composition by carbon number — mean values for 440 butter samples

C No	26	28	30	32	34	36	38	40
Mean	0.2	0.6	1.2	2.6	5.9	10.9	12.8	10.1
C No	42	44	46	48	50	52	54	
Mean	7.1	6.7	7.4	9.1	10.9	9.5	4.6	

Source: Adapted from Rossell, B., (Ed.) *Oils and Fats, Volume 3, Dairy Fats*, Leatherhead Food International, Leatherhead, England, 2003.

Table 2.66. PC and PE contain a similar range of acids, but in different proportions, the PE group being more unsaturated. The SM differ in the presence of long-chain, odd and even, saturated acids. Sphingolipids are involved in intracellular signalling, protect against colon cancer, inhibit cholesterol absorption and synthesis in the intestine, and are antimicrobial. Hellgren (2002) considers that dairy products are probably the main dietary source of these lipids and cites values for fresh milk (118 μmol/kg), cheese with 28% fat (567 μmol/kg), cream (907 μmol/kg), and butter (1170 μmol/kg). Fauquant et al. (2005) have shown that differences in the fatty acid composition of small and large globules result from differences in the composition of the triacylglycerols and not of the phospholipids.

Milk fat contains about 0.40% of unsaponifiable material of which about two-thirds is sterols and the remainder a mixture containing vitamins A, D, and E, and a range of hydrocarbons and of aliphatic alcohols. The sterols are mainly free (>85%) and mainly cholesterol (>95%) and are present in the membrane of the milk fat globule. Other sterols present include lathosterol, 7-dehydrosterol, 24-methylenecholesterol, and fucosterol. The content of cholesterol can be reduced by steam distillation, short path distillation, supercritical fluid extraction, by cyclodextrins, or by enzymic breakdown, but there is no significant market for cholesterol-reduced butter.

The non-sterol components include hydrocarbons (~140 mg/kg of fat, C_{20}–C_{34} odd and even, saturated and monounsaturated compounds), squalene (~140 mg/kg of fat), aliphatic alcohols (~270 mg/kg of fat, mainly saturated C_{19}–C_{31} compounds), carotenoids, etc. (~190 mg/kg of fat) and vitamins A, D, and E and other materials (~460 mg/kg of fat) (Rossell, 2003).

2.4.1.7 Production, trade, and consumption of butter

In the 5-year period 2000 to 2004, the production of butter rose only about 6% from 5.97 to 6.35 million tonnes. Imports and exports were each in the range of only 0.6 to 0.8 million tonnes, so most butter is consumed in the country where it is produced (Table 2.67). The most significant exception is New Zealand with a production in 2004–2005 of 340,000 tonnes, consumption of only 30,000 tonnes, and exports of 310,000 tonnes, mainly to the EU and to countries of the former Soviet Union.

TABLE 2.66 The major phospholipids (PL) in milk and the major fatty acids present in each PL class

PL	% mol	14:0	16:0	18:0	18:1	18:2	Other
PC	34	8	36	11	26	5	14%
PE	32	1	11	12	54	12	10%
SM	25	2	22	4	5	1	22:0 15%, 23:0 27%, 24:0 15% other 9%

Note: Phosphatidylcholine PC, phosphatidylethanolamine PE, and sphingomyelin SM
Other PL present include phosphatidylserine (3%), phosphatidylinositol (5%), and plasmalogens (3%)
Other acids present include 12:0, 15:0, 16:1, 17:0, 18:3, 20:3, and 20:4.
Source: Adapted from Jensen, R.G., *J. Amer. Oil Chem. Soc.*, **50**, 186–192, 1973.

TABLE 2.67 Butterfat production, consumption, imports, and exports (million tonnes) by country/region for the calendar years 2000 to 2004

	Production					Consumption				
	2000	**2001**	**2002**	**2003**	**2004**	**2000**	**2001**	**2002**	**2003**	**2004**
World	5.97	6.01	6.19	6.27	6.35	5.94	6.00	6.12	6.23	6.40
EU-25	1.83	1.81	1.87	1.86	1.84	1.74	1.75	1.71	1.70	1.70
CIS	0.38	0.40	0.39	0.40	0.40	0.44	0.46	0.47	0.48	0.48
USA	0.48	0.46	0.50	0.46	0.46	0.49	0.47	0.49	0.51	0.51
India	1.35	1.41	1.48	1.57	1.65	1.36	1.41	1.49	1.57	1.65
Pakistan	0.46	0.48	0.49	0.49	0.50	0.46	0.48	0.49	0.49	0.50
NZ	0.33	0.33	0.33	0.35	0.34	0.03	0.03	0.03	0.03	0.03
	Imports					**Exports**				
	2000	**2001**	**2002**	**2003**	**2004**	**2000**	**2001**	**2002**	**2003**	**2004**
World	0.63	0.68	0.72	0.74	0.75	0.67	0.66	0.73	0.76	0.75
EU-25	0.06	0.09	0.09	010	0.10	0.15	0.17	0.19	0.26	0.26
CIS	0.09	0.12	0.13	0.14	0.11	0.04	0.07	0.04	0.04	0.04
USA	0.02	0.03	0.03	0.03	0.05	0.01	–	–	0.01	0.01
India	–	–	–	–	–	–	–	–	–	–
Pakistan	–	–	–	–	–	–	–	–	–	–
NZ	–	–	–	–	–	0.30	0.26	0.34	0.32	0.29

Note: CIS = Commonwealth of Independent States (former Soviet Union), NZ = New Zealand.
Source: Adapted from the *Oil World Annual 2005*, ISTA Mielke GmbH, Hamburg, 2005.

TABLE 2.68 Major producers and consumers of butter on a fat basis (million tonnes) in 2004–2005

Country	Production	Exports	Imports	Consumption
World total	6.47	0.80	0.80	6.47
EU-25[a]	1.86	0.28	0.11	1.71
CIS	0.40	0.04	0.13	0.49
India	1.69	–	–	1.69
Pakistan	0.51	–	–	0.51
US	0.48	–	0.04	0.52
NZ	0.34	0.31	–	0.03

[a] France (0.41) and Germany (0.42) are the major European consumers of butter in the EU.
Source: Adapted from the *Oil World Annual 2005*, ISTA Mielke GmbH, Hamburg, 2005.

The EU is also an exporter of butter (280,000 tonnes). The major producers and consumers of butter in 2004–2005 are as shown in Table 2.68.

2.4.1.8 Human milk and other animal milks

The composition of human milk fat is influenced by the diet of the mother and by the days postpartum when the sample is collected. Total lipids range from 2 to 5%. This is mainly triacylglycerol (98 to 99%), but phospholipids (0.6 to 1.1%) and cholesterol (0.4 to 1.3%) are also present.

Jensen (1999) has prepared a substantial review with fatty acid data for samples of human milk fat from many countries (Australia, Canada, China, Denmark, France, Holland, Hungary, Israel, Japan, Nigeria, Spain, and Sudan). These are summarised in Table 2.69, where a distinction is drawn between mothers expected to consume a western diet and those on a non-western diet. Those on the non-western diet have more of the plant-derived n-6 and n-3 PUFA in their milk fats, while those on the western diets have higher levels of *trans* acids, presumably resulting from partially hydrogenated fats in the diet. However, these are mean values derived from a wide range of observed values. Comparing western and non-western diets, the data for 18:1 lie between 23.6 and 55.2% and between 21.5 and 39.2% and, for 18:2, they fall in the range 5.8 to 27.5% and 9.3 to 32.7%, respectively. Human milk fat contains many other acids, and Jensen (1999) lists 214. In contrast to cow and other animal milk fats, the level of saturated acids shorter than 10:0 is very small. Recent interest in the nature of the *trans* acids in human milk fat is reflected in papers by Wolff et al. (1998), Precht et al. (2000), and Jensen et al. (2000). Also, Diersen-Schade and Boettcher (2005) have discussed the requirement of arachidonic acid and DHA for infant development.

TABLE 2.69 Fatty acid composition (% wt, mean values) of breast milk from women consuming western and non-western diets

Fatty acid	Western	Non-western
10:0	1.42	1.00
12:0	5.67	6.14
14:0	6.58	7.22
16:0	21.58	19.60
18:0	6.04	5.90
18:1 *c*	31.08	27.10
18:1 *t*	2.78	0.43
18:2 *c*	11.73	18.14
18:2 *t*	0.61	0.04
20:4 n-6	0.42	0.92
18:3 n-3	1.08	2.07
20:5 n-3	0.09	0.52
22:6 n-3	0.45	0.88

Source: Adapted from Jensen, R.G., *Lipids,* **34**, 1243–1271, 1999.

Winter et al. (1993) have reported the 170 most abundant triacylglycerols of which 22 are present at levels of 1.0% or greater. These have been detailed by Jensen (1999). Two groups dominate: those containing 16:0, 18:1, and 18:1 fatty acids (11.8%mol) and those containing 16:0, 18:1, and 18:2 acids (10.0% mol). Human milk fat is unusual in that much of the palmitic acid is present in the *sn*-2 position. Christie and Clapperton (1982) report that in human milk fat containing 27.0% of palmitic acid the levels of this acid in the *sn*-1, 2, and 3 positions are 18.7, 57.1, and 5.3%, respectively. Martin-Susa et al. (2004) have reported changes in the fatty acids of human milk gangliosides during lactation.

The levels of milk fat from other animals are as follows: buffalo (6.7%), sheep (7.8%), goat (4.1%), camel (3.9%), and cow (3.5%). Information concerning their fatty acid and triacylglycerol composition is given in Table 2.70 and Table 2.71.

2.4.2 Lard

Lard is the body fat of pigs and is typically rich in palmitic (26%) and oleic acid (44%), with lower levels of linoleic (11%) and palmitoleic acid (5%) (Table 2.72). A pig carcass generally contains about 30% of fat, but in fat pigs this may be as high as 50%. By virtue of its fatty acid and triacylglycerol composition, lard normally appears as a white solid fat.

Differences in the fatty acid composition of tallow and lard reflect the fact that while tallow comes from a ruminant animal, where dietary lipids are subject to rumen biohydrogenation, the pig is monogastric and its depot fats more closely resemble the dietary intake. Lard is accordingly richer in linoleic acid than is tallow. The content of linoleic acid may be as low as 2% in pigs fed with rice, but as high as 30% when fed with soya.

A typical fatty acid composition of lard is given in Table 2.72. Codex fatty acid specifications (Table 2.73) indicate values greater than 1% for seven acids (14:0, 16:0, 16:1, 18:0, 18:1, 18:2, and 18:3) along with lower levels of iso-acids (C_{14} to C_{17}), anteiso and n- C_{15} and C_{17} acids, 14:1, 16:2, 17:1, and several C_{20} to C_{22} acids. The content of *trans* acids at around 1% is much lower than in the ruminant fats.

TABLE 2.70 Fatty acid composition (g/100 ml milk and % of total fat) of human, sheep, and goat milk fats

	Human		Sheep		Goat	
Fatty acid	**g/100ml**	**%**	**g/100ml**	**%**	**g/100ml**	**%**
4:0	–	–	0.20	3.0	0.13	3.3
6:0	–	–	0.14	2.1	0.09	2.3
8:0	–	–	0.14	2.1	0.10	2.5
10:0	0.06	1.4	0.40	6.0	0.26	6.6
12:0	0.26	6.2	0.24	3.6	0.12	3.1
14:0	0.32	7.7	0.66	10.0	0.32	8.1
16:0	0.92	22.1	1.62	24.4	0.91	23.2
18:0	0.29	7.0	0.90	13.5	0.44	11.2
16:1	0.13	3.1	0.13	2.0	0.08	2,0
18:1	1.48	35.5	1.56	23.6	0.98	25.0
18:2	0.37	8.9	0.18	2.7	0.11	2.8
18:3	0.05	1.2	0.13	2.0	0.04	1.0
20:4	0.03	0.7	–	–	–	–
Other	0.26	6.2	0.33	5.0	0.35	8.9
Total	4.17	100.0	6.63	100.0	3.93	100.0

Note: The unsaturated acids, and especially 18:1, represent the total of *cis* and *trans* isomers.
Source: Adapted from Rossell, B., (Ed.) *Oils and Fats, Volume 3, Dairy Fats*, Leatherhead Food International, Leatherhead, England, 2003.

TABLE 2.71 Triacylglycerol composition (% wt) by carbon number of goat milk fat (mean of 35 samples) and of ewe milk fat (mean of 45 samples)

C No	Goat	Ewe	C No	Goat	Ewe
24	0.1	–	40	12.6	12.0
26	0.5	0.7	42	12.5	9.0
28	1.2	1.6	44	11.6	8.1
30	2.5	2.5	46	8.1	6.8
32	4.1	3.6	48	5.8	6.7
34	6.2	6.0	50	5.8	7.6
36	9.4	9.6	52	4.9	8.4
38	12.1	12.8	54	2.0	4.5

Source: Adapted from Fontecha, J., et al., *J. Amer. Oil Chem. Soc.,* **75**, 1893–1896, 1998; and Goudjil, H., et al., *J. Amer. Oil Chem. Soc.,* **80**, 219–222, 2003.

TABLE 2.72 Fatty acid composition of beef tallow, mutton tallow, and lard

Fatty Acid	Beef Tallow	Mutton Tallow	Lard
10:0	–	–	0.1
12:0	0.9	–	0.2
14:0	3.9	4.0	1.4
16:0	26.0	22.5	24.9
16:1	4.4	2.4	2.8
18:0	19.8	20.4	14.1
18:1	27.7	39.3	43.1
18:2	3.2	5.8	10.7
18:3	0.1	2.4	1.0
Saturated	50.6	46.8	40.7
Monounsaturated	43.7	42.4	47.2
Polyunsaturated	4.2	8.1	11.7

Source: Adapted from Rhee, K.C., in *Fatty Acids in Foods and Their Health Implications,* Ching Kuang Chow (Ed.), Marcel Dekker, New York, 2000, p. 88.

TABLE 2.73 Codex ranges (% wt) for lard (rendered pork fat) and tallow (*premier jus*) agreed in 1999

Acid	Lard	Tallow	Acid	Lard	Tallow
14:0	1.0–2.5	2–6	8:0–12:0	<0.5[a]	<0.5[a]
16:0	20–30	20–30	14:0 iso	<0.1	<0.3
16:1	2.0–4.0	1–5	14:1	<0.2	0.5–1.5
18:0	8–22	15–30	15:0	<0.2	0.2–1.0
18:1	35–55	30–45	15:0 iso	<0.1	)<1.5[a]
18:2	4–12	1–6	15:0 anteiso	<0.1	)
18:3	<1.5	<1.5	16:0 iso	<0.1	<0.5
			16:2	<0.1	<1.0
			17:0	<1	0.5–2.0
			17:1	<1	<1.0
			17:0 iso	<0.1	)<1.5[a]
			17:0 anteiso	<0.1	)
			20:0	<1.0	<0.5
			20:1	<1.5	<0.5
			20:2	<1.0	<0.1
			20:4	<1.0	<0.5
			22:0	<0.1	<0.1
			22:1	<0.5	Not detected

[a] Total for range of acids indicated.
Note: Arranged as major (left-hand columns) and minor components (right-hand columns).

The fatty acid composition of lard depends not only on diet but also varies with the site from which the fat is taken. Some relevant figures are given in Table 2.74, along with a regiospecific analysis. The major triacylglycerols of pig inner back fat are reported to be MSS (33%), MSM (28%), MSD (12%), SSD (7%), SSS (7%), MMM (5%), MDM (3%), and other (5%), where S = saturated, M = monoene, D = diene, and each three letter grouping includes all triacylglycerols containing the acyl groups indicated.

An unusual feature of lard is the high level of palmitic acid in the *sn*-2 position in its triacylglycerols. Over 70%

TABLE 2.74 Stereospecific analysis of triacylglycerols from various pig tissues

Fat source	*sn*	14:0	16:0	16:1	18:0	18:1	18:2
Inner back	1	0.7	9.8	1.7	38.8	42.7	6.3
	2	3.5	72.1	3.7	3.8	14.0	2.9
	3	0.6	5.4	2.1	11.3	65.4	15.2
Outer back	1	0.9	9.5	2.4	29.5	51.3	6.4
	2	4.1	72.3	4.8	2.1	13.4	3.3
	3	–0.2	0.4	1.5	7.4	72.7	18.2
Perinephric	1	2.5	11.7	2.3	35.2	43.7	4.6
	2	4.8	75.4	4.1	2.8	10.2	2.7
	3	–1.6	5.0	2.3	12.1	68.5	13.7
Mesenteric	1	1.8	15.6	2.6	37.6	39.4	3.0
	2	4.5	78.4	3.9	3.0	8.9	1.3
	3	–0.3	2.3	1.6	19.1	66.6	10.7

Notes: Negative numbers result from small experimental errors.
Data also given for fat from milk, adipose tissue adhering to stomach, heart, adrenal liver, blood, and kidney.
Fatty acid composition of the fat can be calculated as 1/3(1+2+3). Thus, the level of palmitic acid from these four sites is 29.1, 27.4, 30.7, and 32.1%.
Source: Adapted from Christie, W.W. and Moore, J.H., *Biochim. Biophys. Acta,* **210**, 46–56, 1970.

TABLE 2.75 Production, consumption, imports, and exports (million tonnes) of tallow by country/region for the calendar years 2000 to 2004

	Production					Consumption				
	2000	**2001**	**2002**	**2003**	**2004**	**2000**	**2001**	**2002**	**2003**	**2004**
World	6.74	6.78	7.01	7.21	7.30	6.72	6.80	7.00	7.21	7.29
China	2.78	2.84	2.95	3.05	3.13	2.82	2.89	2.94	3.03	3.12
EU-25	1.79	1.75	1.80	1.85	1.84	1.77	1.73	1.79	1.85	1.82
US	0.49	0.49	0.51	0.52	0.53	0.40	0.44	0.44	0.44	0.44
Brazil	0.33	0.34	0.36	0.35	0.36	0.33	0.34	0.36	0.35	0.36
Russia	0.18	0.18	0.18	0.20	0.20	0.18	0.18	0.19	0.20	0.21
	Imports					**Exports**				
World	0.19	0.13	0.13	0.14	0.14	0.19	0.13	0.13	0.14	0.15

Source: Adapted from the *Oil World Annual 2005*, ISTA Mielke GmbH, Hamburg, 2005.

of the acids in this position are palmitic acid. In this respect, it resembles human milk fat. This analytical feature can be exploited in some measure to detect the presence of pig fats as an adulterant. As a consequence of this unusual feature, its physical properties (especially melting behaviour) are changed markedly when the fat is randomised to give an improved shortening.

Compared with vegetable oils, animal fats are rich in cholesterol (3000 to 4000 mg/kg) and deficient in natural antioxidants. Despite their relatively saturated nature, therefore, animal fats have to be stabilised against oxidation by addition of natural or synthetic antioxidants. Because of the low level of tocopherols naturally present, the oxidative stability of lard can be enhanced considerably. It has been reported that lard has an induction period of only 2.5 hours when heated at 100ºC with blown air, but this is extended to 18 hours with added tocopherol (0.01%). BHA and/or BHT are often added to lard at levels of 100 to 200 ppm.

Table 2.75 contains details on the production, consumption, and trade in lard. Imports and exports are not significant as most lard is consumed in the country where it is produced, particularly in China and in EU-25. Lard is a by-product of the pig industry geared to producing pork. Appropriate figures are given in Table 2.76.

2.4.3 Tallow

Tallow is mainly fat from cattle and may be considered as a by-product of the beef industry. It may also contain some fat from sheep, but should be free of pig fat (lard). Production of tallow now exceeds 8 million tonnes, and

TABLE 2.76 Annual production (million tonnes) of red meats and poultry meat

	2001	**2002**	**2003**	**2004**
Beef and veal	59.6	61.0	61.7	62.7
Pork	92.5	95.5	98.0	99.4
Mutton and lamb	11.7	12.0	12.2	12.4
Poultry meats	72.9	75.7	77.6	80.7
Other	4.2	4.2	4.3	4.3
Total	240.9	248.4	253.8	259.5

Source: Adapted from the *Oil World Annual 2005*, ISTA Mielke GmbH, Hamburg, 2005.

tallow, lard, and butter occupy positions 5 to 7 in ranking order of oil and fat production after the four major vegetable oils (soybean, palm, rape/canola, and sunflower) About 25% of the world tallow production is exported/imported and the balance is used in the country/region in which it is produced. Figures provided in Table 2.77 show that the U.S. dominates the production and consumption of this fat, followed by EU-25 and China.

The rendering industry is a very significant part of livestock processing. Edible meat represents only two-thirds of what goes to the abattoir (slaughterhouse) leaving the balance to be processed by the rendering industry. This industry seeks to produce valuable by-products, rich in protein or in fat, which are both safe and acceptable and, thereafter, to dispose of the residue in a manner that is environmentally acceptable, measured against increasingly rigorous standards.

Tallow is available in up to 20 different grades categorised according to titre (melting point of component fatty acids), colour, free acid content, and MIU (moisture, impurities, and unsaponifiable material). Poorer grades, often from other animal sources, are classified as "greases" and are generally included with tallows for recording purposes. Only the highest grades can be used for human food.

Tallow is used in three general areas: as a food such as in margarine and in frying oils (Berger, 1997), as an energy-rich component of animal feed (Harris, 1995), and in the oleochemical industry as a source of acids, esters, alcohols, soaps, and N-compounds used in personal care products, cosmetics, emulsifiers, etc. The division between these three sectors depends mainly on the quality of the tallow, but also on supply/demand balances. In Europe about 50% of tallow production is used for production of soap and other oleochemicals, the balance being used as food or feed. Tallow is frequently in competition with palm stearin, particularly for oleochemical purposes. As with other fats, the food uses of tallow are related to its nutritional, physical, and chemical properties depending, in turn, on the composition of the fatty acids, the triacylglycerols, and the minor components. These figures may be modified in the near future as increasing quantities of tallow are converted to methyl esters for use as biofuel.

U.S. figures are reported by the U.S. Census Bureau. In the month of November 2004, the total U.S consumption of tallow was 146,000 tonnes of which only 8000 tonnes (5.7% of total) was used for edible purposes. Of the balance, 93,000 tonnes (63.5%) was used for animal feed, 24,000 tonnes (16.5%) to produce fatty acids, and the remainder for other nonedible uses.

Typically beef tallow contains palmitic, stearic, and oleic acids at levels of around 26, 22, and 39% (total ~87%). The more significant minor components include myristic acid (3.5%), hexadecenoic acid (3.5%), and linoleic acid (2.5%), while odd-chain acids, branched-chain acids, and acids with *trans* unsaturation (up to 5%, included in the figure for oleic acid cited above) are also present. In common with other ruminant fats tallow is now known to contain conjugated linoleic acids (CLA). A typical level of ~2.5 mg/g compares with values about twice as large in milk and dairy products. These acids have aroused much attention recently because of their reported anticarcinogenic properties.

Table 2.78 contains information on the fatty acid composition of tallow and a stereospecific analysis of a tallow sample reported by Christie et al. in 1991. (Codex ranges are given in Table 2.73.) The distribution of the major fatty acids does not follow any obvious pattern and, in contrast to many seed oils, fatty acids in the 1 and 3 positions are not the same. Oleic acid is concentrated in the 2 position. Palmitic and stearic acids, though not concentrated in the 2 position, are nevertheless present there at significant levels.

TABLE 2.77 Production, consumption, imports, and exports (million tonnes) of tallow by country/region for the calendar years 2000 to 2004

	Production					Consumption				
	2000	**2001**	**2002**	**2003**	**2004**	**2000**	**2001**	**2002**	**2003**	**2004**
World	8.20	7.69	8.07	8.03	8.11	8.15	7.77	8.06	8.12	8.06
US	3.95	3.50	3.83	3.72	3.64	2.94	2.65	2.60	2.61	2.57
EU-25	1.13	1.05	1.08	1.09	1.09	1.20	1.22	1.16	1.13	1.11
China	0.62	0.65	0.68	0.70	0.72	0.95	0.96	0.99	1.00	1.02
Brazil	0.43	0.45	0.46	0.47	0.49	0.49	0.44	0.46	0.47	0.45
Australia	0.50	0.51	0.47	0.46	0.49	0.10	0.10	0.11	0.12	0.12
Mexico	0.10	0.11	0.11	0.11	0.12	0.39	0.42	0.52	0.48	0.52
Canada	0.29	0.30	0.31	0.29	0.34	0.10	0.09	0.10	0.14	0.15
	Imports					**Exports**				
World	2.26	2.07	2.31	2.11	2.14	2.21	2.06	2.35	2.12	2.14

The major exporting countries are the U.S., Australia, and Canada.
The major importing countries/regions are China, Mexico, and EU-25.
Source: Adapted from the *Oil World Annual 2005*, ISTA Mielke GmbH, Hamburg, 2005.

TABLE 2.78 Fatty acid composition and stereospecific analysis of tallow

	TAG	*sn-1*	*sn-2*	*sn*-3
14:0	3.4	2.9	1.5	5.7
16:0	29.5	42.0	24.6	21.9
16:1	1.9	2.1	0.8	2.9
18:0	26.0	34.4	11.3	32.4
18:1	34.9	14.9	55.3	34.6
18:2	1.5	0.2	3.9	0.3
Other	2.8	3.5	2.6	2.2

Source: Adapted from Christie, W.W., et al., *J. Am. Oil Chem. Soc.,* **68**, 695–701, 1991.

From a nutritional viewpoint, tallow is perceived as having several disadvantages based on a number of factors:

1. Tallow is an animal fat and, therefore, not acceptable to vegetarians or to some religious or cultural groups. This objection extends from food to personal care products. For example, for acceptance by some ethnic groups, stearic acid incorporated into such materials must be derived from vegetable sources. Associated with this is the overall concern in some communities over animal welfare.
2. Almost 50% of the fatty acids in tallow are saturated and include myristic acid, the saturated acid with greatest cholesterol-raising effect on blood plasma.
3. The level of essential fatty acids is low.
4. Tallow contains acids with *trans* unsaturation (~5%).
5. It contains cholesterol (~1000 ppm) at levels higher than those found in vegetable oils (negligible), though lower than those in dairy products (2000 to 3000 ppm).
6. It contains little or no natural antioxidant.

On a more positive note, good quality tallow is considered by many to produce a desirable flavour in biscuits and fried foods, and the presence of conjugated linoleic acid (CLA) in ruminant fats may also come to be regarded as a plus factor.

The major triacylglycerols are SSS (18%), SUS (47%) and USU (18%) where S and U represent saturated and unsaturated acyl groups attached to glycerol. The high levels of the more saturated glycerol esters give tallow a high slip melting point and a flat melting curve. This is a useful trait for applications where the structure and body of the products is important. However, when eaten, the high melting point may give an impression of waxiness on the palate.

As expected for a fat with a high content of saturated acids and a low level of polyunsaturated acids, tallow should show high oxidative stability. However, as an animal fat, it contains little or no antioxidant and, therefore, may be less resistant to oxidative deterioration than materials of similar melting point derived from vegetable oils. Tallow contains only low levels of phospholipids (<0.07%) and tocopherols (0.001%). Sterols at 94 to 140 mg/100 g of fat are almost entirely cholesterol.

Tallow can be subjected to the full range of processing techniques to extend its use as a food product. These include blending, hydrogenation, fractionation, and interesterification. For oleochemical purposes, it is often converted to free acids that may be fractionated to give palmitic, stearic, and oleic fractions at varying levels of purity. Deffense (in Rossell, 2001) details a number of fractionations of beef tallow (mp 43 to 46ºC) to produce stearins of higher melting point (54 to 56ºC) and oleins of lower melting point (20ºC) as well as fractions with intermediate values. It is of interest that margarines were first produced using beef olein.

2.4.4 Chicken fats

Despite the high level of poultry meat now consumed around the world, there is little information about the amount of fat derived from this source. Presumably, production is smaller than from the red meats because there is little removal of fat from these animals prior to retail sale. Such chicken fat as is available probably comes from food companies processing and cooking chickens on an industrial scale.

The fatty acid composition of chicken fat and other poultry fats is summarised in Table 2.79. The composition depends on the diet of the chickens and also on what parts of the chicken are used as fat source. It is clear from Table 2.80 that skin lipids differ from muscle lipids and that lipids from white meat differ from those from dark meat.

Attempts have been made to increase the levels of omega-3 acids in broiler meat by adding these acids to the chicken feed (Rymer et al., 2005).

2.4.5 Fish oils

2.4.5.1 Sources and production

For those living near rivers, lakes, or the sea, fish have been part of the human diet for many centuries and there has

TABLE 2.79 Fatty acid composition of chicken fat and some other poultry fats

	Chicken				Duck		Goose
References	**a**	**b**	**c**	**d**	**a**	**b**	**a**
14:0	1.3	0.9	0.7	0.6	0.7	0.7	0.5
16:0	23.2	22.3	25.2	28.6	25.9	26.1	22.0
16:1	6.5	6.0	7.8	2.7	4.2	4.2	3.0
18:0	6.4	6.9	5.9	6.8	8.2	8.2	6.5
18:1	41.6	39.5	40.5	51.7	46.3	46.8	56.9
18:2	18.9	20.0	18.4	4.9	12.6	12.6	10.4
18:3	1.3	1.3	0.7	2.4	1.0	1.0	0.5
20:1	–	1.2	0.5	0.7	–	1.2	–
Other	0.8	1.9	0.3	1.6	1.1	–0.8	0.2

Sources: (a) and (b) Rossell, B., (Ed.) *Oils and Fats, Volume 3, Dairy Fats*, Leatherhead Food International, Leatherhead, England, 2003, pp. 14 and 158, respectively; (c) Ki-Teak Lee and Foglia, T.A., *J. Am. Oil Chem. Soc.,* 77, 1027–1034, 2000; and (d) Nai-Ting Ma, et al., *J. Am. Oil Chem. Soc.,* **81**, 921–926, 2004.

TABLE 2.80 Fatty acid composition of some chicken fats

	Light muscle		Dark muscle	
	No skin	With skin	No skin	With skin
14:0.	0.9	0.9	0.9	0.9
16:0	24.6	23.8	21.6	23.2
16:1	3.5	6.1	5.8	6.1
18:0	11.4	6.4	8.5	6.5
18:1	29.8	38.2	32.4	38.5
18:2	19.3	21.1	23.9	21.6
18:3	0.9	1.0	1.2	1.0
20:1	0.9	1.2	0.3	1.1
20:4	5.3	0.6	2.9	0.6
Other	3.4	0.7	2.5	0.5

Source: Adapted from Christie, W.W., et al., *J. Am. Oil Chem. Soc.,* **68**, 695–701, 1991.

long been a trade in dried fish. A significant fishing industry developed as fishermen learned to fish over wider areas of the seas and when improvements in storage conditions allowed distribution to urban dwellers for many of whom fresh fish and fried fish are now standard parts of their diet.

Today the fishing industry is organised in part to produce fish for consumption as fresh fish and in various processed forms and in part to produce fishmeal (protein) with fish oil only a by-product of this operation. Fish oil is produced mainly from fish caught in open seas. The whole fish is generally used as raw material, though trimmings from the fish processing industry are also a useful source of both meal and oil.

In 1970 the catch of wild marine fish was about 70 million tonnes. This rose to about 93 million tonnes in 1996, but has hardly changed since. In contrast, production of farmed fish has increased rapidly. In 1976 through 1980, the average annual production of farmed fish was 4.1 million tonnes. Twenty years later, between 1996 and 2000, this had risen to 30.7 million tonnes, and the figure forecast for 2016 to 2020 is 70.5 million tonnes. Aquaculture is particularly important in China, and this country accounted for 70% of the world figure in the 5-year period from 1996 to 2000.

The leading producing countries of fish oil are Peru and Chile, followed by Denmark, U.S., Iceland, and Norway. Japan was a large producer of fish oil in the 1980s, but sardines disappeared from Japanese waters in 1992 and the country is now an importer of fish oil.

For the South American fishing nations of Peru and Chile, the major fish species used in commercial production of fishmeal and oil are anchovy, jack mackerel, pacific mackerel, and sardine. In the fishing grounds of Western Europe (Iceland, Norway, Denmark, U.K., and Spain) capelin, Atlantic horse mackerel, sandeel, Norway pout, sprat, herring, and blue whiting are important. Menhaden and pollack are popular among U.S. fishermen (Haraldsson and Hjaltson, 2001, 2006).

New types of fish oils, including salmon oil from Norway and tuna oil from Thailand and Australia, are by-products of the processing of these two fish. They are generally high quality oils and provide a useful source of omega-3 acids. Nichols (2004) has discussed the sources of long-chain omega-3 acids, particularly from Australian and New Zealand waters.

Cod liver oil, with an annual production of 10,000 tonnes, was first marketed as a source of vitamins A and D and now also as a source of important long-chain omega-3 fatty acids, such as EPA and DHA. Shark liver oil (mainly from Southeast Asia, particularly China) is a source of vitamin A, squalene, and alkoxy glycerols (see below).

Annual production of fish oils during the past 10 years has been around 1.1 million tonnes with marked fluctuations in production, mainly due to the climatic phenomenon, *el Nino*. This changes conditions in the ocean, particularly along the coasts of Peru and Chile, causing the fish to move to deeper waters. Production from 2005 onwards is predicted to be significantly lower than 1 million tonnes. The major fishing grounds are in the South Pacific (Peru and Chile) or in the North Atlantic (Denmark, Iceland, and Norway). There is a strong trade in fish oils as shown in Table 2.81.

As shown in Table 2.82, there have been significant changes in the uses of fish oil in the past 10 years. After partial hydrogenation, fish oils were used mainly for the production of margarine and spreads, but this application has declined considerably on dietary grounds. In its place, the use of fish oil for aquaculture feed has increased rapidly from 16 to 55% in 10 years. The problem of feeding decreasing stocks of fish oils to increasing numbers of farmed fish raises a serious issue of sustainability. This has been discussed by Bell et al. (2005) who investigated the possibility of replacing dietary fish oil with a blend of fish oil and linseed oil. The latter is a sustainable source

TABLE 2.81 Annual production and trade (thousand tonnes) in fish oils

	2000	2001	2002	2003	2004
Production					
World	1411	1131	934	989	1109
Peru	593	300	189	206	343
Chile	171	145	146	130	141
Denmark	140	124	109	105	113
Iceland	94	99	80	129	70
Norway	83	65	62	51	56
US	87	127	96	89	81
Japan	70	64	65	67	68
Exports	849	754	527	618	652
Imports	819	774	514	601	659

The major exporting countries are Peru, Iceland, Norway, and the U.S.
The major importing countries/regions are EU-25, Norway, Chile, and Japan.
Source: Adapted from the *Oil World Annual 2005*, ISTA Mielke GmbH, Hamburg, 2005.

TABLE 2.82 Major uses of fish oils (proportion %) in 1990, 1995, and 2000

	1990	1995	2000
Hydrogenated fats	77.5	60.1	36.2
Aquaculture feed	15.8	33.1	55.0
Industrial	6.3	5.7	6.3
Pharmaceutical	0.4	1.1	2.0

Source: Pike, I.H. and Barlow, S.M., *Lipid Technology*, **12**, 58–60, 2000.
Information for 2003, as discussed in the text, was kindly supplied by Dr. Pike.

of α-linolenic acid (the C_{18} omega-3 acid) that may serve as a metabolic source of EPA and DHA. Fish oil in the feed serves as an inexpensive source of energy and as a source of omega-3 acids.

Use for industrial purposes remains around 6%. Pharmaceutical and dietary use of fish oils without partial hydrogenation is based on the very important essential fatty acids, vitamins, and other materials present in such sources. This use is growing rapidly, but remains small in volume terms. Refined fish oils or preparations made from these are available in encapsulated form or they may be incorporated into bread and drinks and into infant formula as a source of long-chain polyunsaturated fatty acids.

Dr. Pike (private communication) has updated the information in Table 2.82 with figures for 2003. In that year, total consumption of fish oil was 980 kt (thousands of tonnes) distributed between aquaculture (700 kt, 72%), direct human consumption (190 kt, 19%), technical uses (50kt, 5%), and other uses (40 kt, 4%). Of that used for human consumption, it is believed that ~150 kt is hardened (hydrogenated) mainly in South America and that the balance (40 kt) is consumed as refined fish oil generally in encapsulated form. This last use is increasing at ~15% per annum. In 2002, 732,000 tonnes of fish oil was used in aquaculture, mainly for salmon (364 kt), trout (168 kt), and marine fish (100 kt). It is forecast that by 2010 these requirements will be supplemented by fish oil fed to carp, shrimp, and carnivorous freshwater fish.

2.4.5.2 Fatty Acids

Fish oils contain a wide range of saturated, monounsaturated, and polyunsaturated acids (Table 2.83), but fish fatty acid composition is generally cited in terms of the major acids only. Typical fatty acid data for some commercial fish oils are given in Table 2.84. Such oils are rich in saturated acids (mainly myristic and palmitic), monounsaturated acids covering the range of hexadecenoic through docosenoic, and omega-3 C_{20} and C_{22} polyunsaturated fatty acids. These last are very important acids for which fish oils are the largest source. The table illustrates the differing distribution of monounsaturated acids.

TABLE 2.83 Fatty acids identified in fish oils

Acid type	Number of carbon atoms
Saturated — straight chain	12, 14–24 odd and even members
Saturated — branched chain	15, 17, 18, 19
Monounsaturated	14, 16, 17, 18, 19, 20, 22, 24
Polyunsaturated	16:2–4, 18:2–4, 20:2–5, 21:5, and 22:3–6

Note: Many of the unsaturated acids occur in several isomeric forms.
Source: Adapted from Haraldsson, G.G. and Hjaltason, B., in *Structured and Modified Lipids*. (Ed.) Gunstone, F.D., New York, Marcel Dekker, 2001, 313–350.

TABLE 2.84 Typical fatty acid composition of some commercial fish oils

	Anchovy	Capelin	Cod Liver	Men-haden	Sardine	Salmon (Farmed)	Tuna
				Saturated			
14:0	9	7	4	9	8	5	3
16:0	17	10	10	19	18	12	22
				Monounsaturated			
16:1	13	10	8	12	10	6	3
18:1	10	14	25	11	13	20	21
20:1	1	17	10	1	4	10	1
22:1	1	15	7	–	3	9	3
				Polyunsaturated (n-3)			
20:5	22	8	10	14	16	7	6
22:5	2	–	1	2	2	3	2
22:6	9	6	10	8	9	11	22
				Other			
	16	13	15	24	17	17	17

Note: "Other" includes 18:0, 18:2, 18:3, and 18:4, and other C_{20} and C_{22} acids.
Source: Adapted from Haraldsson, G.G. and Hjaltason, B., in *Structured and Modified Lipids*. (Ed.) Gunstone, F.D., New York, Marcel Dekker, 2001, 313–350.

The C_{16} and C_{18} members are significant in all the oils detailed in Table 2.83, but the C_{20} and C_{22} acids are confined to oils such as capelin and salmon. The fish oils vary in the level of combined PUFA and also in the distribution between EPA and DHA. This is important when selecting a fish oil as a source of one or other of these PUFA for enhancement and when seeking a dietary source rich in one of these acids, but not in the other.

One problem in handling and storing the highly unsaturated fish oils is their ease of oxidation. It is for this reason that the oil can be supplied in capsules or in microencapsulated form. Hsieh et al. (1989) identified 55 volatile components formed when menhaden fish oil is heated at 65°C. The structures and concentration (ranging from 2 to 8500 ppb) were listed. They included 10 alkanes (8:0 to 17:0), 26 saturated and unsaturated aldehydes, 5 ketones, 6 short-chain carboxylic acids, 3 furan-containing compounds, and 5 aromatic compounds. Most are easily recognised as fatty acid oxidation products. The compound present at the highest concentration is heptan-3-one (8500 ppb), but compounds at lower concentration might be more important in terms of odour.

2.4.5.3 Fish oils as sources of valuable dietary constituents

It has long been known that fish oils contain materials with valuable dietary and pharmaceutical properties as well as having physical properties that make them useful as emollients. Until the 1960s, fish liver oils were used in Europe and the U.S. mainly for their vitamins A and D, but high-quality fish oil is now used as a source of long-chain omega-3 acids. Cod and shark liver oils are both commercially available, the former at levels around 10,000 tonnes/annum. Shark liver oil, produced in Southeast Asia, particularly China, is a valuable source of squalene and of glycerol ethers. Salmon oil and tuna oil are now available as by-products of fish processing (Haraldsson and Hjaltason, 2001).

2.4.5.3.1 Squalene

Squalene ($C_{30}H_{50}$) is an interesting acyclic molecule containing six isoprene units. It is a key intermediate in the bioconversion of mevalonic acid (C_6) to triterpenes (C_{30}) and sterols. In this biosynthetic sequence, the cyclisation of squalene to lanosterol represents the conversion of an acyclic precursor to the tetracyclic system characteristic of sterols.

Squalene is a minor component in several vegetable oils, such as olive, wheat germ, and rice bran oil at levels up to 0.7%, and of yeast lipid. It occurs in amaranthus oil at a higher level (6 to 8%) and can be recovered from this oil or from olive oil deodoriser distillate (10 to 30% sqalene) by short-path, high-vacuum distillation (Sun et al., 1997). However, it is obtained mainly from shark liver oil, which is also a source of diacyl glyceryl ethers. After distillation of squalene, the residue serves as a source of glycerol ethers. The highly unsaturated hydrocarbon oxidises easily and is often used in cosmetic products in its fully hydrogenated form (squalane). Summers et al. (1994) and Kayana and Mankura (1998) have reviewed the hydrocarbons in marine fish.

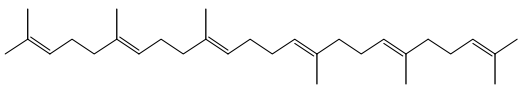


FIGURE 2.1 Squalene $C_{30}H_{50}$.

2.4.5.3.2 Vitamins

Fish liver oils were previously a useful source of vitamins A and D, but synthetic forms of these compounds are now more widely used and interest in fish oils has shifted from the vitamins to the omega-3 acids.

2.4.5.3.3 Glycerol ethers

Shark liver oils contain glycerol ethers largely, but not entirely, in their diacyl form. The ethers have mainly saturated or monounsaturated alkyl chains with three compounds predominating (together 48 to 72%): 16:0 (chimyl), 18:1 (selachyl), and 18:0 (batyl). These are accompanied by homologues of other chain lengths, including odd-chain compounds.

$R^1OCH_2CH(OH)CH_2OH$ glycerol ether
$R^1OCH_2CH(OCOR^2)CH_2OCOR^3$ diacyl glycerol ether
Typically $R^1 = CH_3(CH_2)_7CH{=}CH(CH_2)_7CH_2$- (selachyl, 18:1 alkyl) R^2CO and R^3CO are typical acyl chains

After distillation of the squalene, the residue is hydrolysed and unsaponifiable material separated from liberated fatty acids. The glycerol ethers are then isolated from the unsaponifiable fraction by crystallisation and are used as natural surface-active agents in cosmetics and ointments (Summers et al., 1994; Kayama and Mankura, 1998).

2.4.5.3.4 Long-chain polyunsaturated fatty acids

Fish oils are characterised by their wide range of component acids and, particularly, by the highly unsaturated members. Many of the following may be present at levels exceeding 10%: saturated (14:0, 16:0), monounsaturated (16:1, 18:1, 20:1, 22:1) and omega-3 polyunsaturated fatty acids (20:5, 22:6). Many minor fatty acids are also present. The monoene acids as a group are particularly high in herring (typically 62%), capelin (61%), and cod liver oil (57%). Good sources of EPA and DHA are tuna (typically 6% of EPA and 22% of DHA), sardine (16 and 9%), anchovy (22 and 9%), menhaden (14 and 8%), and farmed salmon (7 and 11%). Sometimes the total level of these two acids is important and on other occasions it is desirable to have high concentrations of only one of them. The ratio of these two acids differs among these sources and it is apparent from these figures that tuna oil is a good source of DHA (Haraldsson and Hjaltason, 2001). Similar figures have been provided by Duo Li et al. (2003) for a range of fresh fish and of commercial canned fish products.

The omega-3 acids of greatest interest are EPA (20:5 eicosapentaenoic acid) and DHA (22:6 docosahexaenoic acid). Sometimes it is desirable to have these acids at higher concentrations than is provided naturally or to enrich one of them at the expense of the other. Alternatively, it may be desired to incorporate one or both of them into an oil rich in medium-chain acids. It is possible to distinguish between these two polyunsaturated fatty acids and other saturated and unsaturated acids and even to distinguish between EPA and DHA themselves by exploiting the specificity of appropriate lipases. The American Heart Association recommends combined intakes of EPA and DHA of 1 g/day for patients with known coronary heart disease (CHD) and 0.5 g/day for individuals without known CHD.

$$CH_3CH_2CH{=}CH(CH_2CH{=}CH)_4CH_2CH_2CH_2COOH$$
EPA 20:5

$$CH_3CH_2CH{=}CH(CH_2CH{=}CH)_5CH_2CH_2COOH$$
DHA 22:6

Although high-quality, long-chain polyunsaturated fatty acids can be and are obtained from appropriate single cell oils (Ratledge, 2004), fish oils remain the most convenient source of EPA and DHA and fish oils are available in capsule form. Nevertheless, there is some concern that supplies of fish oils may be insufficient to meet the potential demand for omega-3 PUFA. A different concern relates to the undesirable presence of dioxins, polycyclic aromatic hydrocarbons (PAH), mercury compounds, and other environmental contaminants in fish oil. Breivik et al. (2005) have described how the levels of these compounds in fish oils can be markedly reduced by short path distillation. In a representative North Atlantic fish oil, the content of PCB (polychlorinated biphenyls) was reduced from 427 to 7.8 ng/g and of dioxins from 4.7 to 0.5 pg/g, expressed as toxic equivalents. It has been claimed that fish oil "virtually without taste and smell" can be obtained through an enzyme-based deodorisation process.

Urea fractionation, molecular distillation, and enzymic processes have all been used to concentrate one or both of these omega-3 acids on a scale that gives useful quantities of products (Gunstone, 1997). Urea fractionation is a highly useful technique to raise the levels of PUFA to 70 to 80%, efficiently and with high recovery. Saturated or monounsaturated acids or alkyl esters can be trapped in crystals of urea leaving a mother liquor enriched in polyunsaturated acids/esters. The drawback of this method is the large amounts of solvent, chemicals, and by-products involved and its cost (Haraldsson and Bjaltasan, 2001; Breivik et al., 1997).

Supercritical fluid chromatography has been used to concentrate polyunsaturated fatty acid esters from tuna oil containing 18.3 (1.9%), EPA (5.3%), and DHA (23.7%). Alkio et al. (2000) used this technique to produce DHA (90% pure) and EPA (50% pure) in quantities of 1000 and 400 kg/year in the form of their ethyl esters. It is claimed that these products are cost competitive with existing products.

Distillation separates alkyl esters according to their chain length, so fractions enriched in esters of C_{20} and C_{22} acids can be obtained. The technique can also be used after enzymatic reaction to separate (volatile) acids or alkyl esters from (nonvolatile) glycerol esters. Thirty tonnes of fish oil ethyl esters, containing 28% combined EPA and DHA, were distilled to afford nearly 10 tonnes of product with ~50% of EPA and DHA combined (57% recovery). Subsequent precipitation with urea (16 tonnes) gave a concentrate with 84% EPA and DHA (31% recovery). This product is commercially available from Norway. Further enrichment is performed by preparative scale HPLC by which individual acids can be obtained at purity levels >95% (Haraldsson and Bjaltasan, 2001).

The specificity of lipases can be exploited in two ways. Using lipases with 1,3-regiospecificity, it is possible to confine alcoholysis (deacylation) to the *sn*-1/3 positions and leave the acyl groups in the *sn*-2 position still attached to glycerol. These are generally enriched in EPA and especially in DHA.

Some lipases discriminate against acids or esters having a double bond close to the carboxyl group and, thus, distinguish between acids like oleic and linoleic with Δ9 unsaturation and acids, such as EPA, with Δ5 unsaturation and DHA with Δ4 unsaturation. For example, Schmitt-Rozieres et al. (2000) examined the enrichment of polyunsaturated fatty acids from sardine cannery effluents by enzymatic selective esterification. Sardine oil was hydrolysed to give mixed acids with EPA 11.9% and DHA 9.5%. When these were crystallised from acetone at −10°C, the mother liquor contained these two acids at the slightly enhanced levels of 12.2 and 11.7%, respectively. This mixture was esterified with butanol in the presence of an appropriate lipase. Lipozyme (*Rhizomucor miehei* lipase) discriminated against DHA and the level of this in the unreacted acid fraction rose to around 80% with no change in the level of EPA. The lipase in *Candida rugosa* raised EPA levels from 30 to 40%.

Halldorson et al. (2003) have described a useful separation of EPA and DHA by reaction of glycerol with fish oil fatty acids in the presence of immobilized *Rhizomucor miehei* lipase under water-deficient, solvent-free conditions at 40ºC. DHA, and to a lesser extent EPA, remain in the unreacted acid fraction, while the more common fatty acids are converted to glycerol esters. Products with high levels of DHA or EPA require an appropriate choice of starting material (Table 2.85). There is some trade off between concentration of PUFA and recovery and a decision has to be made about the optimum substrate and reaction time.

Bornscheuer et al. (2003) have reviewed the use of fish oil in the production of structured lipids containing

TABLE 2.85 Enhancement of DHA and EPA levels from selected fish oils by lipase-catalysed esterification with glycerol over the time period shown

		Original acids (%)		Recovered free acids			
				Weight %		Recovery %	
	Hours	EPA	DHA	EPA	DHA	EPA	DHA
Herring	24	5.5	8.0	1.9	50.6	3.1	67.6
Tuna	24	5.2	24.5	6.0	71.1	28.6	89.8
Sardine	28	16.8	12.3	13.2	50.0	14.1	78.4
Chilean fish oil	12	20.0	7.2	15.8	25.7	13.1	51.5

Note: The original publication contains details of results obtained after six different reaction times, which are mainly less than those selected for the above table.
Source: Adapted from Halldorson, A., et al., *J. Am. Oil Chem. Soc.,* **80**, 915–921, 2003.

long-chain polyunsaturated fatty acids at the *sn*-2 position and medium-chain acids at the *sn*-1/3 positions (Jennings et al., 1999; Xu et al., 2000). For example, menhaden oil and caprylic acid (8:0) react in the presence of *Rhizomucor miehei* lipase to furnish a product with about 30% of caprylic acid and 30% of EPA and DHA combined (Xu et al., 2000).

References

Achaya, K.T., Ghee, vanaspati, and special fats in India, in *Lipid Technologies and Applications,* Gunstone, F.D., Padley, F.B., Eds., Marcel Dekker, New York, 1997, 369-390.

Ackman, R.G., Fish oils, in *Bailey's Industrial Oil and Fat Products*, Vol. 3, Shahidi, F., Ed., New York, Wiley-Interscience, 2005, 279–318.

Alkio, M. et al. Purification of polyunsaturated fatty acid esters from tuna oil with supercritical fluid chromatography. *J. Am. Oil Chem. Soc.*, **77**, 315–321, 2000.

Anon., Numerical data cited in this chapter are taken from Oil World Annual 2004 or from Oil World 2020 published by ISTA Mielke GmbH, Hamburg, Germany in 2004 and 1999, respectively.

Anon., Oil World Annual 2005, ISTA Mielke GmbH, Hamburg, 2005.

Bell, G. et al. Replacement of marine fish oils with vegetable oils in feeds for farmed salmon, *Lipid Technol.*, **17**, 7–11, 2005.

Berger, K.G., Edible tallow as a food ingredient. *Lipid Technol. Newsl.*, **3**, 56–59, 1997.

Bimbo, A.P., Rendering, in *Bailey's Industrial Oil and Fat Products*, Vol. 6, Shahidi, F., Ed., New York, Wiley-Interscience, 2005, 57–102.

Bornscheuer, U.T. et al. Lipase-caalyzed synthesis of modified lipids, in *Lipids for Functional Foods and Nutraceuticals.* Gunstone, F.D., Ed., Bridgwater, U.K., Oily Press, 2003, 149–182.

Breivik, H. and Thorsrad, O., Removal of organic environmental pollutants from fish oil by short-path distillation. *Lipid Technol.*, **17**, 5–58, 2005.

Breivik, H. et al. Preparation of highly purified concentrates of eicosapentaenoic acid and docosahexaenoic acid. *J. Am. Oil Chem. Soc.,* **74**, 1425–1429, 1997.

Cantor, A.H., Decker, E.A., and Collins, V.P., Fatty acids in poultry and egg products, in *Fatty Acids in Foods and Their Health Implications,* Ching Kuang Chow, Ed., Marcel Dekker, New York, 2000, 125–152.

Christie, W.W. and Clapperton, J.L., Structures of the triglycerides of cow's milk, fortified milks (including infant formulae), and human milk, *J. Soc. Dairy Technol.,* **35**, 22–24, 1982.

Christie, W.W. and Moore, J.H., Stereospecific analysis of the triacylglycerols from various pig tissues. *Biochim. Biophys. Acta,* **210**, 46–56, 1970.

Christie, W.W., Nikolova-Damyanova, B., Laakso, P., and Herslof, B., Stereospecific analysis of triacyl-*sn*-glycerols via resolution of diastereoisomeric diacylglycerol derivatives by high-performance liquid chromatography on silica. *J. Am. Oil Chem. Soc.,* **68**, 695–701, 1991.

De Greyt, W.F.J. and Kellens, M.J., Improvement of the nutritional and physicochemical properties of milk fat, in *Structural and Modified Lipids*, Gunstone, F. D., Ed., Marcel Dekker, New York, 2001, 285–312.

De Greyt, W. and Huyghebaert, A., Lipase-catalyzed modification of milk fats, *Lipid Technol.*, **7**, 10–12, 1995.

Deffense, E., Dry multiple fractionation: trends in products and applications, *Lipid Technol.,* **7**, 34–38, 1995.

Deffense, E., Fractionation of animal carcase fats into hard and soft fats, in Rossell, B., Ed., *Oils and Fats, Vol. 2 – Animal Carcase Fats,* Leatherhead Publishing, Leatherhead, England, 2001, pp. 149–174.

Deffense, E. and Hartel, R.W., Fractionation of milk fat, in *Oils and Fats, Vol. 3, Dairy Fats*, Rossell, B., Ed., Leatherhead Food International, Leatherhead, England, 2003, 158–186.

Diersen-Schade, D.A. and Boettcher, J.A., Docosahexaenoic acid and arachidonic acid: implications for infant development, *Lipid Technol.,* **17**, 225–228, 2005.

Duo Li, et al. Omega-3 fatty acids, in *Lipids for Functional Foods and Nutraceuticals*, Gunstone, F.D., Ed., Bridgwater, U.K., Oily Press, 2003, 225–262.

Fernie, C.E., Conjugated linoleic acid, in *Lipids for Functional Foods and Nutraceuticals*, Gunstone, F D., Ed., The Oily Press, Bridgwater, England, 2003, 295–299.

Fauquant, C., et al., Differently sized native milk fat globules separated by micro filtration; fatty acid composition of the milk fat globule membrane and the triglyceride core, *Eur. J. Lipid Sci. Technol.,* **107**, 80–86, 2005.

Fontecha, J. et al. Triglyceride analysis by gas chromatography in assessment of authenticity of goat milk fat, *J. Am. Oil Chem. Soc.,* **75**, 1893–1896, 1998.

Gibon, V. and Tirtiaux, A., Latest trends in dry fractionation, *Lipid Technol.*, **14**, 33–36, 2002.

Gibon, V., Fractionation of lipids for use in foods, in *Modifying Lipids for Use in Food*, Gunstone, F.D., Ed., Woodhead Publishing Company, Cambridge, England, 2006, 201–233.

Goudjil, H. et al. TAG composition of ewe's milk fat. Detection of foreign fats, *J. Am. Oil Chem. Soc.,* **80**, 219–222, 2003.

Gunstone, F.D., The availability of polyunsaturated fatty acids, *Lipid Technol.*, **9**, 91–94, 1997.

Haas, M.J., Animal fats, in *Bailey's Industrial Oil and Fat Products*, Vol. 1, Shahidi, F., Ed., New York, Wiley-Interscience, 2005, 161–212.

Halldorson, A. et al. Separation of EPA and DHA in fish oil by lipase-catalyzed esterification with glycerol, *J. Am. Oil Chem. Soc.,* **80**, 915–921, 2003.

Hammond, E., Filled and artificial dairy products and altered milk fats, in *Modifying Lipids for Use in Food*, Gunstone, F.D., Ed., Woodhead Publishing Company, Cambridge, England, 2006, 462–487.

Haraldsson, G.G. and Hjaltason, B., Fish oils as sources of important polyunsaturated fatty acids, in *Structured and Modified Lipids*, Gunstone, F.D., Ed., New York, Marcel Dekker, 2001, 313–350.

Haraldsson, G.G. and Hjaltasan, B., PUFA production from marine sources for use in food, in *Modifying Lipids for Use in Food*, Gunstone, F D., Ed., Woodhead Publishing Company, Cambridge, England, 2006, 336–368.

Harris, W.J.P, The use of fats in animal feeds, *Lipid Technol*., **7**, 130–133, 1995.

Hellgren, L.I., Sphingolipids in the human diet — occurrence and implications, *Lipid Technol.*, **14**, 129–133, 2002.

Hettinga, D., Butter, in *Bailey's Industrial Oil and Fat Products*, Vol. 2, Shahidi, F., Ed., New York, Wiley-Interscience, 2005, 1–60.

Hjaltason, B. and Haraldsson, G.G., Use of marine PUFA in food, in *Modifying Lipids for Use in Food*, Gunstone, F.D., Ed., Woodhead Publishing Company, Cambridge, England, 2006, 587–602.

Hjaltason, B. and Haraldsson, G.G., Fish oils and lipids from marine sources, in *Modifying Lipids for Use in Food*, Gunstone, F.D., Ed., Woodhead Publishing Company, Cambridge, England, 2006, 56–79.

Hsieh, T.C.Y. et al. Characterisation of volatile components of menhaden fish oil, *J. Am. Oil Chem. Soc.,* **66**, 114–117, 1989.

Iverson, J.L. and Sheppard, A.J., Determination of fatty acids in butter using programmed gas chromatography of the butyl esters, *Food Chem.,* **21**, 223–234, 1986.

Jahreis, G. and Kraft, J., Sources of conjugated linoleic acid in the human diet, *Lipid Technol.*, **14**, 29–32, 2002.

Jennings, B.H. and Akoh, C.C. Enzymatic modification of triacylglycerols of high eicosapentaenoic and docosahexaenoic acids content to produce structured lipids. *J. Am. Oil Chem. Soc*., **76**, 1133–1137, 1999.

Jensen, R.G., Ed., *Handbook of Milk Fat Composition,* Academic Press, San Diego, 1995.

Jensen, R.G., Fatty acids in milk and dairy products, in *Fatty Acids in Foods and Their Health Implication*, 2nd ed., Ching Kuang Chow, Ed., Marcel Dekker, New York, 2000,109–123.

Jensen, R.G. et al. *Trans* fatty acids in human milk, *Eur. J. Lipid Sci. Technol.,* **102**, 640–642, 2000.

Jensen, R.G., Lipids in human milk, *Lipids,* **34**, 1243–1271, 1999.

Jensen. R.G., Composition of bovine milk lipids*, J. Am. Oil Chem. Soc.*, **50**, 186–192, 1973.

Kayama, M. and Mankura, M., Natural oleochemicals in marine fishes, *Inform,* **9**, 794–799, 1998.

Kaylegian, K.E., Contemporary issues in milk fat technology, *Lipid Technol.*, **11**, 132–136, 1999.

Ki-Teak Lee and Foglia, T.A., Synthesis, purification, and characterization of structured lipids produced from chicken fat, *J. Am. Oil Chem. Soc.,* **77**, 1027–1034, 2000.

Lock, A.L. and Bauman, D.E., Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health, *Lipids*, **39**, 1197–1206, 2004.

Maniongui, C. et al. Determination of bovine butterfat triacylglycerols by reversed-phase liquid chromatography and gas chromatography, *J. Chromatog.,* **543**, 81–103, 1991.

Martin-Susa, S. et al. Lactational changes in the fatty acid composition of human milk gangliosides, *Lipids*, **39**, 111–116, 2004.

Nai-Ting Ma et al. Fatty acid profile and aroma compounds of lipoxygenase-modified chicken oil, *J. Am. Oil Chem. Soc.,* **81**, 921–926, 2004.

Nichols, P.D., Sources of long-chain omega-3 oils, *Lipid Technol.*, **16**, 247–251, 2004.

Pike, I.H. and Barlow, S.M., The fats of life — the role of fish, *Lipid Technol.*, **12**, 58–60, 2000.

Precht, D., Quantitative detection of milk fat in chocolate mixtures I: determination of milk fat in cocoa butter, *Fat Sci., Technol.,* **92**, 153–161, 1990.

Precht, D. and Frede, E., Determination of solid fat content in milk fat by gas chromatography triglyceride analysis, *Fat Sci. Technol.,* **96**, 324–330, 1994.

Precht, D. and Molkentin, J., Identification and quantitation of *cis*/*trans* C16:1 and C17:1 fatty acid positional isomers in German human milk lipids by thin-layer chromatography and gas chromatography/mass spectrometry, *Eur. J. Lipid Sci. Technol.,* **102**, 102–113, 2000.

Rajah, K.K., Manufacture of marketable yellow fats: butter, margarine spread, ghee, and vanaspati, in *LFRA Oils and Fats Handbook, Vol. 1, Vegetable Oils And Fats*, Rossell, B., Ed., Leatherhead Food International, Leatherhead, England, 1999, Y1–32.

Ratledge, C., Single cell oils — a coming of age, *Lipid Technol.*, **16**, 34–39, 2004.

Rhee, K.C., Fatty acids in meats and meat products, in *Fatty Acids in Foods and Their Health Implications,* Ching Kuang Chow, Ed., Marcel Dekker, New York, 2000, 83–108.

Rossell, B., Ed., *Oils and Fats, Vol. 3, Dairy Fats*, Leatherhead Food International, Leatherhead, England, 2003.

Rymer, C. and Givens, D.I., n-3 fatty acid enrichment of edible tissue of poultry: a review, *Lipids*, **40**, 121–130, 2005.

Scheeder, M., Lipids from land animals, in *Modifying Lipids for Use in Food*, Gunstone, F.D., Ed., Woodhead Publishing Company, Cambridge, England, 2006, 28–55.

Scheeder, M., Modifying fats of animal origin for use in food, in *Modifying Lipids for Use in Food*, Gunstone, F.D., Ed., Woodhead Publishing Company, Cambridge, England, 2006, 306–335.

Schmitt-Rozieres, M. et al. Enrichment of polyunsaturated fatty acids from sardine cannery effluents by enzymatic selective esterification, *J. Am. Oil Chem. Soc.,* **77**, 329–332, 2000.

Sébédio, J-L., et al., Eds., *Advances in Conjugated Linoleic Acid Research,* Vol. 2, AOCS Press, Champaign, IL, 2003.

Summers, G. et al. Handling and processing shark livers for the recovery of squalene and diacyl glycerol ethers. DSIR Crop Research Seafood Report No 1 (ISSN 1170-540X), New Zealand, 1994.

Sun, H. et al. Fractionation of squalene from amaranth seed, *J. Am. Oil Chem. Soc.,* **74**, 413–418, 1997.

van Aken, G.A. et al. Composition and crystallisation of milk fat fractions, *J. Am. Oil Chem. Soc.,* **76**, 1323–1331, 1999.

Winter, C.H. et al. Fatty acid composition of human milk triglyceride species, *J. Chromatog.,* **616**, 9–24, 1993.

Wolff, R.L. et al. Evaluation of sequential methods for the determination of butterfat fatty acid composition with emphasis on *trans*-18:1 acids. Application to the study of seasonal variations in French butters, *J. Am. Oil Chem. Soc.,* **72**, 1471–1483, 1995.

Wolff, R.L., Contribution of *trans*-18:1 acids from dairy fat to European diets, *J. Am. Oil Chem. Soc.,* **71**, 277–283, 1994.

Wolff, R.L. et al. *Trans*-18:1 acid content and profile in human milk lipids. Critical survey of data in connection with analytical methods, *J. Am. Oil Chem. Soc.,* **75**, 661–671, 1998.

Xu, X. et al. Modification of menhaden oil by enzymatic acidolysis to produce structured lipids: optimization by response surface design in a packed bed reactor. *J. Am. Oil Chem. Soc.,* **77**, 171–176. 2000.

Yurawecz, M.P., et al., Eds. *Advances in Conjugated Linoleic Acid Research,* Vol. 1, AOCS Press, Champaign, IL, 1999.

2.5 Waxes

2.5.1 General aspects

Strictly speaking, the word "wax" should refer to an ester of a monohydric long-chain alcohol with a long-chain acid. However, generally, the term "wax" is used in the broad sense of the word to include surface lipids, which have properties that are broadly similar to honeycomb material. The types of compounds that can be included under this broad definition are listed in Table 2.86.

Surface waxes are exposed to the environment and, therefore, are chemically rather stable. Thus, there is an absence of functional groups, which might be susceptible to attack by atmospheric agents. Furthermore, the very long carbon chains of most wax components reduces their volatility. In addition, many of the compounds present in surface waxes are rather stable metabolically and are not readily susceptible to microbial degradation (Kolattukudy, 1976).

Certain general structural features of natural waxes have been described by Kolattukudy (1976), and these are summarized in Table 2.87. However, it must also be stressed that the structure and composition of surface waxes vary considerably from organism to organism. Thus, with regard to Table 2.87, the longer aliphatic chains are more abundant in plant waxes than in animal surface waxes, whereas bird waxes may contain appreciable amounts of chains of less than 16 carbons. With regard to branching, methyl branches are the most common, but, in birds, ethyl and propyl branches are found. Although polyunsaturated carbon chains are nearly always absent from surface waxes, substantial proportions of di-unsaturated hydrocarbons have been found in insects. In this case, autoxidation may be reduced by the simultaneous presence of cuticular phenolics. So far as the general composition of surface waxes is concerned, very long chain hydrocarbons are common in insects and plants, but rare in animals. Higher plant waxes contain the most complex mixture of components, while insects and birds have the simplest.

The biosynthesis of individual types of waxes has been thoroughly described for various classes of organisms in Kolattukudy (1976). So far as degradation is concerned, natural waxes often possess sufficient resistance to breakdown to appear in sewage sludge or fossil remains. However, although they have much slower turnovers than internal lipids, waxes are degraded by microorganisms and do not (fortunately) accumulate in excessive amounts in the environment.

Surface waxes serve a number of functions, usually associated with protection. In plants and insects, they prevent desiccation and, in birds, they serve to waterproof feathers. While a few components present in surface lipids can prevent growth of pathogens, the total surface wax layer certainly functions to prevent microbial entry into the organism. Some surface lipids serve as chemical communicants, such as the hydrocarbon sex attractants and kairomone of insects. Although internal waxes are infrequently found in Nature, where they do occur, they act for energy storage (e.g., in jojoba seeds or marine organisms). For a fuller review of all aspects of natural waxes, refer to Kolattukudy (1976).

2.5.2 Mammalian and bird waxes

The greatest amounts and variety of waxes in terrestrial mammals are associated with the skin. The epidermal cells play a role in synthesizing surface lipid, but the major input is provided by the sebaceous glands. These are connected via a duct into a hair canal, from which sebaceous lipid travels to the surface over a period of about a week.

When mammalian surface lipids are considered, there are considerable differences between species. In humans, triacylglycerols are major components, but they are low or absent in other mammals. Sterol and wax esters are usually major, with diesters becoming major (65% in mouse) in some animals. Further details of human surface lipids are given in Section 11.4, and for different animals, refer to Downing (1976).

The main lipid classes for human stratum corneum and sebum are shown in Table 2.88. Thus, sebum contains triacylglycerols and wax monoesters as major components. In contrast, the skin surface layer has ceramides as

TABLE 2.86 Classes of chemicals found in what are generally classified as natural

Class of compound	Occurrence
1. Hydrocarbons	
(a) n-Alkanes	Bacteria, fungi, algae, higher plants, insects and higher animals including mammals[a]
(b) Branched alkanes	Bacteria, fungi, algae, higher plants, insects and higher animals including mammals
(c) Olefins	Bacteria, algae, higher plants (minor), insects and higher animals (minor)
(d) Cyclic alkanes	Plants
(e) Isoprenoid hydrocarbons (other than squalene)	Marine organisms and certain birds
2. Ketones	
(a) Monoketones	Higher plants, bacteria
(b) β- Diketones	Higher plants
3. Secondary alcohols	Higher plants, insects, bacteria (minor)
4. Alkanediols	
(a) Alkane-1, 2-diols (diesters)	Birds, mammals, plants (minor)
(b) Alkane-2, 3-diols (diesters)	Birds
(c) Alkane-α, ω-diols	Plants
5. Acids	
(a) Alkanoic and alkenoic acids	Every living organism[b]
(b) α- or ω-Hydroxy acids	Mammals (as diesters), higher plants
6. Wax esters	
(a) Primary alcohol esters	Bacteria, fungi, algae, higher plants, insects and higher animals including mammals
(b) Secondary alcohol esters	Insects
(c) Diester waxes	
Alkanediol diesters	Birds, mammals, insects
Diesters of hydroxy acids	Mammals, insects
(d) Triesters	
Triacylglycerols	Plants, animals
Triesters of alkane- 1,2-diol, ω- hydroxy acid and fatty acid	Mammals[c]
Esters of hydroxymalonic acid, fatty acid and alcohol	Birds
Triesters of hydroxy acids, fatty acid and fatty alcohol	Insects[d]
Triesters of fatty acid, hydroxy acid and diol	Insects
(e) Polyesters of hydroxy fatty acids	Higher plants[e]
7. Primary alcohols	Bacteria, algae, higher plants, insects and higher animals including mammals
8. Aldehydes	Higher plants
9. Terpenoids	Plants and animals[f]

Note: In bacteria, fungi and algae, there is no evidence of the occurrence of surface waxes, but waxy materials have been isolated from them. In order to indicate the widespread occurrence of such compounds, these organisms are included in this table.

[a] The source of the hydrocarbons has not been established; proof of synthesis within the mammalian tissues is not available except in wool wax.

[b] Free fatty acids (very long) occur in the surface of waxes of mainly higher plants and humans.

[c] Thus far, it has been identified only in the rhino mutant mouse.

[d] Only beeswax that has been subjected to careful analysis has revealed the presence of the diesters and triesters indicated.

[e] Insoluble biopolyester called "cutin" is the structural component of the plant cuticle.

[f] Pentacyclic triterpenes are major components of certain plant waxes, while sterols occur in most higher animals.

From Kolattukudy (1976).

TABLE 2.87 General structural features of natural waxes

Chain length	Very long chains (up to C_{62}) are common
Branching	Branched carbon chains common, with methyl branches frequent
Unsaturation	Polyunsaturated chains nearly always absent; double bonds, when present, at different positions from those of internal lipids
Functional types	Saturated hydrocarbons, olefins, wax esters, aldehydes, ketones, primary and secondary alcohols and terpenoids can be present; the bulk of the surface lipid is distinctly different from the major internal lipids of the same organism

a major fraction. The ceramides of the stratum corneum from various species have been characterised and shown to be diverse (see Rawlings, 1996). The biosynthesis of epidermal lipids, their biological significance and factors affecting composition have been reviewed by Rawlings (1996).

In birds, waxes are produced by the uropygial gland situated on the rump at the bottom of the tail feathers. The wax is then transferred to the feathers during the act of preening. Among uropygial secretions, monoester waxes predominate. In the fatty acid moieties, methyl branches are common, while the alcohol part usually contains a homologous series of unbranched and monomethyl-branched chains. Additionally, the uropygial gland wax composition varies significantly from order to order (cf. Table 2.89). Extensive details of compositions are given in Jacob (1976) and the biosynthesis of bird waxes is discussed by Buckner and Kolattukudy (1976).

2.5.3 Insect waxes

A waxy layer, whose primary function is to prevent desiccation, covers the surface of insects. Hydrocarbons comprise a majority of the cuticular lipids, while wax esters, sterol esters, alcohols and nonesterified fatty acids are also common components. In addition to preventing desiccation, insect waxes also function to prevent abrasion, to act as a barrier against microbial penetration, to reduce the absorption of toxic environmental chemicals (including insecticides), and, in some cases, certain components may act in chemical communication (Jackson and Blomquist, 1976).

Some examples of the compositions of different insect waxes are given in Table 2.90. It will be seen that the overall composition varies considerably between species, but with hydrocarbons usually representing the major component. It will also be noticed that there are large differences between the life stages of a given insect. This is particularly so for the stonefly, where the aquatic naiad has a much higher percentage of triacylglycerols.

The predominant n-alkanes of insects are odd chain lengths in the range C_{21} to C_{33}. Branched alkanes and alkenes are also usually odd numbered. In primary alcohol wax esters, even-chain fatty acids and alcohols usually predominate, but secondary alcohol wax esters are unusual components. Triacylglycerols, when significant,

TABLE 2.88 The main lipid classes of the stratum corneum and sebum

Sebum lipids		Stratum corneum lipids	
Triacylglycerols	41%	Ceramides	50%
Wax Monesters	25%	Fatty Acids	25%
Fatty Acids	16%	Cholesterol	19%
Squalene	12%	Cholesterol Sulphate	4%
Sterol Esters	3%	Phospholipids	1%
Free Sterol	1%	Glucosyl Ceramides	1%
Unidentified	2%		

From Rawlings (1996).

TABLE 2.89 Composition (%) of the uropygial gland secretions of different birds

	Heron (*Aredea cincrea*)			Kestrel (*Falco tinnunculus*)		Oyster-Catcher (*Haematopus ostralegus*)		Eagle Owl (*Bubo bubo*)	
Chain type	**Triacylglycerol fatty acids**	**Wax fatty acids**	**Wax alcohols**	**Wax acids**	**Wax alcohols**	**Wax acids**	**Wax alcohols**	**Wax acids**	**Wax alcohols**
Unbranched	98	100	100	1	32	1	50	3	23
Monomethyl	–	–	–	37	57	63	48	2[a]	48
Dimethyl	–	–	–	45	10	35	–	26	29
Trimethyl	–	–	–	16	–	–	–	3	–

[a] This fraction contains 42% monoethyl fatty acids.
Data from Jacob (1976).

TABLE 2.90 Major classes of surface lipids from nine insects

	Lipid content (wt%)								
	Big stonefly (*Pteronarcys californica*)		Mormon cricket (*Anabrus simplex*)	Cockroaches (*Periplaneta australasiae, P.brunnea, P.fulginosa*)	Blowfly (*Lucilia cuprina*)		Eri silkworm (*Cynthia ricini*)	Mealworm (*Tenebrio molitor*)	Migratory grasshopper (*Melanoplus sanguinipes*)
	Naiad	*Adult*			*Pupa*	*Adult*			
Hydrocarbon	3	12	48–58	91	33	62	5	10	60
Wax esters	1	4	9–11	–	–	–	16	13	28
Triacylglycerols	78	7	–	7	25	16	–	–	1
Free fatty acids	12	49	15–18	2	16	7	tr	5	6
Aliphatic alcohols	–	–	2–3	–	–	–	70	58	2
Sterols	1	18	–	1	2	–	tr	1	1
Others	5	10	15–18	–	15	24	8	13	–

From Jackson and Blomquist (1976).

usually have C_{16} and C_{18} saturated and unsaturated acyl groups. Sterols are often found in small amounts, with cholesterol the most common compound. Primary alcohols and nonesterified fatty acids are both usually even-chain compounds.

2.5.3.1 Occurrence and characteristics

A general source of information is Stanley-Samuelson and Nelson (1993). The biosynthesis of the individual insect wax components is discussed by Jackson and Blomquist (1976). Chemical analysis of insect waxes is reviewed by Lockey (1985) and Nelson (1978), and recent aspects of this and biosynthesis can be found in Nelson and Blomquist (1996). Over a hundred insect species have now been studied and many of their hydrocarbons function as sex phenomones, antiaphrodisiacs and kairomones, in addition to their role as waterproofing chemicals. In addition, recent work shows that the hydrocarbons may be present internally in larger amounts than on the surface and that changes in composition may occur sooner for the internal fraction. Insect hydrocarbons are being used increasing as chemotaxonomic characters (Nelson and Blomquist, 1996).

2.5.4 Plant waxes

Epicuticular wax of plants lies on and is interspersed with the insoluble part of the leaf cuticle known as cutin (see Section 1.2.12). The major components are indicated in Table 2.91. Thus, n-alkanes, monoesters, polyesters of ω-hydroxy acids, primary alcohols, acids, secondary alcohols and ketones, and β-di-ketones can be generally regarded as widespread and major constituents (Tulloch, 1976) (cf. Table 2.92).

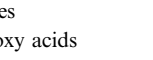
The chain lengths of the hydrocarbons and primary alcohols of some frequently analysed waxes are shown in Table 2.93. Hydrocarbons of C_{27}, C_{29}, and C_{31} are the most common, while the free alcohols are usually of C_{26}, C_{28}, and C_{30}. Free acids have chain lengths that are generally similar to those of the alcohols from the same species, although the waxes of the apple and the rose have free acids that contain a much higher proportion of C_{16} to C_{22} components than the free alcohols. Jojoba accumulates a storage wax and is commercially important (see Sections 2.3.56 and 11.8).

The biosynthesis and degradation of plant waxes was reviewed by Kolattukudy et al. (1976) and Kolattukudy (1980) and more recently by von Wettstein-Knowles (1996), who has also covered genetic aspects. A recent review of the biosynthesis of plant cuticular wax is that by Kunst and Samuels (2003). These authors include aspects of transport of the components to the plasma membrane, export from the cell and, then, movement across the cell wall to the cuticle. Physical aspects of waxes, as transport barriers in plant cuticles, are reviewed by Riederer and Schreiber (1996), while methods for analysis are covered by Walton (1990) and Linskens and Jackson (1991). Junipér (1996) discusses the role of plant surface waxes during interactions with insects and the chapter by Bianchi (1996) is also a useful general source of information about plant waxes.

2.5.5 Marine waxes

The phytoplankton contain little or no wax esters and these compounds are primarily an animal product; exceptions are the marine cryotomonad, *Chroomonas salina,* which makes large amounts of wax ester under certain growth conditions (Henderson and Sargent, 1989), and the dinoflagellate, *Peridinium foliaceum* (Lee and Patton, 1989). Seven phyla have been found to contain species where wax esters are major lipid components (>10% of the total lipid). Some examples of families where wax analyses have been made are shown in Table 2.94. In addition to those animal phyla listed in this table, the

TABLE 2.91 Cuticular wax components

Compounds	General structure	Notes
Hydrocarbons		
n-Alkanes	$H_3C[CH_2]_n\ CH_3$	Common; usually C_{29}, C_{31}
Isoalkanes	$[CH_3]_2CHR$	Not as common as n-alkanes
Anteisoalkanes	$H_3CCH_2CH(CH_3)R$	
Internally branched alkanes	$R^1CH(CH_3)R^2$	
Cyclic alkanes	$C_6H_{11}R$	Very small amounts
Alkenes	$R^1CH{=}CHR^2$	Sometimes important
Terpenoid hydrocarbons, e.g. farnesene, pristane		
Aromatic hydrocarbons		Anthracene or phenanthrene type with an attached alkane chain
Ketones	R^1COR^2	Not as common as alkanes
Ketols	$R^1CHOH[CH_2]_nCOR^2$	α-, β- and δ-ketols, all rare
Secondary alcohols	$R^1CH(OH)R^2$	About as common as ketones
β- Diketones	$R^1COCH_2COR^2$	Usually minor, sometimes major
Monoesters	R^1COOR^2	Common. Contain even-chain saturated acid and alcohol components
Phenolic esters		Rare
Diesters		Rare
Polyesters		Only important in gymnosperms
Primary alcohols	RCH_2OH	Common; even chains predominate
Aldehydes	RCHO	Usually minor; not as common as alcohols
Acids		
Alkanoic acids	RCOOH	Very common; even-chain saturated usually
Dicarboxylic acids	$HOOC[CH_2]_nCOOH$	Uncommon
γ- Lactones		Rare. δ-Lactones also rare
ω- Hydroxy acids		Not common
Diols		
α, ω-Diols	$HOCH_2[CH_2]_nCH_2OH$	Not very common
α-, β-Diols	$HOCH_2CH(OH)[CH_2]_nCH_3$	Rare
Terpenes		Many types found; not as common as other wax components
Flavones		Occur occasionally

From Kolattukudy (1980).

TABLE 2.92 Composition of waxes of some Gramineae

	Major Components (% of total)				
Species	**Hydrocarbons**	**Esters**	**Alcohols**	**β--Diketones**	**β- Hydroxy-diketones**
Agropyron cristatum	15	20	48	–	–
Avena sativa	5	10	45	5	3
Elymus cinereus	8	9	8	34	21
Festuca ovina	5	5	7	70	1
Lolium perenne	5	5	70	–	–
Secale cereale	5	10	15	40	13
Stipa tenacissima	20	10	10	–	–
Triticum aestivum	10	15	20	20	10

Source: Tulloch (1976).

Ctenophora, Chaetognatha, and Annelida contain species in which wax esters are major components, i.e., >10% total lipid (Lee and Patton, 1989). Copepods, which are the dominant zooplankton in most areas of the ocean, contain wax esters in an oil sac. The size of the sac changes with the amount of food digested and is presumed to have a reserve energy function (Sargent et al., 1976; Bauermeister and Sargent, 1979). Fish also often

TABLE 2.93 Composition of hydrocarbons and free alcohols from some plant waxes

	Grape leaf		Apple fruit		Pea leaf		Sugarcane stem	
Chain length	**H**	**A**	**H**	**A**	**H**	**A**	**H**	**A**
22	–	1	–	2	–	–	–	–
24	4	7	–	6	–	2	1	–
25	10	–	1	–	–	–	7	–
26	6	21	–	33	–	58	5	14
27	17	–	13	3	–	1	56	–
28	8	42	1	32	–	39	3	73
29	30	1	81	5	1	–	13	–
30	3	16	1	13	–	–	2	7
31	12	–	1	–	98	–	4	–
32	–	4	–	–	–	–	–	6
33	–	–	–	–	–	–	2	–

Note: H = hydrocarbons, A = alcohols
From Tulloch (1976).

TABLE 2.94 Occurrence of wax esters in marine animals[a]

Phylum	**Order/Family**	**Wax esters (% lipid)**
Coelenterata	Acinaria (sea anemones)	15–40
	Madreporaria (scleractinian corals)	92
	Zoantharia (zoanthid corals)	30
Mollusca	Cranchiidae (squid)	32
Arthropoda	Amphipoda	12–80
Copepoda	Actideidae	11–44
	Calanidae	21–92
	Eucalanidae	11–69
	Euchaetidae	31–82
	Heterorhabdidae	67–69
	Lucicutidae	52–63
	Metridiidae	41–76
Decapoda	Incl. Euphausia (krill)	15–69
Chordata	Elasmobranchii (e.g., frill sharks)	58
	Beryciformes	90
	Clupeiformes (e.g., bristlemouths)	16–85
	Crossopterygii (e.g., hatchet fishes)	10–97
	Gadiformes (e.g., cod)	25–60
	Myctophiformes (e.g., lantern fishes)	12–90
	Perciformes (e.g., dolphins, mullets)	18–92
	Zeiformes	76
	Odontoceti (e.g., dolphins, sperm whale)	30–80

For full information, refer to Sargent et al. (1976).

contain significant amounts of wax esters, and it has been calculated that much of the organic matter produced in the euphotic zone will be transformed to wax esters (Sargent et al., 1976).

In contrast, although hydrocarbons occur in all marine organisms, they generally account for 1% or less of the total lipid. Notable exceptions are the liver oils from some sharks, which may contain over 30% of their lipid as squalene.

The chain lengths of some examples of marine wax esters are shown in Table 2.95. Most fish species, marine mammals and deep-water zooplankton have C_{32}, C_{34}, C_{36}, and C_{38} as major components, while upper water zooplankton (e.g., *Calcanus hyperboreas)* often have C_{42} as an additional significant component. When the fatty acid and alcohol components from wax esters are examined, some differences in chain length are obvious (Table 2.96). Thus, 16:0 and 18:1 chains are common for alcohols, while the acids are mainly 16:1 and 18:1 with 22:6 often being a significant component.

Most marine organisms contain odd-chain-length n-alkanes in the range C_{13} to C_{33}. The predominant hydrocarbons of most marine algae are polyunsaturated (particulary all-*cis*-3,6,9,12,15,18-henicosahexaene). Branched-chain hydrocarbons occur in some marine bacteria and cyanobacteria. Two terpenoid hydrocarbons with a wide distribution are squalene and pristane. Squalene is a major component of the liver fat of certain sharks and the eulachon. Pristane is a major hydrocarbon in copepods and other zooplankton.

2.5.5.1 Occurrence and characteristics

The biosynthesis and degradation of marine wax esters and hydrocarbons has been reviewed by Sargent et al., (1976). As far as functions are concerned, the wax esters may serve as a source of metabolic energy or of metabolic water, for buoyancy, as biosonar (e.g., in the head regions of whales and porpoises), or for thermal insulation. Further details can be found in Sargent et al. (1976) and in Lee and Patton (1989). The biochemistry of marine copepods is covered by Sargent and Henderson (1986) and Sargent and Falk-Petersen (1988).

TABLE 2.95 Wax ester chain-length composition (wt%) of some marine animals

Carbon number	*Gaussia princeps* (copepod; whole animal)	*Cyclothone acclinidene* (bristlemouth; whole animal)	*Gnathophausia ingens* (mysid shrimp; egg)	*Lampanyctus ritteri* (lantern fish; muscle)	*Mugil cephalus* (mullet; roe)	*Physeter catodon* (sperm whale; head oil)
26	–	–	–	–		2
28	–	1	–	–	–	6
30	1	6	tr	1	5	11
31	1	–	1	–	6	1
32	12	9	10	10	24	19
33	5	2	7	–	10	3
34	47	18	22	72	19	26
35	tr	3	9	–	5	3
36	15	13	15	16	12	20
37	2	3	–	–	4	1
38	11	17	25	1	12	8
39	1	3	–	–	1	–
40	3	17	8	–	2	–
42	2	8	–	–	–	–

Adapted from Sargent et al. (1976).

TABLE 2.96 Fatty acid and alcohol compositions (wt%) from wax esters of marine animals

Fatty acid	*Gaussia princeps* (copepod; whole animal)		*Cyclothone acclinidene* (bristlemouth; whole animal)		*Gnathophausia ingens* (mysid shrimp; egg)		*Lampanyctus ritteri* (lantern fish; muscle)		*Mugil cephalus* (mullet; roe)		*Physeter catodon* (sperm whale; head oil)	
	Alcohol	Acid	Alcohol	Acid	Alcohol	Acid	Alcohol	Acid	Alcohol	Acid	Alcohol	Acid
12:0	–	–	–	2	–	–	–	–	–	–	tr	6
14:0	8	tr	3	2	8	tr	5	tr	10	1	10	11
14:1	tr	–	–	–	–	–	tr	–	–	–	1	7
15:0	tr	tr	–	tr	–	–	1	tr	6	tr	2	tr
16:0	55	1	22	3	53	1	82	2	54	4	27	4
16:1	tr	9	tr	12	7	12	1	4	14	23	10	21
16:2	–	tr	–	3	–	–	–	1	–	5	–	–
16:3	–	–	–	–	–	–	–	–	–	2	–	–
18:0	1	tr	1	1	6	tr	tr	1	6	1	3	1
18:1	12	71	9	42	12	51	7	72	5	13	39	35
18:2	1	2	1	2	–	1	–	3	–	4	–	–
18:3	–	1	6	–	–	1	tr	tr	–	3	–	–
18:4	–	–	–	–	–	–	–	–	–	3	–	–
20:1	5	2	27	6	1	2	tr	14	–	–	7	9
20:4	–	tr	–	4	–	1	–	–	–	4	–	–
20:5	–	6	–	6	–	7	–	tr	–	9	–	–
22:1	12	–	14	–	–	–	tr	2	–	–	–	–
22:5	–	–	–	–	–	–	–	–	–	5	–	–
22:6	–	2	–	13	–	12	–	tr	–	6	–	–
24:1	3	–	4	–	–	–	–	1	–	–	–	–

Adapted from Sargent et al. (1976).

References

Bauermeister, A. and Sargent, J.R. (1979). Wax esters: major metabolites in the marine environment. *Trends Biochem. Sci.,* **4**(9), 209–211.

Bianchi, G. (1996) Plant Waxes. In *Waxes: Chemistry, Molecular Biology and Functions*, Ed. R.J. Hamiliton, The Oily Press, Dundee, pp. 175–222.

Buckner, J.S. and Kolattukudy, P.E. (1976). Biochemistry of bird waxes. In *Chemistry and Biochemistry of Natural Waxes*, Ed. P.E. Kolattukudy, Elsevier, Amsterdam, pp. 147–200.

Downing, D.T. (1976). Mammalian waxes. In *Chemistry and Biochemistry of Natural Waxes*, Ed. P.E. Kolattukudy, Elsevier, Amsterdam, pp. 17–48.

Downing, D.T. et al. (1986) The role of sebum and epidermal lipids in the cosmetic properties of skin. *Int. J. Cosmetic Sci.* **8**, 115–123.

Henderson, R.J. and Sargent, J.R. (1989). Lipid composition and biosynthesis in aging cultures of the marine

cryptomonad, *Chroomonas salina. Phytochemistry*, **28**, 1355–1361.
Jackson, L.L. and Blomquist, G.J. (1976). Insect waxes. In *Chemistry and Biochemistry of Natural Waxes*, Ed. P.E. Kolattukudy, Elsevier, Amsterdam, pp. 201–233.
Jacob, J. (1976). Bird waxes. In *Chemistry and Biochemistry of Natural Waxes*, Ed. P.E. Kolattukudy, Elsevier, Amsterdam, pp. 93–146.
Juniper, B.E. (1996) Waxes on plant surfaces and their interactions with insects. In *Waxes: Chemistry, Molecular Biology and Functions*, Ed. R.J. Hamilton, The Oily Press, Dundee, pp. 157–174.
Kolattukudy, P.E. Ed. (1976) *Chemistry and Biochemistry of Natural Waxes,* Elsevier, Amsterdam.
Kolattukudy, P.E. (1980). Cutin, suberin and waxes. In *The Biochemistry of Plants*, Eds. P.K. Stumpf and E.E. Conn, vol. 4, Academic Press, New York, pp. 571–645.
Kolattukudy, P.E. et al. (1976). Biochemistry of plant waxes. In *Chemistry and Biochemistry of Natural Waxes,* Ed. P.E. Kolattukudy, Elsevier, Amsterdam, pp. 289–347.
Kunst, L. and Samuels, A.L. (2003) Biosynthesis and secretion of plant cuticular wax. *Prog. Lipid Res.*, **42**, 51–80.
Lee, R.F. and Patton, J.S. (1989) Alcohols and waxes. In *Marine Biogenic Lipids, Fats and Oils*, Ed. R.G. Ackman, CRC Press, Boca Raton, FL, pp. 73–102.
Linskens, H.F. and Jackson, J.F. Eds. (1991). *Essential Oils and Waxes: Modern Methods of Plant Analysis*, vol. 12, Springer, Berlin.
Lockey, K.H. (1988) Lipids of the insect cuticle-origin, composition and function. *Comp. Biochem. Physiol.,* **89B**, 595–645.
Nelson, D.R. (1978) Long-chain methyl-branched hydrocarbons: occurrence, biosynthesis and function. *Adv. Insect Physiol.,* **13**, 1–33.
Nelson, D.R. and Blomquist, G.J. (1996) Insect waxes. In *Waxes: Chemistry, Molecular Biology and Functions* Ed. R.J. Hamilton, The Oily Press, Dundee, pp. 1–90.
Rawlings, A.V. (1996) Skin waxes: their composition, properties, structures and biological significance. In *Waxes: Chemistry, Molecular Biology and Functions*, Ed. R.J. Hamilton, The Oily Press, Dundee, pp. 223–256.
Riederer, M. and Schreiber, L. (1996) Waxes — the transport barriers of plant cuticles. In *Waxes: Chemistry, Molecular Biology and Functions,* Ed. R.J. Hamilton, The Oily Press, Dundee, pp. 131–156.
Sargent, J.R. and Henderson, R.J. (1986). Lipids. In *The Biological Chemistry of Marine Copepods*, Eds. E.D.S. Corner and S.C.M. O'Hara, Clarendon, Oxford, pp. 59–108.
Sargent, J.R. et al. (1976). Marine waxes. In *Chemistry and Biochemistry of Natural Waxes*, Ed. P.E. Kolattukudy, Elsevier, Amsterdam, pp. 49–91.
Sargent, J.R. and Falk-Petersen, S. (1988). The lipid biochemistry of calanoid copepods. *Hydrobiologia*, **167/168**, 101–114.
Stanley-Samuelson, D.W. and Nelson, D.R. Eds. (1993) *Insect Lipids: Chemistry, Biochemistry and Biology.* University of Nebraska Press, Lincoln.
Tulloch, A.P. (1976). Chemistry of waxes of higher plants. In *Chemistry and Biochemistry of Natural Waxes*, Ed. P.E. Kolattukudy, Elsevier, Amsterdam, pp. 235–287.
Von Wettstein-Knowles, P. (1996) Biosynthesis and genetics of waxes. In *Waxes: Chemistry, Molecular Biology and Functions*, Ed. R.J. Hamilton, The Oily Press, Dundee, pp. 91–130.
Walton, T.J. (1990). Waxes, cutin and suberin. In *Methods in Plant Biochemistry*, Eds. J.L.Harwood and J.R. Boyer, vol. 4, Academic Press, London, pp. 105–158.

2.6 Egg lipids

Egg yolks are a well-known source of lipids and are frequently used for the isolation of phosphatidylcholine (Wells and Hanahan, 1969). Between 32 and 35% (depending on species) of the egg's weight is contributed by the yolk, of which about one-third is lipid. A typical composition — that of hen yolk lipids — is shown in Table 2.97. It will be seen that triacylglycerols and phosphatidylcholine are the major constituents (Long, 1961). The lipids appear to be largely complexed to proteins *in vivo*.

The yolk of hen's eggs has been resolved into four major fractions using ultracentrifugation procedures (Cook and Martin, 1969). These are the very low-density lipid (VLDL) fraction (70%), a water-soluble fraction (8%), the low-density fraction of the granule (4%), and the phosvitin-lipovitellin fraction (18%) of total yolk solids. The very low-density fraction can be split further into two subfractions (see Kuksis, 1992). The lipid composition of yolk VLDL is essentially the same as the plasma VLDL from which it was derived. Triacylglycerol is the main component, accounting for about two-thirds of the total lipid with phospholipid at 25% and cholesterol at about 5% (Speake et al., 1998). About 95% of the egg's lipids are in the VLDL fraction. The phosvitin-lipovitellin fraction contains α-lipovitellin (41%), β-lipovitellin (38%), and phosvitin (21%). The protein content of these fractions is high. Aspects of hepatic synthesis and oocyte uptake of precursors have been reviewed (Kuksis, 1992; Speake et al., 1998).

There is much interest in dietary cholesterol and eggs are recognized as being a major source of this sterol (see Section 11.1). The cholesterol of eggs is predominantly unesterified and represents about 5% of the total lipid (0.3g per hen's egg).

TABLE 2.97 The lipid composition of hen's egg yolk

Lipid class	Amount (g) /egg	Percentage Lipid (wt/wt)
Neutral lipids	3.71	61.7
Sterols (cholesterol)	0.29	4.8
Phospholipids	2.01	33.5
Phosphatidylcholine	1.47	24.5
Lysophosphatidylcholine	0.12	0.8
Sphingomyelin	0.05	0.1
Phosphatidylethanolamine	0.30	5.0
Lysophosphatidylethanolamine	0.04	0.7
Inositol phospholipids	0.01	tr
Plasmalogen	0.02	tr
Aminoacylphospholipid	tr	tr

Source: Data from Long (1961).

The fatty acid composition of yolk lipids has been estimated. The major fatty acids are palmitic and oleic, but their enrichments in triacylglycerols and phospholipids differ. Triacylglycerols contain about 46% oleate, 25% palmitate, 15% linoleate and 6% stearate as major acids. Phospholipids contain about 30% oleate, 28% palmitate, 14% linoleate and 15% stearate as major acids (Long, 1961). The phospholipids also contain the bulk of the very long chain PUFAs, such as arachidonate and docosahexaenoate (Speake et al., 1998). It should be borne in mind that the fatty acid composition of yolk lipids is dependent on the hen's diet. Indeed, efforts to increase the n-3 PUFA content have been made successfully in recent years. In contrast, dietary fat has little effect on yolk total lipid or cholesterol content (Kuksis, 1992).

There is now much information on the detailed molecular species composition of major classes of yolk lipids. For triacylglycerol (Table 2.98), the saturates are minor components and three species dominate, the monoene, triene and polyene fractions. By contrast, the dienes are mainly the palmitoyl-dioleoyl species (Table 2.99). As mentioned above, the exact balance of species can be changed significantly by the hen's diet. However, the final fatty acid composition of hen's yolk does not reflect the dietary percentage directly, owing to rapid metabolism and limits to the extent of incorporation for individual acids (see Anderson et al., 1989; Kuksis, 1992; Watkins, 1991).

The major phospholipid components of yolk are phosphatidylcholine and phosphatidylethanolamine. These differ significantly in their fatty acid contents and molecular species distributions (Table 2.100). Both are rich in polyunsaturated (and essential) fatty acids, but phosphatidylethanolamine is notable by having major proportions of arachidonate and docosahexaenoate. Further details, including analytical results for other acyl lipids, are given in Kuksis (1992). A review by Speake et al. (1998) gives a good deal of detail, including information on metabolism.

TABLE 2.98 Effect of species and diet on the fatty acid composition of yolk triacylglycerols

	Chicken	Duck		Goose	
	Farmed	Farmed	Wild	Farmed	Wild
16:0	28	38	35	26	24
16:1 n-7	1	1	1	2	3
18:0	17	12	9	5	4
18:1 n-9	25	24	28	56	44
18:2 n-6	16	6	7	6	4
18:3 n-3	tr	tr	tr	1	18
20:4 n-6	6	15	12	tr	tr
22:6 n-3	6	2	4	tr	tr

Note: tr, <0.5%
Data from Speake et al. (1998).

TABLE 2.99 Major molecular species of egg yolk triacylglycerols

Molecular species	Fraction (mol %)
Saturates (0.7%)	
16:0–16:0-16:0	18
16:0-–18:0–16:0	47
16:0–18:0–18:0	29
Monoenes (16.4%)	
16:0–18:1–16:0	24
16:0–18:1–18:0	43
18:0–18:1–16:0	14
Dienes (38.5%)	
16:0–18:1–18:1	74
Trienes (28.4%)	
16:0–18:2–18:1	51
18:1–18:2–16:0	10
18:1–18:1–18:1	19
Polyenes (16:0%)	
16:1–18:2–18:1	24
16:0–18:2–18:2	18
18:1–18:2–18:1	44

Source: Kuksis (1992).

TABLE 2.100 Major molecular species (mol %) of egg yolk phosphatidylcholine and phosphatidylethanolamine

Molecular species	Phosphatidylcholine	Phosphatidylethanolamine
16:0-18:1	38.2	11.5
18:0-18:1	9.3	10.0
16:0-18:2	21.8	6.2
18:0-18:2	11.2	12.0
18:0-20.4	3.4	28.5
Others	16.1	31.8

From Kuksis (1992).

References

Anderson, G.J. et al. (1989). Rapid modulation of the *n*-3 docosahexaenoic acid levels in the brain and retina of the newly hatched chick. *J. Lipid Res.*, **30,** 433–441.

Cook, W.H. and Martin, W.G. (1969) Egg lipoproteins. In *Structural and Functional Aspects of Lipoproteins in Living Systems,* Eds. E. Tria and A.M. Scanu, Academic Press, London, pp. 579–615.

Johnson, A.R. and Davenport, J.B. (1971) *Biochemistry and Methodology of Lipids*, London, Wiley-Interscience.

Kuksis, A. (1992) Yolk lipids. *Biochim. Biophys. Acta*, **1124**, 205–222.

Long, C. Ed. (1961) *Biochemists' Handbook*, E. & F.N. Spon, London.

Speake, B.K. et al. (1998). Transport and transformation of yolk lipids during development of the avian embryo *Prog. Lipid Res.*, **37**, 1–32.

Watkins, B.A. (1991) Importance of essential fatty acids and their derivatives in poultry. *J. Nutr.*, **121**, 1475–1485.

Wells, M.A. and Hanahan, D.J. (1969) Phospholipase A_2 from *C. adamanteus venom*. *Methods Enzymol.,* **14**, 178–184.

2.7 Milk lipids

The lipid content of mammalian milk varies from 16 g/litre (horse) to 105 g/litre (deer). Human milk contains about 38 g of fat per litre (Long, 1961). When the fat content is expressed in terms of the total calories available, then most mammals, including humans, have a rather similar value, with about half of the total calories being provided by lipid (see also Section 2.4).

The principal lipids of milk are triacylglycerols, which may represent up to 99% of the total lipids (Table 2.101). Human milk contains significant quantities of phospholipids, sterols, sterol esters, unesterified fatty acids, and monoacylglycerols. In contrast, the proportion of phospholipids in bovine milk is low (Table 2.101).

The major phospholipids of milk from those mammals examined so far are phosphatidylethanolamine, phosphatidylcholine, and sphingomyelin. These each comprise about 30% of the total phospholipids (Morrison, 1970; Jensen, 1996).

The fatty acid composition of different milk fats is shown in Table 2.102. It is clear that palmitic and oleic acids are the main components. Other acids usually present as significant components are capric, lauric, myristic, palmitoleic, and linoleic acids. Although medium-chain acids (C_8 to C_{12}) usually represent 8 to 15% of the total acids of milk triacylglycerols, they may comprise up to 65% in rabbits and about 100% of the total in elephants. The chain-termination mechanism by which medium-chain fatty acids are released from fatty acid synthase is of some interest to biochemists (see Section 11.1.1; Dils and Parker, 1982). When present, the medium-chain fatty acids tend to be concentrated at the *sn*-3 position. Surprisingly, palmitate is enriched at the *sn*-2 position (Table 2.103) in contrast to the usual concentration of unsaturated fatty acids there.

TABLE 2.101 Lipid class percentage composition of mammalian milks

	Human	[a] Human (Day 3)	[a] Human (Day 84)	Rat	Bovine
Free sterols	7.5	1.3	0.4	2.5	0.4
Sterol esters	1.4	5.0	1.0	1.5	tr
Triacylglycerols	81.0	97.6	99.0	83.3	95.5
Diacylglycerols	2.7	–	–	3.5	3.0
Monoacylglycerols	–	–	–	1.9	0.2
Unesterified fatty acids	2.8	–	–	6.2	0.4
Phosphoglycerides	4.6	1.1	0.6	0.5	0.5

Adapted from Morrison (1970) and [a]Jensen (1996). The values ([a]) were corrected for the products of hydrolysis (see Jensen, 1996).

The fatty acid compositions of phosphatidylcholines and sphingomyelins from several mammals are shown in Table 2.104. In contrast to triacylglycerols, the fatty acids of these phospholipids are mainly long and very long chain. Palmitic, stearic, oleic, and, sometimes, linoleic acids are major components of the phosphatidylcholine fraction. In contrast, sphingomyelin has much smaller amounts of unsaturated components. Palmitic, stearic, arachidic, behenic, and lignoceric acids are major components. In sheep milk sphingomyelin, tricosanoic acid is a major constituent, while in the other species shown, nervonic acid is the major unsaturated fatty acid (Table 2.104).

A summary description of the formation of the milk fat globule and its movement into the lumen of the mammary gland has been given (Gurr and Harwood, 1991, and

TABLE 2.102 Fatty acid composition (wt%) of the milk fats of various species

Species	6:0	8:0	10:0	12:0	14:0	16:0	18:0	16:1	18:1	18:2	18:3
Red Kangaroo	–	–	–	–	1.0	22.4	6.7	5.1	55.2	6.3	2.7
Rhesus macaque		5.4	7.0	2.4	1.9	20.9	4.7	4.7	30.8	10.7	2.6
Human	–	–	1.3	3.1	5.1	20.2	5.9	5.7	46.4	13.0	1.4
Domestic rabbit	–	22.4	20.1	2.9	1.7	14.2	3.8	2.0	13.6	14.0	4.4
Bat (*T. brasillensis*)	0.3–0.6	3.1–5.8	10.9–16.6	10.5–11.4	10.1–14.1	29.1–31.8	2.4–4.0	1.8–2.1	14.9–15.3	5.8–9.6	–
Rat	–	–	tr	tr	3.0	23.8	5.6	1.9	49.8	14.3	-
Fin whale	–	–	–	0.1	5.5	22.9	3.9	0.5	24.7	1.1	0.7
Dog	–	–	tr–1	tr–1.0	4.0	27.3	4.4	6.4	41.8	12.6	1.9
Black bear		–	tr–0.3	tr–0.8	3.4	26.2	tr–5.9	4.4	49.8	11.0	1.4
Ferret	–	–	–	0.4	4.3	23.2	tr	4.9	56.6	7.6	2.1
Cat	–	–	1.0	1.9	7.2	24.6	10.4	5.1	37.4	8.5	2.0
Grey seal	–	–	–	tr	3.2	17.9	1.8	15.9	30.6	0.6	tr
Horse	tr	1.8	5.1	6.2	5.7	23.8	tr–2.3	7.8	20.9	14.9	12.6
Pig	–	–	0–0.7	tr–0.5	4.0	32.9	3.5	11.3	35.2	11.9	0–0.7
Llama	–	0.4	0.5	0.4	6.0	36.8	18.9	7.3	24.1	2.0	1.6
Reindeer	1.1	0.4	1.0	1.3	10.7	29.0	11.6	–	15.0	3.7	–
Goat	2.9	2.7	8.4	3.3	10.3	24.6	12.5	2.2	28.5	2.2	–
Sheep	2.8	2.7	9.0	5.4	11.8	25.4	9.0	3.4	20.0	2.1	1.4

Source: Data from Morrison (1970).

TABLE 2.103 Effects of different diets on the fatty acid composition and structure of human milk triacylglycerols

	Diets								
	OL n = 4			OM n = 3			VG n = 4		
Fatty acid	***TG***	***sn-2***	***sn1+3***	***TG***	***sn-2***	***sn1+3***	***TG***	***sn-2***	***sn-1 + 3***
12:0	4.5	5.4	4.0	2.2	2.3	2.1	3.2	5.9	3.8
14:0	8.6	10.4	7.6	13.4	7.5	16.8	14.8	6.8	18.8
16:0	17.2	48.2	1.5	21.4	61.2	1.5	13.1	40.5	–
18:0	5.7	1.5	7.8	5.9	1.4	8.1	3.0	0.7	4.6
18:1	36.3	16.7	46.1	38.0	16.0	50.5	25.6	12.8	31.7
18:2	26.5	16.7	31.4	14.7	16.7	16.7	36.7	29.2	45.4

Note: OL is ovo-lacto diet (eggs and diary products); OM is omnivore; VG is vegan (no animal proteins).
From Jensen (1996).

TABLE 2.104 Fatty acid composition (mol %) of milk phosphatidylcholines (PC) and sphingolipids (Sph) from various species

	Sheep		Pig		Ass		Human	
Fatty acid (major only)	**PC**	**Sph**	**PC**	**Sph**	**PC**	**Sph**	**PC**	**Sph**
14:0	4.6	2.5	1.8	0.4	6.1	3.9	4.5	2.0
16:0	38.2	22.5	39.9	15.1	52.2	28.0	33.7	12.8
16:1	0.5	1.0	6.3	0.3	3.1	3.4	1.7	0.6
18:0	10.6	8.1	10.3	6.9	6.5	4.7	23.1	11.8
18:1	26.6	6.2	21.8	0.5	10.9	2.9	14.0	1.0
18:2	4.3	0.5	15.9	–	14.6	–	15.6	0.3
18:3	2.8	–	1.5	–	6.0	–	1.3	–
20:0	–	0.5	–	10.5	–	7.0	–	8.9
20:1	–	0.7	–	0.6	–	1.3	–	0.5
20:3	0.3	–	0.3	–	0.2	–	2.1	–
20:4	0.3	–	1.3	–	0.4	–	3.3	–
22:0	–	7.5	–	17.0	–	13.7	–	19.5
23:0	–	27.2	–	3.5	–	13.5	–	4.0
24:0	–	17.0	–	20.2	–	14.6	–	19.5
24:1	–	2.0	–	22.0	–	16.1	–	15.4

From Morrison (1970).

TABLE 2.105 Effect of diets on fatty acid composition of human milk

	Western Diets		Non-western diets	
	Wt %	**Range**	**Wt %**	**Range**
12:0	4.9	1.7–12.3	8.1	2.4–16.5
14:0	5.6	2.0–11.8	9.6	5.3–15.9
16:0	20.3	19.3–25.1	21.5	14.1–25.8
18:0	7.5	5.8–9.7	5.6	0.8–8.2
Total Sat.	(41.3)		(47.9)	
18:1 n7	3.4	3.2–3.8	2.9	2.3–3.8
18:1 trans n9	3.6	3.1–4.7	–	
18:1 n9	31.0	22.6–38.7	30.5	17.9–47.0
Total mono.	(43.0)		(35.0)	
18:2 n6	12.6	9.6–16.8	13.8	8.8–23.8
20:4 n6	0.5	0.4–0.7	0.5	0.1–0.7
18:3 n3	0.7	0.3–1.9	0.5	0.1–1.0
20:5 n3	0.1	0–0.2	0.2	0.1–1.1
22:6 n3	0.2	0.1–0.6	0.6	0.1–1.4
Total PUFA	(15.7)		(17.1)	

From Jensen (1996).

references therein). At birth, quite large changes in lipid metabolism occur. Whereas the fetus relied extensively on glucose, the sole source of nutrition for the newborn is milk, from which about 50% of the energy comes from fat. The enzymes for fatty acid synthesis are suppressed and the baby's metabolism becomes geared to using fat directly from the diet. Human milk contains quite a high proportion of polyunsaturated fatty acids and this may be a response to the needs of the still-developing nervous tissues. There are, however, differences in milk fat composition (Tables 2.103 and 2.105) depending on the mother's diet. Despite the low content of polyunsaturated fatty acids in cow milk, there is only limited evidence that babies given formulae based or cow's milk develop less well than those primarily breast fed. However, infant formulae over recent years first contained added vegetable oils (supplementing linoleate) and, then, *n*-3 PUFAs. Because of the high β-oxidation rates of α-linolenic acid, it is considered beneficial to add *n*-3 PUFAs to infant formulae in the form of fish oils (containing eicopentaenoic and docosahexaenoic acids). Currently, there is much discussion about the optimal ratio of *n*-3 and *n*-6 dietary fatty acids, including those for infants and a good summary of this question is given in Gurr et al. (2002).

Many of the compounds responsible for the flavour of milk and milk products are derived from milk lipids. Lactones and methyl ketones are derived from specific minor triacylglycerols. Other flavour volatiles have also been

described (Morrison, 1970). In small amounts, these various compounds give rise to desirable flavours, but, if present in larger quantities, they may produce off-flavours.

A comprehensive review of milk lipids is given by Morrison (1970) and a recent discussion of dietary implications of milk fats is that of Gurr (1989). Useful references giving information on the composition and structure of milk lipids are Jensen (1988), Jensen and Clark (1988), Jensen et al. (1990, 1991), Christie (1994) and Jensen (1996).

References

Christie, W.W. (1995) Composition and structure of milk lipids. In *Advanced Dairy Chemistry*, Ed. P.F. Fox, Part 2, Chapman & Hall, London, pp. 1–36.

Dils, R.R. and Parker, D.S. (1982) Metabolic aspects of lactation and the supply of nutrients to the young. In *Biochemical Development of the Fetus and Neonate*, Ed. C.T. Jones, Elsevier, Amsterdam, pp. 573–590.

Gurr, M.I. (1989) Dairy fats: nutritional nasties or dietary delights? In *Fats for the Future*, Ed. R.C. Cambie, Ellis Horwood, Chichester, pp. 41–61.

Gurr, M.I. and Harwood, J.L. (1991) *Lipid Biochemistry*, 4th ed., Chapman and Hall, London.

Gurr, M.I. et al. (2002) *Lipid Biochemistry,* 5th ed., Blackwell Science, Oxford.

Jensen, R.G. (1988) *Lipids of Human Milk*, CRC Press, Boca Raton, FL.

Jensen, R.G. and Clark, R.W. (1988) Lipid composition and properties. In *Fundamentals of Dairy Chemistry*, Eds., Wong, N.P., Jenness, R., Keeney, M., and Marth, E.H., 3rd ed., Van Nostrand Reinhold, New York, pp. 171–214.

Jensen, R.G. (1996) The lipids in human milk. *Prog. Lipid Res.*, **35**, 53–92.

Jensen, R.G. et al. (1990) Lipids of bovine and human milks: a comparison. *J. Dairy Sci.*, **73**, 223–240.

Jensen, R.G. et al. (1991) The composition of milk fat. *J. Diary Sci.,* **74**, 3228–3243.

Long, C. Ed. (1961) *Biochemists' Handbook*, E. & F.N. Spon, London.

Morrison, W.R. (1970) Milk lipids. In *Topics in Lipid Chemistry*, Ed. F.D. Gunstone, vol.1, Logos Press, London, pp. 51–106.

2.8 Liver and other tissue lipids

With the exception of lean skeletal muscle, all major organs of the body contain appreciable lipid. The lipid is present partly as storage lipid (triacylglycerol) in, for example, the liver, but mainly as phospholipid (and cholesterol) in the tissue's membranous structures. A simple distribution is shown in Table 2.106.

Mammalian liver is generally quite rich in lipid. Fat represents about 50% of the total calories available for metabolic purposes, with a range of 28% (horse) to 69% (rat) (Girard and Ferre, 1982). In terms of lipid content/kg fresh weight, figures for five species range from about 60 g lipid/kg fresh weight for humans, oxen, and rats through 91 for chicken to 116 g lipid/kg fresh weight of cat liver.

The high content of myelinated nerves in brain tissue means that phospholipid and sterol contents will be relatively high. In other tissues, sterols are much less important (Table 2.106).

When the total fatty acids of liver are examined, it is found that land animals contain 30 to 40% of the total acids as saturated (mainly palmitic and stearic). In contrast, aquatic animals contain a much higher proportion of unsaturated acids, with a saturated total of 10 to 20%. Very long-chain unsaturated fatty acids are typical, with 12 to 30% C_{20} unsaturated and 8 to 18% C_{22} unsaturated acids in aquatic animals (Long, 1961).

A thorough analysis of the lipid composition of normal human liver has been made by Kwiterovich et al. (1970). They found that the contents of total cholesterol and total phospholipids were 3.9 ± 0.8 and 25.1 ± 2.7 mg/g wet weight, respectively. As expected, triacylglycerol content was much more variable and was 19.4 ± 15.9 mg/g wet weight. Human liver glycolipids were analysed, with dihexosides being the major neutral ceramides, and GM_3 (hematoside) the major ganglioside. The phospholipid compositions of several mammalian species are shown in Table 2.107. It will be seen clearly that phosphatidylcholine and phosphatidylethanolamine are the major components.

When different sheep or ox tissues are compared for their phospholipid composition (Table 2.108), although phosphatidylcholine and phosphatidylethanolamine are always major components, their plasmalogen derivatives are much more variable. The heart contains large amounts of the latter. Phosphatidylinositol, phosphatidylserine, and sphingomyelin are always significant components. Diphosphatidylglycerol, which is localized in mitochondria, is found in appreciable quantities in those tissues, such as heart muscle, which contain large numbers of these organelles.

Not surprisingly, dietary factors can play a significant role in influencing the lipid composition of different tissues, particularly those where lipid storage is significant. Aspects of diet are dealt with in Section 11.1. In Table

TABLE 2.106 Lipid content of different mammalian tissues, as g/kg fresh weight

	Total lipid	Phospholipid	Total sterol
Brain (man)	104	31–78	26–44
(rat)	97	45 (46)[b]	15
Muscle (lean)	0.6	– (9)[b]	–
Gastric mucosa (tripe)	20–30	–	–
Intestine	65	–	–
Kidney (rat)	96[a]	24–27 (33)	<0.1
Liver (rat)	60	30	2.5

[a] Total fatty acids.
[b] Values in parentheses are from data in White (1973).
Data from Long (1961).

TABLE 2.107 Phospholipid composition (%) of livers from several mammalian species

	Human	Rat	Sheep	Bovine	Mouse
Phosphatidylcholine[a]	43.6	50.8	40.5	55.7	45.6
Phosphatidylethanolamine[a]	27.9	25.2	31.5	13.0	25.1
Phosphatidylinositol	8.6	7.2	8.2	7.9	7.7
Phosphatidylserine	3.1	3.2	2.3	4.2	3.9
Phosphatidylglycerol	–	–	5.3	–	0.1
Diphosphatidylglycerol	3.7	4.8	1.1	4.1	4.7
Sphingomyelin	4.6	4.2	5.0	5.8	4.7
Others	8.5	5.6	6.1	9.3	8.2

[a] Includes plasmalogen derivatives when analysed.
From White (1973).

TABLE 2.108 Phospholipid composition of various sheep or ox tissues

	Sheep				Ox		
	Brain	Heart	Liver	Kidney	Spleen	Lung	Skeletal muscle
Phosphatidylcholine	37.3	25.5	39.7	35.8	40.5	39.5	42.5
Choline plasmalogen	0.9	20.4	0.8	3.2	3.0	0.3	–
Phosphatidylethanolamine	7.7	12.4	27.9	10.6	25.0	21.2	26.6
Ethanolamine plamalogen	16.5	11.0	3.6	7.4	9.8	0.2	–
Phosphatidylinositol	2.1	4.7	8.2	4.6	1.6	3.3	5.6
Phosphatidylserine	9.2	2.2	2.3	3.2	2.1	9.4	4.1
Sphingomyelin	12.8	5.5	5.0	12.1	12.5	16.1	4.5
Phosphatidylglycerol	–	–	5.3	–	–	2.0	0.3
Diphosphatidylglycerol	2.0	5.8	1.1	5.9	1.5	1.0	8.9

From White (1973).

TABLE 2.109 Influence of diet on the lipid composition of different rat tissue samples

		Diet				
Sample		Fat-free	Hydrogenated soybean fat	Corn oil	Milk fat	Beef tallow
Liver	Neutrals [a]	35 ± 1	46 ± 2	45 ± 2	46 ± 1	48 ± 2
	CE	5 ± 1	5 ± tr	5 ± 2	5 ± 1	8 ± 1
	PL	26 ± 1	35 ± 1	33 ± 1	31 ± 1	32 ± 1
Heart	Neutrals	59 ± 1	63 ± 2	49 ± 2	41 ± 1	42 ± 2
	CE	2 ± tr	5 ± 1	4 ± 1	4 ± 1	3 ± tr
	PL	22 ± 1	18 ± 1	33 ± 1	26 ± 1	22 ± 1

Note: Results as g/kg fresh weight tissue; 15-week feeding regime.
[a] Neutrals = primarily triacylglycerols, but also cholesterol and non-esterified fatty acids; CE = cholesterol esters; PL = phosphoglycerides.
Data from Fasman (1975).

2.109, an example of data showing alterations in lipid composition during a feeding regime in rats is given. Of note is that structural lipid content is also altered, in many cases, quite significantly. As discussed by Gurr (1992), it is very important to bear in mind that different animals react quite differently and that animal models may not always be appropriate with regard to, for example, human obesity or heart disease.

References

Fasman, G.D. Ed. (1975) *CRC Handbook of Biochemistry and Molecular Biology*, 3rd ed., CRC Press, Cleveland, OH.

Girard, J. and Ferre, P. (1982) Metabolic and hormonal changes around birth. In *The Biochemical Development of the Fetus and Neonate*, Ed. C.T. Jones, Elsevier, Amsterdam, pp. 517– 551.

Gurr, M.I. (1992) Dietary lipids and coronary heart disease: old evidence, new perspective. *Prog. Lipid Res.*, **31**, 195–243.

Kwiterovich, P.O. et al. (1970) Glycolipids and other lipid constituents of normal human liver. *J. Lipid Res.,* **11**, 322–330.

Long, C. Ed. (1961) *The Biochemists' Handbook*, E. & F.N. Spon, London.

White, D.A. (1973) Phospholipid composition of mammalian tissues. In *Form and Function of Phospholipids*, Eds. G.B. Ansell, R.M.C. Dawson and J.N. Hawthorne, Elsevier, Amsterdam, pp. 441–482.

2.9 Cereal lipids

There has been increasing interest in cereal lipids because of their importance in food technology (e.g., in wheat flour) or as potential sources of significant amounts of dietary polyunsaturated fatty acids (Price and Parsons, 1974). Morrison and co-workers have conducted a systematic study of the distribution of acyl lipids in different parts of wheat and maize seed. In addition, there has been considerable interest in the rather unusual content of lipids in starch (Morrison, 1981).

The lipid content of total cereal seeds varies considerably. Furthermore, the various anatomical parts of seeds have very different lipid contents. Thus, in wheat seeds, the germ contains 25 to 30% lipid while the endosperm has only 1% lipid. Considerable amounts of wheat germ lipid are lost during milling (Galliard and Barnes, 1980). Cereal germ oils are also a very good source of tocopherols.

Maize has been examined in detail (Tan and Morrison, 1979b). Pericarp lipids seem to be the remnants of those of spherosomes (i.e., triacylglycerol, non-esterified fatty acids, sterol esters, diacylglycerol). Maize germ has a very high lipid content (~45%), which is mostly triacylglycerol with small amounts of phosphoglycerides. Indirect analysis of the aleurone layer by difference (Tan and Morrison, 1979a) showed that it contained most of the endosperm triacylglycerol.

Wheat showed many similarities to maize except that the germ of wheat was much smaller and there was less lipid degradation in the developing wheat endosperm. Pericarp lipids were mostly triacylglycerol, diacylglycerol and sterol esters (as in maize pericarp) (Hargin and Morrison, 1980). The aleurone and germ contain similar amounts and proportions of lipids. In comparison to maize, more phospholipid (14 to 18% total lipid) is found. Endosperm nonstarch lipids are mainly triacylglycerol, which is in spherosome structures. Glycosylglycerides are present, presumably from the amyloplast membranes (Hargin et al., 1980).

Sorghum lipids resemble maize and there is considerable similarity between wheat, rye, triticale, and (to a lesser extent) oat lipids. Rice lipids resemble those of barley or oats except that there is sometimes very little glycolipid or phospholipid in milled rice (Choudhury and Juliano, 1980a, b). For representative analyses of maize and wheat lipids, see Table 2.110 and refer to Morrison (1978) for reviews.

Cereal starches contain lipids that are very difficult to extract. The analyses in Table 2.111 are for internal starch lipids, although the presence of surface and nonstarch lipids can modify the properties of starch significantly (Morrison, 1981). The internal lipids are almost exclusively monoacyl lipids (86 to 94%). The presence of significant quantities of non-esterified fatty acids and other neutral lipids (Becker and Acker, 1976) may indicate that the starches that were analysed were not pure and the neutral lipids arose from non-starch or surface lipids (Morrison, 1983). In contrast, maize and rice starch internal lipids have more non-esterfied fatty acid than lysophospholipid (Morrison and Milligan, 1982). Precautions to be taken for the accurate estimation of starch lipids have been discussed (Morrison, 1988) and methods for the use of alcohol/water mixtures in extracting internal lipids described (Morrison and Coventry, 1985). The lipids can then be quantified by appropriate chromatographic methods (Morrison, 1992). For wheat starch, where the lipids are almost exclusively lysophospholipids, it is

TABLE 2.110 Lipid composition of fractions from wheat and maize seeds

	H51 Maize[a] (µg/ kernel)		LG11 Maize[b] (µg/mg seed)		Wheat[c]	
Lipid class	**Germ**	**Endosperm**	**Germ**	**Non-starch endosperm**	**Germ**	**Non-starch endosperm**
SE	7479	1008	818	49	8–10	4–17
TG			36953	344	196–244	46–70
DG			1244	33	0-13	10–18
FFA			305	133	3–6	8–21
DGDG	24	320	436	12	0–7[e]	32–74
DGMG	5[d]	296[d]	172	12		4–24
N-AcylPE	8	5	110	3	tr	8–50
PE	51	20	194	1	4–7	1–9[f]
PC	252	154	748	3	20–28	5–20
PI	96	14	247	3	6–9	n.d.

[a] Weber (1979).
[b] Tan and Morrison (1979a, b).
[c] Hargin and Morrison (1980).
[d] Includes SQDG; a number of other minor lipids were quantified (see references).
[e] Includes other glycosylglycerides.
[f] Includes PG.

Note: SE = sterol esters; TG = triacylglycerol; DG = diacylglycerol; FFA = non-esterified fatty acid; DGDG = digalactosyldiacylglycerol; DGMG = digalactosylmonoacylglycerol; *N*-acylPE = *N*-acylphosphatidylethanolamine; PC = phosphatidylcholine; PI = Phosphatidylinositol; SQDG = diacylsulfoquinovosylglycerol; PG = phosphatidylglycerol.

TABLE 2.111 Lipids in cereal starches (mg/100g)

Lipid	Wheat A-granules	Maize	Rice
SE, TG, DG	1–2	3	2–12
FFA	15–54	379	221–355
MG, ASG[a]	1–2	9	19–31
MGMG, DGMG[a]	4–12	13	20–40
LPG	30–41	7	38–48
LPE	41–75	20	86–112
LPC	451–734	262	453–513
Others	27–62	9	–

[a] Probably from contaminating non-starch lipids.
Note: See Table 2.110; MG = monoacylglycerol; ASG = acylated sterol glycoside; LPG = monacylphosphatidylglycerol; LPE = monoacylphosphatidylethanolamine; LPC = monoacylphosphatidylcholine; MGMG = monogalactosylmonoacylglycerol. From Morrison (1983).

convenient to determine starch phosphorus and then calculate lysophospholipid content using an appropriate conversion factor, after making allowance for the small amount of non-lipid phosphorus (Terter and Morrison, 1992). In maize starches from different varieties, there seemed to be a relationship between amylose and lipid contents (South et al., 1991) (see also Morrison, 1993).

The fatty acids of cereal lipids are generally linoleate, palmitate, oleate, α-linolenate, and stearate in order of abundance. Considerable variations in the fatty acid compositions of bulk lipids have been achieved by breeding in barley, maize, and oats. The fatty acid content and distribution in cereals has been reviewed (cf. Barnes, 1983). If the acyl composition of individual lipids is analysed, then 200 to 500 mg of starch are needed per analysis (Morrison and Karkalas, 1990).

Considerable interest has been shown in the study of lipid metabolism during the germination of cereal seeds, and this subject has been reviewed (Tomos and Laidman, 1979). Moreover, the activity of endogenous lipases causes considerable spoilage of wholewheat flours. This is due to bran-localized lipases and a germ-localized lipoxygenase (Galliard, 1986).

References

Barnes, P.J. Ed. (1983) *Lipids in Cereal Technology,* Academic Press, London.

Becker, G. and Acker, L. (1976) Die Lipide der Getreidestarken. In *Handbuch der Stärke in Einzeldarstellung* (Ed. M. Ulmann), vol. VI-5, Paul Parey, Berlin.

Choudhury, N.H. and Juliano, B.O. (1980a) Lipids in developing and mature rice grain. *Phytochemistry,* **19**, 1063–1069.

Choudhury, N.H. and Juliano, B.O. (1980b) Effects of amylase content on the lipids of mature rice grain. *Phytochemistry,* **19**, 1385–1389.

Galliard, T. (1986) Oxygen consumption of aqueous suspensions of wheat wholemeal, bran and germ: involvement of lipase and lipoxygenase. *J . Cereal Sci.,* **4**, 33–50.

Galliard. T. and Barnes, P.J. (1980) The biochemistry of lipids in cereal crops. In *Biogenesis and Function of Plant Lipids*, Eds. P. Mazliak, P. Benveniste, C. Costes, and R. Douce, Elsevier, Amsterdam, pp. 191–198.

Hargin, K.D. and Morrison, W.R. (1980) The distribution of acyl lipids in the germ, aleurone, starch, and nonstarch endosperm of four wheat varieties. *J. Sci. Food Agric.,* **31**, 877–888.

Hargin, K.D., et al. (1980) Triglyceride deposits in the starchy endosperm of wheat. *Cereal Chem*., **57**, 320–325.

Morrison, W.R. (1978) Cereal lipids. *Adv. Cereal Sci. Technol.,* **2**, 221–348.

Morrison, W.R. (1981) Starch lipids: a reappraisal. *Stärke*, **33**, 408–410.

Morrison, W.R. (1983) Acyl lipids in cereals. In *Lipids in Cereal Technology*, Ed. P.J. Barnes, Academic Press, London, pp. 11–32.

Morrison, W.R. (1988) Lipids in cereal starches: a review. *J. Cereal Sci.* **8**, 1–15.

Morrison, W.R. (1992) Analysis of cereal starches. In *Modern Methods of Plant Analysis,* vol. 14, Ed. H-F. Linskens, pp. 199–205, Springer-Verlag, Heidelberg.

Morrison, W.R. (1993) Cereal starch granule development and composition. In *Seed Storage Compounds*, Eds. P.R. Shewry and K. Stobart, pp. 175–190. Clarenden Press, Oxford.

Morrison, W. R. and Coventry, A.M. (1985) Extraction of lipids from cereal starches with hot aqueous alcohols. *Staerke* **37**, 83–87.

Morrison, W.R. and Karkalas, J. (1990) Starch. In *Methods in Plant Biochemistry,* Vol. 2, Eds. P.M. Day and J.B. Harborne, Academic Press, London, pp. 323–352.

Morrison, W.R. and Milligan, T.P. (1982) Lipids in maize starches. In *Maize: Recent Progress in Chemistry and Technology*, Ed. G.E. Inglett, Academic Press, New York, pp. 1–18.

Price, P.B. and Parsons, J.G. (1974) Lipids of six cultivated barley (*Hordeum vulgare*) varieties. *Lipids*, **9**, 560–566.

South, J.B. et al. (1991) A relationship between the amylose and lipid content in maize. *J. Cereal Sci*., **14**, 267–278.

Tan, S.L. and Morrison, W.R. (1979a). The distribution of lipids in the germ, endosperm, pericarp and tip cap of amylomaize, LG-11 hybrid maize and waxy maize. *J. Am. Oil Chem. Soc.,* **56**, 531–535.

Tan, S.L. and Morrison, W.R. (1979b). Lipids in the germ, endosperm and pericarp of the developing maize kernel. *J. Am. Oil Chem. Soc.,* **56**, 759–764.

Terter, R.F. and Morrison, W.R. (1992) Swelling and gelatinization of cereal starches. III Some properties of wax and normal nonwaxy barley starches. *Cereal Chem.,* **69**, 654–658.

Tomos, A.D. and Laidman, D.L. (1979). The control of mobilization and metabolism in the aleurone tissue during germination. In *Recent Advances in the Biochemistry of Cereals*, Eds. D.L. Laidman and R.G. Wyn Jones, Academic Press, London, pp. 119–146.

Weber, E.J. (1979). The lipids of corn germ and endosperm. *J. Am. Oil Chem. Soc.,* **56**, 637–641.

2.10 Leaf lipids

Both the lipid and the fatty acid compositions of plant leaves show a remarkable consistency between species. Furthermore, these compositions are usually only slightly altered by external factors and developmental period (Hitchcock and Nichols, 1971). Nevertheless, growth under extreme conditions of, for example, phosphorus deficiency may cause substitution of lipid classes — in this case with glycosylglycerides substituting for phosphoglycerides (Hartel et al., 2000; Jouhet et al., 2004). In addition, plants can be classified as 18:3-, 16:3-, or 18:4- depending on the fatty acid composition of the chloroplast monogalactosyldiacyglycerol (Dobson, 2000; Gurr et al., 2002). The 16:3- and 18:3- plants are also distinguished by differences in their lipid metabolism (Heinz and Roughan, 1982).

Up to 7% of the dry weight of leaves is lipid, with the galactosylglycerides being major constituents (Table 2.112). The plant sulfolipid (sulfoquinovosyldiacylglycerol) is also a significant component, representing about 5% of the total acyl lipids. The remainder of the lipid consists principally of phosphoglycerides with phosphatidylcholine, phosphatidylglycerol, and phosphatidylethanolamine as major components, while phosphatidylserine, phosphatidic acid, and phytoglycolipid are usually found in trace amounts. Other minor lipids include triacylglycerol, diacylglycerol, and glucocerebroside (Hitchcock and Nichols, 1971). The waxy cuticle also contains typical lipid constituents (see Sections 1.2.11 and 1.2.12), while carotenoids, chlorophylls, and plastoquinone are major lipids of the photosynthetic membranes (Table 2.113).

The overall distribution of lipids in leaves reflects the high content of chloroplasts in the tissue. The thylakoid membranes of the latter have a rather simple acyl lipid composition (cf. Harwood, 1980), which resembles that of cyanobacterial membranes. Plants grown in the dark have pale yellow leaves, which contain etioplasts rather than chloroplasts. The lipid composition of such etioplasts is different from normal green leaves in that glycosylglycerides are less important and there are changes in the fatty acid composition (Table 2.114). These differences are also seen if the white and green parts of variegated leaves are compared (Table 2.114).

The major fatty acids of leaves are palmitic, linoleic, and α-linolenic. The main monoenoic acids are oleic and palmitoleic, with consistent amounts of *trans*- Δ^3-hexadecenoic acid in green leaves. This unusual fatty acid is located at the 2-position of phosphatidylglycerol (Harwood, 1980). Other fatty acids are only present in minor amounts, with the exception of 16:3 plants, such as spinach or tobacco, which contain all *cis*-7,10,13-hexadecatrienoic acid (Table 2.114).

As mentioned above, fatty acids of unusual structure that characterize seed oils from a variety of plants are seldom found in the lipids of the corresponding leaf tissue. However, there are two exceptions to this rule. First, some members of the family Malvaceae accumulate cyclopropenoid fatty acids in their leaves as well as their seeds (Shenstone and Vickery, 1961). Secondly, the γ-linolenic and octadecatetraenoic acids that characterize the seed oils of the Boraginaceae are also found esterified to the leaf lipids (Jamieson and Reid, 1968, 1969). Stymne et al. (1987) have shown that both γ-linolenic and octadecatetraenoic acid are present at the *sn*-2 position of monogalactosyldiacylglycerol, where octadecatetraenoic acid was the major component (74%) in *Borago officinalis*.

In view of the fact that leaf lipids are dominated by those of the chloroplasts, it is worth emphasizing certain features of the latter's thylakoids. In contrast to animal and most bacterial membranes, phosphoglycerides are minor components. Phosphatidylglycerol is the only phosphoglyceride of importance. Over 75% of the acyl lipid content is glycosylglyceride (Table 2.115). In addition, the fatty acid composition of chloroplast acyl lipids is unusually rich in polyunsaturates. α-Linolenic acid, the main fatty acid, is particularly enriched in the two galactosylglycerides. Phosphatidylglycerol also contains high amounts of α-linolenic acid, but also has large amounts of palmitic and *trans*- Δ^3-hexadecenoic acids, while diacylsulfoquinovosylglycerol also contains palmitic acid as a major component (Table 2.116).

For useful detailed references on plant lipids, refer to the proceedings of the biennial International Symposia on

TABLE 2.112 The acyl lipid composition of plant leaves

	Lipids (% of total acyl lipids)							
Plant	**MGDG**	**DGDG**	**SQDG**	**PC**	**PG**	**PE**	**PI**	**Others**
Clover	46	28	4	7	6	5	1	3
Sugarbeet	26	14	9	24	9	12	5	1
Maize	42	31	5	6	7	3	1	2
Barley	43	26	5	11	6	4	1	4
Rye grass	39	29	4	10	7	5	2	4
Broad bean	38	30	6	7	6	4	2	7

Note: MGDG = monogalactosyldiacylglycerol; DGDG = digalactosyldiacylglycerol; SQDG = sulphoquinovosyldiacylglycerol; PC = phosphatidylcholine; PG = phosphatidylglycerol; PE = phosphatidylethanolamine; PI = phosphatidylinositol.
From Harwood (1980).

TABLE 2.113 Pigment composition of spinach chloroplast membranes

	Thylakoids[a]		
	Moles of lipid/100mol of chlorophyll[b]	μg/mg of protein[c]	Envelope (μg/mg of protein)[c]
Chlorophyll a	70	–	–
Chlorophyll b	30	–	–
Carotenoids			
β-Carotene	6	–	1.42
Violaxanthin	3	4.52	6.50
Lutein + Zeaxanthin	10	6.05	2.50
Antheraxanthin	–	–	0.79
Neoxanthin	3	–	0.40

[a] Typical thylakoid membranes are about 50% lipid, of which 25% is pigment.
[b] Lichtenthaler and Park (1963).
[c] Douce and Joyard (1979).

TABLE 2.114 Fatty acid composition of leaf tissue

	Fatty acids (% of total acids)							
Plant	**16:0**	***9c*-16:1**	***3t*-16:1**	**16:3**	**18:0**	**18:1**	**18:2**	**18:3**
Maize	8	1	3	n.d.	2	7	8	66
Barley	13	3	3	n.d.	2	6	6	64
Broad bean	12	1	4	n.d.	2	7	14	56
Broad bean (etiolated)	16	2	n.d.	n.d.	4	4	29	41
Chlorophytum (green part)	12	1	2	n.d.	4	3	24	48
Chlorophytum (white part)	19	1	n.d.	n.d.	5	4	39	24
Pea	12	1	2	n.d.	1	2	25	53
Spinach[a]	13	n.d.	3	5	tr	7	16	56

[a] Data from Debuch (1961).
Note: n.d., none detected; tr., trace
From Harwood (1980).

TABLE 2.115 The lipid composition of chloroplast membranes

		Percentage of total lipids					
Plant	**Fraction**	**MGDG**	**DGDG**	**SQDG**	**PG**	**PC**	**PI**
Spinach[a]	Envelope	22	32	5	8	27	1
	Thylakoid	51	26	7	9	3	1
Wheat[b]	Envelope	22	43	11	10	14	–
	Thylakoid	45	36	8	10	2	–

[a] From Douce, R., Holz, R.B., and Benson, A.A. (1973) *J. Biol. Chem.,* **248**, 7215–7222.
[b] From Bahl, J., Francke, B. and Monegar, R. (1976) *Planta*, **129**, 193–201.
Note: See Table 2.112 for abbreviations.

TABLE 2.116 The fatty acid composition of chloroplast lipids

		Fatty acid composition (% of total fatty acids)						
Plant	**Lipid**	**16:0**	**16:1[a]**	**16:3**	**18:0**	**18:1**	**18:2**	**18:3**
Spinach	MGDG	tr	–	25	–	1	2	72
	DGDG	3	–	5	–	2	2	87
	SQDG	29	1	–	1	7	26	36
	PG	11	32	–	–	2	4	47
Barley	MGDG	3	1	–	1	1	3	91
	DGDG	9	2	–	1	3	7	78
	SQDG	32	3	–	1	2	5	55
	PG	18	27	–	3	2	11	38

[a] 16:1 = *cis*-9-hexadecenoic acid in MGDG, DGDG and SQDG and *trans*-3-hexadecenoic acid in PG.
18:1 = oleic acid; 18:2 = linoleic acid; 18:3 = α--linolenic acid.
Note: See Table 2.112 for abbreviations.

Plant Lipids (e.g., Harwood and Quinn, 2000; Murata et al., 2003; www.mete.mtesz.hu/pls/proceedings). Research using mutants of *Arabidopsis* with changed lipid compositions is reviewed by Ohlrogge et al. (1991) and, in particular, by Wallis and Browse (2002). Methodological aspects are described by Harwood (1980), Harwood and Boyer (1990) and Christie (2003).

References

Bahl, J. et al. (1976) Lipid composition of envelopes, prolamellar bodies and other plastid membranes in etiolated, green and greening wheat leaves. *Planta*, **129**, 193–201.

Christie, W.W. (2003) *Lipid Analysis*, 3rd ed., The Oily Press, Bridgwater, U.K.

Debuch, H. (1961) The fatty acids from green leaves and the presence of trans-3-hexadecenoic acid. *Z. Naturforsch*., **16B**, 561–567.

Dobson, G. (2000) Leaf lipids of *Ribes nigrum*: a plant containing 16:3, α-18:3 and 18:4 fatty acids. *Biochem. Soc. Trans.,* **28,** 583–586.

Douce, R. and Joyard, J. (1979) Structure and function of the plastid envelope. *Adv. Bot. Res.*, **7**, 1–116.

Douce, R. et al. (1973) Isolation and properties of the envelope of spinach chloroplasts. *J. Biol. Chem.,* **248**, 7215–7222.

Gurr, M.I. et al. (2002) *Lipid Biochemistry,* 5th ed., Blackwell Science, Oxford.

Hartel, H. et al. (2000) DGDI-independent biosynthesis of extraplastidic galactolipids after phosphate deprivation in *Arabidopsis*. *Proc. Natl. Acad. Sci*., **97**, 10649–10654.

Harwood, J.L. (1980) Plant acyl lipids: structure, distribution and analysis. In the *Biochemistry of Plants,* vol. 4, Eds. P.K. Stumpf and E.E. Conn, Academic Press, New York, pp. 1–55.

Harwood, J.L. and Bowyer, J.R. Eds. (1990) *Methods in Plant Biochemistry,* vol. 4, *Lipids, Membranes and Aspects of Photobiology.* Academic Press, London.

Harwood, J.L. and Quinn, P.J. Eds. (2000) *Recent Advances in the Biochemistry of Plant Lipids.* Portland Press, London.

Heinz, E. (1977) Enzymatic reaction in galactolipid biosynthesis. In *Lipids and Lipid Polymers in Higher Plants*, Eds. M. Tevini and H.K. Lichtenthaler, Springer-Verlog, Berlin, pp. 102–120.

Heinz, E. and Roughan, P.G. (1982) *De novo* synthesis, desaturation and acquisitions of monogalactosyldiacyglycerol by chloroplasts of "18:3" and "16:3" plants. In *Biochemistry and Metabolism of Plant Lipids,* Eds. J.F.G.M. Wintermans and P.J.C. Kuiper, Elsevier, Amsterdam, pp. 169–182.

Heinz, E. and Roughan, P.G. (1983) Similarities and differences in lipid metabolism of chloroplasts isolated from 18:3 and 16:3 plants. *Plant Physiol*., **72**, 273–279.

Hitchcock, C. and Nichols, B.W. (1971) *Plant Lipid Biochemistry*, Academic Press, London.

Jamieson, G.R. and Reid, E.H. (1968) Analysis of oils and fats by gas chromatography. V. Fatty acid composition of the leaf lipids of *Myosotis scorpioides. J. Sci. Food Agric.,* **19**, 628–631.

Jamieson, G.R. and Reid, E.H. (1969) Leaf lipids of some members of the boraginaceae family. *Phytochemistry,* **8,** 1489–1494.

Jouhet, J. et al. (2004) Phosphate deprivation induces transfer of DGDG galactolipid from chloroplast to mitochondria. *J. Cell. Biol*. **167**, 863–874.

Lichtenthaler, H.K. and Park, R.B. (1963) Chemical composition of chloroplast lamellae from spinach. *Nature,* **198**, 1070–1072.

Murata, N. et al. Eds. (2003) *Advanced Research on Plant Lipids.* Kluwer, Dordrecht.

Ohlrogge, J.B. (1999) Plant metabolic engineering: are we ready for phase 2? *Curr. Plant Biol.*, **2**, 121–122.

Ohlrogge, J.B. et al. (1991) The genetics of plant lipids. *Biochim. Biophys. Acta,* **1082**, 1–26.

Shenstone, F.S. and Vickery, J.R. (1961) Occurrence of cyclopropene acids in some plants of the order Malvales. *Nature,* **190**, 168–169.

Stymne, S. et al. (1987) Desaturation of fatty acids on complex lipid substrates. In *The Metabolism, Structure and Function of Plant Lipids*, Eds. P.K. Stumpf, J.B. Mudd, and W.D. Nes, Plenum, New York, pp. 405–412.

Wallis, J.G. and Browse, J. (2002) Mutants of *Arabidopsis* reveal many roles for membrane lipids. *Prog. Lipid Res.,* **41**, 254–278.

2.11 Algal lipids

There is increasing commercial interest in the exploitation of algae (both freshwater and marine) as sources of foodstuffs and other important natural products. One subject of particular importance is that of polyunsaturated fatty acids — since many algae are rather rich in these components (Cohen and Ratledge, 2005).

Algae are an extremely diverse group of organisms. It is unsurprising, therefore, that their lipids are varied. Most attention has been paid to algal fatty acid content. Freshwater algae contain similar fatty acids to terrestrial plants (see Section 2.10 for leaf composition). However, the proportions of these acids vary considerably and, in general, freshwater algae contain higher proportions of C_{16} fatty acids and less of C_{18} components than do plant leaves. Representative fatty acid compositions of some freshwater and salt-tolerant algae are shown in Table 2.117. It will be noted that, whereas C_{20} fatty acids are absent (or only found in trace amounts) in freshwater species, in those from high-salt environments, such as the Dead Sea, arachidonate and (*n*-3)eicosapentaenoate are major components (Pohl and Zurheide, 1979; Harwood and Jones, 1989).

Marine algae contain a bewildering array of major fatty acids. Some representative examples of compositions for phytoplankton and macroalgae are shown in Table 2.117. The major saturated fatty acid is invariably palmitate and, in contrast to higher plants, palmitoleate is the major monoene. C_{18} fatty acids are much less abundant than in leaves, and the C_{20} polyunsaturated acids (particularly arachidonate and eicosapentaenoate) are very important. In some individual lipid classes, these latter acids can account for a very high proportion of the total acyl groups (see Harwood and Jones, 1989).

TABLE 2.117 Total fatty acid compositions (% total) of some algae

	16:0	16:1	16:2	16:3	16:4	18:1	18:2	18:3	18:4	20:4	20:5	22:6
Marine phytoplankton												
Monochrysis lutheri (Chrysophyceae)	13	22	5	7	1	3	1	tr	2	1	18	7
Olisthodiscus spp. (Xanthophyceae)	14	10	2	2	1	4	4	6	18	2	19	2
Lauderia borealis (Bacillariophyceae)	12	21	3	12	1	2	1	tr	–	1	3	-
Amphidinium carterae (Dinophyceae)	24	1	1	tr	–	5	1	2	15	–	14	25
Dunaliella salina (Chlorophyceae)	41	15	tr	–	–	11	8	19	–	–	–	–
Hemiselmis brunescens (Cryptophyceae)	13	3	3	tr	tr	2	tr	9	30	tr	14	–
Marine macroalgae												
Fucus vesiculosus (Phaeophyceae)	21	2	tr	–	tr	26	10	7	4	15	8	–
Chondrus crispus (Rhodophyceae)	34	6	tr	–	–	9	1	1	4	18	22	–
Ulva lactuca (Chlorophyceae)	18	2	tr	1	18	9	2	17	24	1	2	tr
Freshwater species												
Scenedesmus obliquus	35	2	tr	tr	15	9	6	30	2	–	–	–
Chlorella vulgaris	26	8	7	2	–	2	34	20	–	–	–	–
Chlamydomonas reinhardtii	20	4	1	4	22	7	6	30	3	–	–	–
Salt-tolerant species												
Ankistrodesmus spp.	13	2	1	1	14	25	2	29	2	–	1	–
Isochrysis spp.	12	6	–	–	15	4	6	17	–	–	2	13
Nannochloris spp.	9	20	7	9	–	4	1	1	–	–	27	–

From Harwood and Jones (1989).

Interestingly, in view of its postulated role in granal stacking (see Bolton et al., 1978), *trans* Δ-3-hexadecenoate is found in phosphatidylglycerol of algae that have a quite different chloroplast morphology to higher plants, e.g., brown and red algae and diatoms (see Harwood and Jones, 1989).

Cyanobacteria have a much simpler lipid and fatty acid composition than eukaryotic algae. Murata and Nishida (1987) have divided cyanobacteria into four groups based on the original classification of Kenyon et al. (1972). The fatty acid compositions of examples from each of the four groups are shown in Table 2.118. Group 1 cyanobacteria only synthesize saturated and monounsaturated acids. The other three groups all synthesize the polyunsaturated linoleate and, in addition, accumulate γ-linolenate, α-linolenate, and (*n*-3) octadecatetraenoate, respectively.

Cyanobacteria also differ from eukaryotic algae in having a very simple lipid class composition. Only the typical "chloroplast" lipids (monogalactosyldiacylglycerol, digalactosyldiacylglycerol, sulphoquinovosyldiacylglycerol, and phosphatidylglycerol) are found, with, sometimes, minor amounts of monoglucosyldiacylglycerol (Table 2.119). In nitrogen-fixing cyanobacteria, unusual glycolipids have been reported. These are most easily studied in the heterocysts of the filamentous heterocystous strains. Chemical structures for the main glycolipid components have been reported (Bryce et al., 1972) and minor components described (see Murata and Nishida. 1987).

For eukaryotic algae. the glycosylglycerides are major components and all the usual phosphoglycerides are found. Some representative examples of different compositions are shown in Table 2.120.

Certain classes of algae may accumulate significant amounts of more unusual complex lipids. DGTS (diacylglycerol-*O*-(*N,N,N*-trimethyl)homoserine) is an unusual compound found in Chlorophyta, but first reported in *Ochromonas danica* (see Harwood and Jones, 1989). Its β-alanine derivative diacylglycerol-*O*-hydroxymethyl-(*N,N,N*-trimethyl)-β-alanine (DGTA) has been identified in Phaeophyta (see Eichenberger, 1990).

Phosphatidyl-*O*-[*N*-(2-hydroxyethyl) glycerine (PHEG) has been identified in *Fucus serratus* (Eichenberger et al., 1995) while diacylglyceryl carboxyhydroxymethylcholine

TABLE 2.118 Representative examples of the fatty acyl compositions of cyanobacteria

		Fatty acid composition (% total)						
Group	**Organism**	**16:0**	**16:1**	**18:1**	**18:2**	**α-18:3**	**γ-18:3**	**18:4**
1	*Anacystis nidulans*	46	46	3	0	0	0	0
2	*Anabaena variabilis*	32	22	11	17	16	0	0
3	*Synechocystis 6714*	28	4	5	17	0	31	0
4	*Tolypothrix tenuis*	22	3	16	15	6	13	11

Data from Murata and Nishida (1987).

TABLE 2.119 The acyl lipid compositions of cyanobacteria and *Prochloron* spp.

	Lipid (% total)				
Organism	**MGDG**	**MGlcDG**	**DGDG**	**SQDG**	**PG**
Anabaena variabilis	54	1	17	11	17
Anacystis nidulans	57	n.m.	11	11	21
Prochloron spp.	55	3	11	26	5

Note: n.m. = not measured. MGlcDG, monoglucosyldiacylglycerol.
Data from Murata and Nishida (1987).

TABLE 2.120 The acyl lipid compositions of some algae

Lipid (%)	***Chattonella antiqua***	***Dunaliella parva***	***Acetabularia mediterranea***	***Chlamydomonas reinhardtii***	***Chondrus crispus***	***Fucus vesiculosus***
PC	5	9	0.1	–	30.1	4.2
PE	3	–	1.2	5	1.5	6.2
PI	1	2	0.6	2	tr	2.9
PG	3	6	3	10	7.7	2.2
DPG	–	–	–	–	1.9	5.4
MGDG	29	21	37	47	16.9	15.0
DGDG	18	11	20	16	14.8	11.3
SQDG	29	7	20	7	15.5	22.0
Other glycolipids	–	1	–	–	–	–
DGTS	6	15	20	16	–	–
DGTA	–	–	–	–	–	24.1
Nonpolar lipids	–	15	–	–	3.4	4.9
Free fatty acids	–	13	–	–	2.2	–

Note: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, Phosphatidylinositol; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; SQDG, sulphoquinovosyldiacylglycerol; DGTS, diacylglycerol trimethylhomoserine ether lipid; DGTA, diacylglycerol hydroxymethyltrimethyl-β-alanine lipid.
Source: Harwood and Jones (1989).

(DGCC) and diacylglyceryl glucuronide (DGGA) were found in the lipid fraction of *Pavlova lutheric* (Eichenberger and Gribi,1997). Many freshwater algae contain chlorosulfolipids (Mercer and Davies, 1979). These unusual lipids have been reviewed (Haines, 1973a, b). Halogenated fatty acids and their derivatives in different organisms, including algae, have been reviewed in detail by Dembitsky and Srebnik (2002). Certain diatoms have been found to contain other novel sulphur-containing lipids (see Kates, 1987).

The metabolism of algal lipids was reviewed by Harwood and Jones (1989) and updated recently (Guschina and Harwood, 2006). The latter includes the identification of unusual lipids including oxylipins, which may have a number of important physiological functions. Some aspects of the use of algae as a commercial source of lipids, particularly hydrocarbons, are covered by Scragg and Leathers (1988). *Botrycoccus braunii* and *Dunaliella salina* produce hydrocarbons at levels of 15 to 75%, while the red alga *Porphyridium cruentum* has been proposed as a possible source of arachidonic acid. The importance of such essential fatty acids when microalgae are used as feedstocks in aquaculture is reviewed by Volkman (1989), who also covers additional general aspects of lipid analysis and content of algae. Recently, there has been considerable interest in the exploitation of algae in the production of *n*-3 and *n*-6 PUFAs (e.g., Wen and Chen, 2003; Sijtsma and de Swaaf, 2004) and this is reviewed by Cohen and Ratledge (2005). The occurrence and metabolism of the very long-chain PUFAs, arachidonic, eicosapentaenoic and docosahexaenoic acids, are described in detail by Guschina and Harwood (2006).

References

Bolton, P. et al. (1978) The lipid composition of a barley mutant lacking chlorophyll b. *Biochem. J.*, **174**, 67–72.

Bryce, T.A. et al. (1972) Monohexoside derivatives of long-chain polyhydroxy alcohols — novel class of glycolipid specific to heterocystous algae. *Phytochemistry*, **11**, 295–302.

Cohen, Z. and Ratledge, C. (2005) *Single Cell Oils*, AOCS, Champaign, IL.

Dembitsky, V.M. and Srebnik, M. (2002) Natural halogenated fatty acids: their analogues and derivatives. *Prog. Lipid Res.,* **41,** 315–367.

Eichenberger, W. (1990) Identification of new plant lipids: structure of a second betaine lipid from algae. In *Plant Lipid Biochemistry, Structure and Utilization*, Eds. P.J. Quinn and J.L. Harwood, Portland Press, London, pp. 9–16.

Eichenberger, W. and Gribi, C. (1997) Lipids of *Pavlova lutheri*: cellular site and role of DGCC. *Phytochemistry*, **45**, 1561–1567.

Eichenberger, W. et al. (1995) Phosphatidyl-*O*-[N-(2-hydroxyethyl) glycine] (PHEG) a novel glycerophospholipid from brown algae (Phaeophyceae). *J. Plant Physiol.* **146**, 398–404.

Guschina, I.A. and Harwood, J.L. (2006) Lipids and lipid metabolism in eurkaryotic algae. *Prog. Lipid Res.* **45**, 160–186.

Haines, T. (1973a) Halogen-containing and sulphur-containing lipids of Ochromonas. *Annu. Rev. Microbiol.*, **27**, 403–411.

Haines, T. (1973b) Sulfolipids and halosulfolipids. In *Lipids and Biomembranes of Eukaryotic Microorganisms*, Ed. J.A. Erwin, Academic Press, New York, pp. 197–232.

Harwood, J.L. and Jones, A.L. (1989). Lipid metabolism in algae. *Adv. Bot. Res.,***16**, 1–53.

Kates, M. (1987). Lipids of diatoms and of halophilic *Dunaliella* species. In *The Metabolism, Structure and Function of Plant Lipids*, Eds. P.K. Stumpf, J.B. Mudd, and W.D. Nes, Plenum, New York, pp. 613–621.

Kenyon, C.N. et al. (1972) Fatty-acid composition and physiological properties of some filamentous blue-green algae. *Arch. Microbiol.,* **83,** 216–236.

Mercer, E.I. and Davies, C.L. (1979) Distribution of chlorosulfolipids in algae. *Phytochemistry*, **18,** 457–462.

Murata, N. and Nishida, I. (1987). Lipids of blue-green algae (Cyanobacteria) In *The Biochemistry of Plants,* vol. 9*,* Eds. P.K. Stumpf and E.E. Conn, Academic Press, New York, pp. 315–347.

Pohl, P. and Zurheide, F. (1979) Fatty acids and lipids of marine algae and the control of their biosynthesis by environmental factors. In *Marine Algae in Pharmaceutical Science*, Eds. H.A. Hoppe, T. Levring, and Y. Tanaka, Walter de Gruyter, Berlin, pp. 473–523.

Scragg, A.H. and Leathers, R.A. (1988). Production of fats and oils by plant and algal cell cultures. In *Single Cell Oil*, Ed. R.A. Moreton, Longman, Harlow, U.K., pp. 71–98.

Sijtsma, L. and de Swaaf, M.E. (2004) Biotechnological production and application of the ω-3 polyunsaturated fatty acid decosahexaenoic acid. *App. Microbiol. Biotechnol.,* **64**, 146–153.

Volkman, J.K. (1989) Fatty acids of microalgae used as feedstocks in aquaculture. In *Fats for the Future*, Ed. R.C. Cambie, Ellis Horwood, Chichester, U.K., pp. 263–283.

Wen, Z.Y. and Chen, F. (2003) Heterotrophic production of eicosapentaenoic acid by microalgae. *Biotech Adv.* **21**, 273–294.

2.12 Fungal lipids

The lipid content of many fungi has been reported, and has proved to be highly variable depending on not only the individual species but also the growth conditions. In addition, the lipids of mycelium and yeast cells, reproductive structures as well as those of cells in different stages of development, all vary. In general, phospholipids, sterols (and their esters, triacylglycerols and fatty acids) are major components (Tables 2.121 and Table 2.122). For a thorough survey of fungal lipids, the reader is referred to two books by Weete (1974, 1980) and to reviews covering yeast lipids (Rattray, 1988) and other fungi (Losel, 1988).

2.12.1 Overall lipid content

Vegetative hyphae contain 1 to 56% of their dry weight as lipids, depending on species, developmental stage, and growth conditions. Although certain species, such as the yeast *Saccharomyces cerevisiae* (ATCC 7754), have a very high lipid content, fungi generally average about 17% lipids. Several genera seem to be capable of producing high amounts of lipids. These include *Claviceps, Penicillium, Aspergillus, Mucor, Fusarium* and *Phycomyces* (Woodbine, 1959). Thus, lipids can represent up to 64% of the dry weight of *Pencillium spinulosum* (see Losel, 1988). The fluctuating proportions of total cellular lipid present in membranes correspond closely to the amount of these structures as seen in electron micrographs. The relative proportions of these and of storage lipids also vary considerably with growth conditions and development stage (Losel, 1988). In comparison, yeasts usually average 5 to 15% of their dry weight. "Fat" yeasts produce 30% or more lipids and, apart from the *Saccharomyces* spp. mentioned above, other "fat" yeasts include some *Rhodotorula* and *Lipomyces* spp. (Hunter and Rose, 1971; Rattray et al., 1975; Cohen and Ratledge, 2005).

The lipid content of fungal spores varies considerably with species and the conditions of spore formation. Values between 1 and 45% of spore dry weight have been reported. When individual genera are examined, there are

TABLE 2.121 Lipid composition of some yeasts

Species	Lipid content (% dry wt)	Percentage composition				
		TG	FA	Sterol	SE	PL
Debaryomyces hanseni	7	27	4	6	1	60
Hansenula anomala	13	77	tr	3	15	7
Lipomyces starkeyi	16	60	22	3	1	9
Saccharomyces cerevisiae (agar plate)	7	7	4	4	22	62
Saccharomyces cerevisiae (batch culture)	9	40	6	–	20	30
Yarrowia lipolytica (*Candida lipolytica*)	36	52	5	–	4	9
Candida utilis	11	55	1	2	1	38
Rhodotorula rubra	15	63	6	2	1	25

Note: TG = triacylglycerols; FA = nonesterified fatty acids; SE = sterol esters; PL = phospholipids; tr = trace.
Source: Rattray (1988).

TABLE 2.122 Lipid composition of some fungi

Class	Species	Lipid content (% dry wt)	NL	Polar	PL	TG	Sterols	SE
Dictyosteliomycetes	*Dictyostelium discoideum*	12	21	79	–	–	8	–
Chrytridiomycetes	*Blastocladiella emersonii*	8–11	12–47	25–82	35–69	8–28	5–13	18[a]
Oomycetes	*Achyla americana* (oospores)	33	98	2	0.4	89	0.3	0.8
	Pythium ultimum	4–18	55–91	9–45	–	40–58	–	–
Zygomycetes	*Mucor spp.*	3–15	55–65	35–45	20–35	–	4–16	5–10
	Rhizopus arrhizus	3–15	35–68	32–65	–	2–18	2–18	1–12
Ascomycetes	*Aspergillus niger*	4	31	69	41	5	19	4
	Neurospora crassa	9–19	–	–	93–98	<1	–	1–4
Hymenomycetes	*Agaricus bisporus*	5–6	68–74	22–26	–	12–13	2.3	2[a]
Urediniomycetes	*Puccinia graminis*	5–18	64–92	8–36	–	–	1–7	–

[a] Only one sample analysed.
Note: NL = nonpolar lipids; Polar = polar lipids; PL = phospholipids; TG = triacylglycerols; SE = sterol esters.
Source: Losel (1988).

still considerable variations in total lipid between species. For example, the lipids of *Ustilago* spp. vary between 4 and 22%. Spores from mesophilic species of *Mucor* have a lower lipid content than those of thermophilic or thermotolerant species (Sumner and Morgan, 1969). In some species, such as the rust fungi, the endogenous lipid is used as a source of energy during germination. However, not all the lipid is present as globules that are easily available for mobilization; sometimes most of the lipid is phospholipid and carotenoids (as in *Neurospora crassa*) and these compounds are little utilized during germination.

2.12.2 Cell wall lipids

Most of the fungal cell wall is composed of polysaccharides (80 to 90%), with the remainder consisting mainly of lipid and protein. There seems to be no correlation between cell wall lipid amounts and the genus examined. Most of the cell wall lipid is "bound" and relatively difficult to extract. In *S. cerevisiae*, neutral lipids and phosphoglycerides are major components, while *Candida albicans* contains sterol esters, sterols, fatty acids, triacylglycerols, and phospholipids. Thus, in contrast to *Saccharomyces* spp., the *Candida* spp. contain mainly sterols and sterol esters (40 to 60% total). In fungi, the nature of the wall lipid is undefined, although glycolipids have been noted in some cases. Hydrocarbons and sporopollenin, a carotenoid polymer highly resistant to decomposition, may occur on the surface of spores (see Losel, 1988).

2.12.3 Changes in lipid content with growth conditions

There are several major factors involved in determining the influence of the environment on fungal lipids. These include temperature, pH, carbon source, inorganic nutrients, aeration and growth factors. A particularly comprehensive account of factors influencing fatty acid composition in yeasts is by Rattray (1988). The use of yeasts and fungi as a source of oils is covered by Cohen and Ratledge (2005).

The response of fungi to growth temperatures varies with species. Many yeasts and yeast-like fungi contain more lipid when they are grown at temperatures below their optimum (Hunter and Rose, 1971). However, in other fungi (e.g., *Rhodotorula gracilis)* there are opposite changes. Fungi can be classified into mesophilic, psychrophilic, thermophilic, and thermotolerant species depending on their optimal growth temperatures. There do not appear to be any consistent differences in lipid content between these four groups. However, there is an increase in unsaturation of fatty acids of mesophilic fungi when compared to thermophilic fungi (Table 2.123) and this is consistent with a general increase in unsaturation at lower temperatures (Table 2.123).

The most important carbon source for fungal growth is glucose, while the best disaccharide is maltose. These and other sugars are easily converted to lipid. However, there are significant differences in the amount of lipid synthesis from different sugars. For example, in *Penicillium chrysogenum,* sucrose > fructose > glucose are sources for fat production. In addition, the actual concentration of the sugar has been shown to be important in many cases (cf. Weete, 1980; Rattray et al., 1975). Often, optimal lipid synthesis occurs at around 40% sucrose or glucose in the growth medium, but individual fungi vary considerably. With the increasing use of microorganisms for the utilization of inexpensive industrial waste products, many fungi have been studied with regard to the efficiency of their conversion of carbon substrates to lipid. These include, for example, fungi that can degrade alkanes (cf. Gerasimova et al., 1975).

There are two aspects to fungal requirements for inorganic nutrients. First, there are, clearly, absolute requirements for certain elements. Secondly, the levels of individual nutrients may alter lipid metabolism. For example, phosphorus levels may alter the phospholipid composition *of Candida utilis,* and increasing sodium chloride (from 0 to 10%) in the

TABLE 2.123 Unsaturation indices in fungi grown at different temperatures

Class	Fungus	Growth temperature (°C)	Unsaturation (Δ-/mol fatty acid)	Reference
Phycomycetes	*Mucor mucedo*	25	1.16–1.47	Sumner and Morgan (1969)
	M. miehei	25	0.95	Sumner and Morgan (1969)
		48	0.78	Sumner and Morgan (1969)
	M.strictus	10	1.13–1.26	Sumner et al. (1969)
		20	0.98–1.24	Sumner et al. (1969)
	Rhizopus sp.III	36	0.76–0.84	Sumner et al. (1969)
		48	0.60–0.86	Sumner and Morgan (1969)
Ascomycetes	*Candida lipolytica*	25	1.00	Kates and Baxter (1962)
	C. scottii	10	1.72	Kates and Baxter (1962)
	Chaetomium thermophile	45	0.65	Mumma et al. (1970)
	C. globosum	25	0.96	Mumma et al. (1970)
	Stilbella thermophile	45	0.56	Mumma et al. (1970)
	Stilbella sp.	25	1.47	Mumma et al. (1970)

medium increases the lipid content (from 0.3 to 6.3%) in *Candida* spp. The most important nutritional parameter for fungal lipid synthesis is the ratio of carbon to nitrogen. High fat accumulation is associated with low protein synthesis and is, therefore, favoured by high ratios of carbon/nitrogen. Indeed, the highest accumulation of fats by many fungi is obtained in nitrogen-deficient media. In general, lipid accumulation is favoured more when nitrogen is supplied from an inorganic source, since organic nitrogen tends to stimulate growth and protein synthesis. For a recent study of the effect of growth media on lipid composition, see Griffiths et al. (2003a), who studied the very important plant pathogen, *Phytophthora infestans*.

The effect of pH on fungal lipid accumulation is not clear. For some species, increasing medium pH increases lipid synthesis, but for others there is no effect (Weete, 1980). On the other hand, the degree of aeration is important because of the requirement for oxygen in sterol and unsaturated fatty acid synthesis. In addition, the degree of aeration has profound effects on fungal growth and differentiation. For example, in *Mucor rouxii*, growth under aerobic conditions results in filamentous growth, while anaerobic conditions cause the cells to be yeast-like. Under the latter conditions there are relatively high concentrations of short- to medium-chain (C_8 to C_{14}) fatty acids and hardly any unsaturation.

Although many fungi are able to grow successfully on media containing a carbon source and mineral salts, some also require vitamins or certain amino acids. In general, vitamin deficiencies result in a reduction of lipid content. However, certain yeasts, when grown on inositol-deficient media, accumulate large amounts of triacylglycerols (cf. Weete, 1980).

2.12.4 Fungal fatty acids

The fatty acid compositions of fungi have been extensively reviewed (Shaw, 1966; Wassef, 1977; Weete, 1980; Rattray, 1988; Losel, 1988). Fungi produce saturated and unsaturated aliphatic acids of 10 to 24 carbons in chain length. Palmitic is the major saturated acid, oleic the main monoenoic acid and linoleic the most common polyunsaturated acid.

In Phycomycetes, the presence of γ-linolenic acid is notable since the higher fungi (Ascomycetes, Fungi Imperfecti, and Basidiomycetes) produce only α-linolenate. Some higher homologues in the *n*-6 series, such as 20:3 and 20:4, may also be found. The Phycomycetes also tend to produce more C_{20} and C_{22} acids than the higher fungi, and several of their aquatic species have quite distinct fatty acid patterns. A few examples of fatty acid patterns are given in Table 2.124. Because of the recent interest in γ-linolenic acid for human nutrition, commercial processes have been developed by using moulds of the Mucorales for producing significant amounts of γ-linolenate-enriched oil (see Ratledge, 1989a).

Further discussions of this aspect and the use of *Mortierella* species for γ-linolenic and eicosapentaenoic acids are given by Ratledge (1989b). The industrial production of lipids by yeasts is reviewed by Davies and Holdsworth (1992), with physiological aspects of lipid accumulation summarized by Moreton (1988) and other topics, including economics, by Ratledge (1988).

So far as the Ascomycetes are concerned, there seem to be no differences in fatty acid composition that distinguish the subgroups ascosporogenous (true yeasts) from asporogenous (yeast-like) yeasts. *Saccharomyces* and *Candida* are the most-studied examples of these two groups. *Saccharomyces* spp. contain rather high levels of 16:1 and, with few exceptions, do not synthesize polyunsaturated fatty acids. In general, 16:1 > 18:1 > 16:0 and these acids account for >90% of the total. The relative amounts of acids in *C. utilis* vary with strain and culture conditions, but 16:0, 18:1, and 18:2 are principal fatty acids as in most asporogenous yeasts.

Several yeasts or yeast-like fungi secrete lipids. These extracellular lipids include hydroxy-, acetoxy- or long-chain fatty acids. For example, *Saccharomyces melanga* produces 3-D-hydroxypalmitic acid. Extracellular glycolipids called sophorosides are also produced by some yeast-like fungi, e.g., *Torulopsis magnaliae*. In contrast to

TABLE 2.124 Fatty acid compositions of some fungi

Species	Fatty acids (%total)								
	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4
Phycomycetes									
Blastocladiella emersonii	–	1	13	3	3	39	17	11	16
Entomophthora muscae	–	6	14	17	3	38	5	2	14
Mucor strictus	–	6	22	2	9	35	12	14	–
Phycomyces blakesleeanus	–	tr	11	2	21	30	35	2	–
Rhizophylyctis rosea (aquatic)	–	tr	14	–	6	74	3	3	–
Ascomycetes									
Candida albicans	–	1	12	8	7	36	25	10	–
Saccharomyces cervisiae	–	3	18	52	2	20	–	–	–
Torulopsis candida	–	tr	28	4	9	43	12	3	–
Fungi Imperfecti (Deuteromycetes)									
Aspergillus niger	–	tr	16	1	7	21	38	16	–
Penicillium chrysogenum	–	3	13	–	12	19	43	6	–
Homobasidiomycetes									
Calvatia gigantea	12	3	9	1	tr	9	62	tr	1[a]
Lactarius vellerens	–	tr	7	tr	54	22	14	2	–
Polyporus hirsutus	1	1	20	7	2	22	50	2	tr[a]
Heterobasidiomycetes									
Ustilago scitaminca	1	2	20	3	7	30	32	2	–

[a] 20:0.
Source: Weete (1980).

the Ascomycetes, Fungi Imperfecti do not usually contain large quantities of 16:1. Their major fatty acids are 16:0, 18:1, and 18:2, with significant amounts of α-18:3.

The basidiomycete fungi are divided into Homobasidiomycetes (saprophytic, e.g., mushrooms, puff balls) and the Heterobasidiomycetes (parasitic, e.g., the rust and the smut fungi). The Homobasidiomycetes have 16:0, 18:1, and 18:2 as their main fatty acids (Table 2.124), with linoleate usually predominating. In some mushrooms, hydroxy fatty acids are significant and may be the major acids (Prostenik et al., 1978). The rust fungi contain *cis*-9,10-epoxy octadecanoic acid, which is used to confirm them in culture.

2.12.5 Glycerol-based lipids in fungi

Triacylglycerols are the most abundant type of lipid in many fungi. However, their quantities vary considerably according to species, stage of development, and growth conditions (see previous sections and Table 2.125 and Table 2.126). The alkyl and alkenyl ethers are not common constituents of yeasts or mycelial fungi. Certain unusual nonpolar, such as diesters or 1-alkenyl esters of dihydric alcohols (e.g., ethanediol, butane-1,4-diol), may be present in a few species (Weete, 1980).

A comparison of the fatty acid content of the principal storage lipid, triacylglycerol, with that of a major membrane lipid, phosphatidylcholine, for several yeasts is made in Table 2.127. Although the same fatty acids are usually present in both lipids, their proportions vary considerably. As a generalization, the membrane lipid is much more unsaturated than the storage lipid.

The glycerophospholipids of fungi (Griffin et al., 1970), yeasts (Hunter and Rose, 1971; Rattray et al., 1975) and various organisms (Kates and Wassef, 1970) have been reviewed. The glycerophospholipid content of fungi averages 41% of the total lipids and is higher in the log phase than in

TABLE 2.125 Lipid composition of some yeasts

Species	Lipid content (% dry wt)	Percentage composition				
		TG	FA	Sterol	SE	PL
Debaryomyces hanseni	7	27	4	6	1	60
Hansenula anomala	13	77	tr	3	15	7
Lipomyces starkeyi	16	60	22	3	1	9
Sacchromyces cerevisiae (agar plate)	7	7	4	4	22	62
Saccharomyces cerevisiae (batch culture)	9	40	6	–	20	30
Yarrowia lipolytica (*Candida lipolytica*)	36	52	5	4		9
Candida utilis	11	55	1	2	1	38
Rhodotorula rubra	15	63	6	2	1	25

Note: TG: triacylglycerols; FA = nonesterified fatty acids; SE = sterol esters; PL = phospholipids; tr = trace.
Source: Rattray (1988).

TABLE 2.126 Lipid composition of some fungi

Class	Species	Lipid content (% dry wt)	NL	Polar	PL	TG	Sterols	SE
Dictyosteliomycetes	*Dictyostelium disoideum*	12	21	79	–	–	8	–
Chytridiomycetes	*Blastocladiella emersonii*	8–11	12–47	25–82	35–69	8–28	5–13	18*
Oomycetes	*Achyla Americana* (oospores)	33	98	2	0.4	89	0.3	0.8
	Pythium ultimum	4–18	55–91	9–45	–	40–58	–	–
Zygomycetes	*Mucor spp.*	3–15	55–65	35–45	20–35	–	4–16	5–10
	Rhizopus arrhizus	3–15	35–68	32–65	–	2–18	2–18	1–12
Ascomycotina	*Aspergillus niger*	4	31	69	41	5	19	4
	Neurospora crassa	9–19	–	–	93-98	<1	–	1–4
Hymenomycetes	*Agaricus bisporus*	5–6	68–74	22–26	–	12–13	2–3	2*
Urediniomycetes	*Puccinia graminis*	5–18	64–92	8–36	–	–	1–7	–

* Only one sample analysed.
Note: NL = neutral lipids; Polar = polar lipids; PL = phospholipids; TG = triacylglycerols; SE = sterol esters.
Source: Losel (1988).

TABLE 2.127 Comparison of the fatty acid compositions of the nonpolar lipid or triacylglycerol fraction with phosphatidylcholine from different yeasts

		Fatty acid (%)					
Organism	Lipid	14:0	16:0	16:1	18:1	18:2	18:3
Candida albicans	NL	4	52	–	33	6	3
	PC	1	15	25	47	9	–
Lipomyces starkeyi	NL	–	34	7	51	4	–
	PC	–	9	16	52	20	–
Saccharomyces cerevisiae	TG	3	16	42	27	–	–
	PC	tr	6	58	35	–	–
Trichosporon pullulans	TG	tr	15	tr	57	24	1
	PC	–	6	tr	8	83	3

Note: NL = nonpolar lipid; TG = triacylglycerol; PC = phosphatidylcholine; tr = trace.
Source: Rattray (1988).

TABLE 2.128 Phospholipid composition of some yeasts

		Composition (%)					
Organism	Total (% lipid)	PS	PI	PC	PE	DPG	PA
Ascosporogenous yeasts							
Hansenula polymorpha	31	17	22	24	26	–	11
Lipomyces starkeyi	16	11	12	43	25	5	1
Saccharomyces cerevisiae	32	5	12	20	56	7	–
Schizosaccharomyces pombe	43	12	14	50	13	6	–
Asporogenous and basidiosporogenous yeasts							
Candida albicans	15	12		40	25	5	–
Candida utilis	30	10	12	17	48	15	–
Rhodotorula rubra	25	16	7	16	29	12	6
Trichosporan cutaneum	40	12	11	29	15	6	7

Note: PS = phosphatidylserine; PI = phosphatidylinositol; PC = phosphatidylcholine; PE = phosphatidylethanolamine; DPG = diphosphatidylglycerol; PA = phosphatidic acid.
Source: Rattray (1988).

the stationary phase of growth. Sample compositions are shown in Table 2.128 and Table 2.129. It will be seen that the major lipids are phosphatidylcholine and phosphatidylethanolamine, with phosphatidylinositol often a major component. Unlike many other organisms, fungi often contain phosphatidylserine in significant amounts (usually 5 to 10%). Some detail is known of the subcellular distribution of different phospholipids. Most information is known for isolated mitochondria. In addition, the effects of growth temperature, morphogenesis and reproduction, and of genetic or nutritional factors on phospholipid class composition have been reported (see Losel, 1988). On the other hand, there is a wealth of detail of the phospholipids in individual membranes from yeasts, such as *S. cerevisiae* (see Rattray, 1988).

TABLE 2.129 Phospholipid compositions of some fungi

Organism	Composition (%)					
	PS	PI	PC	PE	DPG	PA
Dictyostelium discoideum						
spores	–	13	29	44	3	7
early culmination	–	11	11	41	4	7
Phytophora parasitica	1	7	39	20	3	3
Choanephora cucubitarum[a]	3	3	28	39	8	7
Glomus mosseae (mycelium)[a]	17	–	16	34	14	–
Aspergillus niger	5		51	29	2	–
Neurospora crassa	8	6	45	36	2	3
Cephalosporum lalciforme	9	2	50	15	6	–
Uromyces phaseoli	8	6	54	27	3	–

Note: PS = phosphatidylserine; PI = phosphatidylinositol; PC = phosphatidylcholine; PE = phosphatidylethanolamine; DPG = diphosphatidylglycerol; PA = phosphatidic acid.

[a] Contain significant amounts of phosphatidylglycerol (13% *C.cucurbitarum*, 19% *G.mosseae*).

Source: Losel (1988).

In *S. cerevisiae,* phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylserine are major phospholipids. Phosphatidylglycerol and diphosphatidylglycerol are present in mitochondrial membranes. Yeast phospholipid metabolism has been reviewed by Carman and Henry (1999). Several pesticides have an effect on lipid composition and metabolism of fungi. Recent studies include the action of mandelamide on *Phytophthora infestans* and of iprodione on *Botrytis cinerea.* Other relevant references can be found in these two papers (Griffiths et al., 2003b, 2003c).

2.12.6 Other fungal lipids

Sphingolipids, such as cerebrosides and ceramides, are minor but widely distributed constituents of fungi. Certain species contain characteristic sphingosine-containing lipids, such as the inositol-containing phosphorylceramides of *S. cerevisiae* (cf. Weete, 1980). Other minor lipids include hydrocarbons, while sterols (and sometimes carotenoids) are often major components. A full description of such lipids is outside the remit of this section and, for further details, the reader is directed to Weete, (1980), Brennan et al. (1974), Goodwin (1973), and Losel (1988).

When grown aerobically, most fungi can synthesize sterols. Exceptions often occur with pathogens of plants and animals, particularly the Oomycetes. Fungal sterols and their biosynthesis have been well reviewed (Elliott, 1977; Goodwin, 1973; Wassef, 1977; Weete, 1980). The exact pattern of sterol accumulation depends very much on the species. For example, desmosterol is found in many oomycetes, but seldom in other fungi (Losel, 1988). As with acyl lipids, the amounts of sterols vary with environmental factors. Sterol esters often appear to function as a store for membrane sterol precursors. In yeasts, lanosterol, zymosterol, dehydroergosterol and ergosterol are common (Rattray, 1988).

Carotenoids have been found in 60% of all fungi examined (see reviews by Goodwin, 1973; Weete, 1980). The major component is β-carotene. Xanthophylls appear to be absent from lower fungi, but have a wide, if sporadic, distribution in higher fungi. In many of the Discomycetes, xanthophylls are the major carotenoids. A vast range of terpenoids have been reported also (see Losel, 1988).

References

Brennan, P.J. et al. (1974) The lipids of fungi. *Prog. Chem. Fats Other Lipids,* **14**, 49–89.

Carman, G.M. and Henry, S.A. (1999) Phospholipid biosynthesis in the yeast *Saccharomyces cerevisiae* and its interrelationship with other metabolic processes. *Prog. Lipid Res.,* **38**, 361–399.

Cohen, Z. and Ratledge, C. Eds. (2005) *Single Cell Oils*, AOCS, Champaign, IL.

Davies, R.J. and Holdsworth, J.E. (1992) Synthesis of lipids in yeasts: biochemistry, physiology, production. *Adv. Appl. Lipid Res.,* **1,** 119–159.

Elliott, C.G. (1977) Sterols in fungi: their functions in growth and reproduction. *Adv. Microb. Physiol.,* **15**, 121–173.

Gerasimova, N.M. et al. (1975) Study of composition of lipids of *Cunninghamella elegans* cultured on N-alkanes. *Microbiology,* **44**(3), 408–411.

Goodwin, T.W. (1973) Comparative biochemistry of sterols in eurkayotic microorganisms. In *Lipids and Biomembranes of Eurkaryotic Microorganisms*, Ed. J.A. Erwin, Academic Press, New York, pp. 1–40.

Griffin, P.F.S. et al. (1970) Free lipids and carbohydrates of *Agaricus bisporus* mycelium. *Biochem. J.,* **119**, 11P-12P.

Griffiths, R.G. et al. (2003a) Effect of culture conditions on the lipid composition of *Phytophthora infestans. New Phytol.,* **158**, 337–344.

Griffiths, R.G. et al. (2003b) A mandelamide pesticide alters lipid metabolism in *Phytophthora infestans. New Phytol.,* **158**, 345–353.

Griffiths, R.G. et al. (2003c) Lipid composition of *Botrytis cinerea* and inhibition of its radiolabelling by the fungicide iprodione. *New Phytol.*, **160**, 199–207.

Hunter, K. and Rose, A.H. (1971) Yeast lipids and membranes. In *The Yeasts,* Vol. 2, Eds. A.H. Rose and J.S. Harrison, Academic Press, New York, pp. 211–270.

Kates, M. and Baxter, R.M. (1962) Lipid composition of mesophilic and psychrophilic yeasts (*Candida* species) as influenced by environmental temperature. *Can. J. Physiol.,* **40**, 1213–1227.

Kates, M. and Wassef, M.K. (1970) Lipid chemistry. *Annu. Rev. Biochem.,* **39**, 323–358.

Losel, D.M. (1988) Fungal Lipids. In *Microbial Lipids,* Vol. 1, Eds. C. Ratledge and S.G. Wilkinson, Academic Press, London, pp. 699–806.

Moreton, R.S. (1988) Physiology of lipid accumulating yeasts. In *Single Cell Oil*, Ed. R.S. Moreton, Longman, Harlow, U.K., 1–32.

Mumma, R.O., Fercus, C.L., and Sekwa, R.D. (1970) Lipids of thermophilic fungi: lipid composition comparisons between thermophilic and mesophilic fungi. *Lipids,* **5**, 100–103.

Prostenik, M. et al. (1978) Lipids of higher fungi. III. The fatty acids and 2-hydroxy fatty acids in some species of Basidiomycetes. *Chem. Phys. Lipids*, **22,** 97–103.

Ratledge, C. (1988) Biochemistry, stoichiometry, substrates and economics. In *Single Cell Oil,* Ed. Moreton, R.S., Harlow, U.K.: Longman, pp. 33–70.

Ratledge, C. (1989a) Biotechnology of oils and fats. In *Microbial Lipids*, Vol. 2, Eds. C. Ratledge and S.G. Wilkinson, Academic Press, London, pp. 567–668.

Ratledge, C. (1989b) Microbial oils and fats: perspectives and prospects. In *Fats for the Future*, Ed. R.C. Cambie, Ellis Horwood, Chichester, U.K., pp. 153–171.

Rattray, J.B.M. (1988) Yeasts. In *Microbial Lipids,* Vol. 1, Eds. Ratledge, C. and Wilkinson, S.G., Academic Press, London, pp. 555–697.

Rattray, J.B.M. et al. (1975) Lipids of yeasts. *Bacteriol. Rev.,* **39**, 197–231.

Shaw, R. (1966) Polyunsaturated fatty acids of microorganisms. *Adv. Lipid Res.,* **4,** 107–174.

Sumner, J.L. and Morgan, E.D. (1969) Fatty acid composition of sporangiospores and vegetative mycelium of temperature-adapted fungi in the order Mucorales. *J. Gen. Microbiol.,* **59**, 215–221.

Sumner, J.L. et al. (1969) Effect of growth temperature on the fatty acid composition of fungi in the order Mucorales. *Can. J. Microbiol.,* **15,** 515–520.

Wassef, M.K. (1977) Fungal lipids. *Adv. Lipid Res.,* **15**, 159–232.

Weete, J.D. (1974) *Fungal Lipid Biochemistry,* Plenum, New York.

Weete, J.D. (1980) *Lipid Biochemistry of Fungi and Other Organisms,* Plenum, New York.

Woodbine, M. (1959) Microbial fat: microorganisms as potential fat producers. *Prog. Ind. Microbiol.,* **1,** 179–245.

2.13 Bacterial lipids

Any discussion of the occurrence of lipids in bacteria is complicated by the wide variety of such compounds that are found, often only in a few species. Nevertheless, some broad generalizations can be made, and these will be highlighted in the following section; however, it must always be remembered that there is an exception to every rule. For a more complete coverage of bacterial lipids, the reader is referred to Razin and Rottem (1982), Finnerty (1978), Cronan (1978), Raetz (1978), Shaw (1975), Goldfine (1972) and Ratledge and Wilkinson (1988, 1989). Heath et al. (2001) have thoroughly reviewed antibacterial agents, which target lipid biosynthesis. This article concentrates on fatty acid biosynthesis, but includes sections on phospholipid and Lipid A synthesis, as well as possible new developments.

2.13.1 Fatty acid distribution

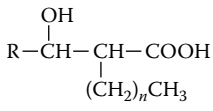
Fatty acids in the C_{12} to C_{20} chain-length range account for the majority of bacterial fatty acids, which are usually saturated or monounsaturated. Bacteria generally do not contain polyunsaturated fatty acids and some isolated reports of their occurrence are probably due to contamination from the growth medium. Notable exceptions are some cyanobacteria, which contain linoleate and linolenate (Section 2.11), gliding bacteria, which have large amounts of arachidonate in their acyl lipids (Fautz et al., 1979), and a range of eubacteria found in marine environments (e.g., Intriago and Floodgate, 1991; Matsui et al., 1991).

Besides the ubiquitous even-chain saturated and unsaturated fatty acids, bacteria characteristically contain odd-chain and branched fatty acids as well as 3-hydroxy- and cyclopropane derivatives. These fatty acids are present in lipopolysaccharide, cell-wall lipoprotein and lipoteichoic acid, as well as membrane glycerolipids (Table 2.130). As a generalization, the Gram-positive bacteria contain higher proportions of branched-chain acids than do Gram-negative bacteria. In those Gram-positive bacteria, where branched chains are prevalent, there is usually a corresponding decrease in the proportions of unsaturated acids (O'Leary and Wilkinson, 1988).

Exceptionally long-chain, branched fatty acids termed mycolic acids and of general formula **(1)** are found in members of the *Mycobacterium–Nocardia–Corynebacterium* group, *Rhodococcus* and the "aurantiaca" toxin (Brennan, 1988) (see also Section 1.2.13).

TABLE 2.130 Major fatty acids in bacteria

Saturated	Tetradecanoic acid
	Hexadecanoic acid
	Octadecanoic acid
Unsaturated	Octadecenoic acid (11*c*)
Branched chain	13-Methyltetradecanoic acid
	14-Methylhexadecanoic acid
Substituted	3-Hydroxytetradecanoic acid
	cis-9,10-Methylene-hexadecanoic acid
	cis-11,12-Methyleneoctadecanoic acid



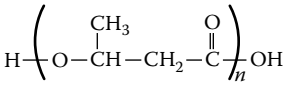
(1)

n = 5–23 (odd numbers only)

R may be up to C_{59} and include other functional groups.

2.13.2 Glycerol-based acyl lipids

In contrast to animals and plants, bacteria seldom accumulate triacylglycerols or other "fats." The monoacyl and diacyl derivatives are important metabolic intermediates and will be present in small but detectable amounts. Thus, the lipids of bacteria are essentially associated with one or other of the cell membranes, with the exception of storage polymers (notably poly(3-hydroxybutyrate) (PHB)) or hydrocarbon inclusions (e.g., from hydrocarbon-grown *Acinetobacter* strains). PHB occurs in many prokaryotes — both Gram-positive and Gram-negative. Its biosynthesis is reviewed by Schweizer (1989) and some commercial applications are discussed by Ratledge (1989). The structure of PHB is shown in **(2)**. Economically, the production of PHB is much more expensive than that of cheap chemically-produced polypropylenes and polyethylenes. However, its biodegradable nature is a big advantage in medical applications and it is likely to become more important in the future.



(2)

where n = 600–25000

Poly(3-hydroxyalkanoate)s have also been reported to accumulate in inclusion bodies in different bacteria, such as *Pseudomonas* spp. (de Smet et al., 1983). In contrast to the inclusion bodies for PHB and poly(3-hydroxyalkanoate)s, where the surrounding membrane is an intracytoplasmic one, the hydrocarbons of *Acinetobacter* spp. are contained by a monolayer limiting membrane with a lipid composition different from that of other cellular membranes (Scott and Finnerty, 1976).

Glycerophospholipids are usually the major constituents of bacterial membranes. These lipids are usually enriched with saturated fatty acids at the *sn*-1 position and with unsaturated acids at the *sn*-2 position. In cyanobacteria, the distribution is governed by chain length rather than unsaturation, with *sn*-1 C_{18} and *sn*-2 C_{16} being the rule (Zepke et al.,1978). The cyanobacteria are also exceptional in that they synthesize (and accumulate) only one phospholipid, phosphatidylglycerol.

With the exception of the Actinomycetes (e.g., mycobacteria), phosphatidylglycerol is present in all species of bacteria. It is frequently the major phospholipid. The *sn*-3 hydroxyl of the glycerol head group may be esterified with an amino acid (usually alanine, ornithine, or lysine) to form an aminoacylphosphatidylglycerol (Goldfine, 1982). These derivatives are more common in Gram-positive species. The *sn*-2 hydroxyl of the glycerol head group may be glycosidically linked to glucosamine or the *sn*-3 hydroxyl group can be acylated. However, these substitutions are not very common.

Cardiolipin (or diphosphatidylglvicerol) usually occurs along with phosphatidylglycerol. The proportions of these two lipids can be easily changed by growth conditions. The presence of cardiolipin as a major constituent in bacteria is in striking contrast to eukaryotes, where it is only present in the inner mitochondrial membrane.

The major glycerophospholipid of Gram-negative bacteria is phosphatidylethanolamine. It is also a major component of some Gram-positive species, such as *Bacillus.* In contrast, phosphatidylcholine is seldom a major Iipid in bacteria. In a few genera, the ethanolamine head group may be partly methylated (giving the mono- and dimethyl derivatives).

Some examples of phospholipid composition in Gram-positive and Gram-negative bacteria are shown in Table 2.131 and general features of the lipid composition of Gram-negative bacteria are shown in Table 2.132.

Phosphatidylinositol is uncommon in bacteria and is found in a few Gram-positive species only. In Actinomycetes and a few other bacteria, mannosides of phosphatidylinositol may be present. Other glycerophospholipids, such as phosphatidic acid and phosphatidylserine, have a widespread occurrence in bacteria, but only in small amounts. Both of these lipids play an important role as metabolic intermediates (Finnerty, 1978; Raetz, 1978).

TABLE 2.131 3Examples of phospholipid compositions of bacteria[a]

	Major Phospholipids (% total)[b]		
	PE	**PG**	**DPG**
Gram-positives			
Micrococcus spp.	0	26–88	10–67
Planococcus spp.	8–14	23–38	4445
Staphyloccus spp.	0	52–90	1–10
Streptococcus spp.	0	8–38	8–62
Gram-negatives			
Escherichia coli	74	19	3
Klebsiella pneumoniae	38–82	5–13	6–14
Vibrio spp.	23–81	10–54	tr–2
Haemophilus spp.	78–85	15–18	0–3
Pseudomonas spp.	40–87	9–76	0–35
Acetobacter spp.	25–31	20–31	4–13
Neisseria spp.	51–77	19–22	2–20

[a] For further information, see O'Leary and Wilkinson (1988) and Wilkinson (1988).

[b] Others include 36 to 38% lysyl PG in *Staphyloccus* spp., 5 to 16% phosphoglycolipids in *Streptococcus* spp. and 32 to 44% phosphatidylcholine in *Acetobacter* spp.

TABLE 2.132 Summary of the lipid content of Gram-negative bacteria[a]

Usual features	
Main fatty acids	16:0,16:1 (Δ-9), 18:1(Δ-11), 3-OH 14:0
Main phospholipids	PE with variable proportions of PG, DPG
Minor/rare acyl lipids	Hydrocarbons, acylglycerols, waxes
Absent	Steroids
Unusual compositions	
Fatty acids	
Mainly straight-chain odd-chain	e.g., *Desulphobulbus* spp.
Mainly branched-chain	e.g., *Pseudomonas putrefaciens*
Polyenoic acids significant	e.g., *Vibrio marinus*
Phospholipids	
PE absent	e.g., *Pseudomonoas vesiculans*
PC a major component	e.g., *Agrobacterium* spp.
Plasmanyl lipids significant	e.g., *Stigmatella* spp.
Other polar lipids	
Glycolipids significant	e.g., *Some Pseudomonas* spp.
Ornithine amide lipids present	e.g., *Acetobacter* spp.
Sphingolipids present	e.g., *Bacteriodes* spp.
Sulphonolipids present	e.g., *Flavobacterium* spp.

[a] For more details, refer to Wilkinson (1988).
Note: PE, phosphatidylethanolamine; PG, phosphatidylglycerol; DPG, disphosphatidylglycerol; PC, phosphatidylcholine.

Instead of a phosphate unit at the *sn*-3 position, a carbohydrate moiety may be present. Such glycosylglycerides have quite a widespread, but usually minor occurrence in bacteria. The cyanobacteria have already been mentioned as having only one phospholipid. They have a very simple lipid pattern, with the remaining compounds being three glycoglycerides: monogalactosyldiacylglycerol, digalactosyldiacylglycerol, and sulfoquinovosyldiacylglycerol. Thus, in contrast to other bacteria and like the photosynthetic membranes of plants and algae, these organisms contain glycosylglycerides as their major membrane lipids.

Glycosylglycerides are more important in Gram-positive than Gram-negative bacteria. Except in green photosynthetic bacteria and *Treponema* spp., where they are the most common lipids, the monoglycosyldiacylglycerols are less common than the diglycosyl lipids. The disaccharide moiety of the latter is usually either two glucose, galactose, or mannose sugars linked (12) or (16) (Section 1.2.5 and Table 1.11).

The glycosyldiacylglycerols also give rise to some unusual phosphoglycolipids and constitute the hydrophobic moiety of lipoteichoic acids, and the lipo-conjugates of most Gram-positive bacteria (Section 1.2.13). A distinctive feature of the streptococci is the variety and abundance of phosphoglycolipids linking the glycolipids with the lipoteichoic acids. Typically the phosphoglycolipids are derived from α-kojibiosyldiacylglycerol by substitution at one or both of the 6 positions on glucose by a (1) 3-*sn*-phosphatidyl group, (2) *sn*-glycerol-1-phospho group, or (3) fatty acyl group (see O'Leary and Wilkinson, 1988). In mycobacteria, phosphatidylinositol mannosides may act as membranous anchors with lipoarabinomannins as the functionally important biopolymers, while glycosyldiacylglycerols, phosphatidylglucosyldiacylglycerols and glycerophosphoglycosyldiacylglycerols act as membranous anchors with the lipoteichoic acids as the corresponding extensions (Brennan, 1988). In a few species (e.g., *Mycobacterium tuberculosis*), both anchors may coexist.

Some phosphoglycolipids are also present in certain Gram-positive organisms. Phosphatidylglycolipids are found in streptococci, *sn*-glycero-3-phosphoglycolipids in mycoplasmas, and the *sn*-glycero-1-phosphophosphatidylglycolipids in several Gram-positive genera.

2.13.3 Other glycolipids

Acylated sugars, such as acylglucoses of mycobacterial "cord factor," a diacylated glucose attached to D-glyceric acid in the cell envelope of *Nocardia caviae* (Pommier and Michel, 1981) and the rhamnolipid of *Pseudomonas aeruginosa* are specific examples of sparsely occurring glyolipids. Other examples are the mycosides of mycobacteria. These are glycosides of *p*-substituted phenols with normal and branched-chain fatty acids esterified to the oligosaccharide. The glycosides are involved in the pathogenicity of *Mycobacteria.* In comparison to other bacteria, *Mycobacteria* (and the related *Nocardia* and *Corynebacteria*) contain large amounts of lipid, which is mainly in the cell wall.

The mycobacterial cell wall contains three main components — (1) a skeleton composed of arabinogalactan mycolate covalently linked through a phosphodiester bond to peptidoglycan, (2) peptides that are removed by proteinolysis, and (3) free lipids that are easily extracted by solvents.

The skeleton consists of a branched polymer of D-arabinose and D-galactose in a 5/2 ratio, where about every 10th arabinose contains a mycolic acid esterified to the 5′-hydroxyl. These mycolic acids are C_{60} to C_{90} fatty acids that are 2-branched and 3-hydroxylated. They may also contain cyclopropane rings, methyl branches, and methoxy groups. In *Nocardia* similar arabinoglactan mycolates are present, known as nocardomycolic acids (40 to 60 carbons with a C_{14} or C_{16} branch). In *Corynebacterium* spp., corynomycolic acids (28 to 40 carbons with a C_{24} banch) are found (Minnikin, 1982). A comprehensive review of the structure, biosynthesis, and function of myclic acids is that by Barry et al. (1988). A potential use of such acids is as biosurfactants and a recent paper on this subject, including a new method of synthesis, is that by Lee et al. (2005).

Some 25 to 30% of the weight of mycobacterial cell walls consists of extractable lipids. These are a mixture of cord factors, mycosides, sulfolipids and wax D. Cord factors are a mixture of trehalose 6,6′-mycolates. The

constituent mycolic acids are of the same composition as those found in wax D and are characteristic of the organism from which they are isolated (Figure 2.2). The sulpholipids consist of trehalose, which is sulphated at the 2 position and acylated at several positions on both sugar residues. The acyl groups are a mixture of palmitic acid and very long-chain (31 to 46 carbons) methyl-branched acids called phthioceranic acid (Minnikin, 1982). The outermost layer of the mycobacterial cell wall contains mycosides, which are either phenolic glycolipids (mycosides A and B) or glycolipid peptides (mycoside C). Their complex structure is shown in Figure 2.3. For a comprehensive review of the lipids of mycobacteria and other actinomycetes, refer to Brennan (1988).

The cell wall layer of mycobacteria contributes to their resilience and contains many compounds that contribute to their pathogenicity. Of the latter, the dimycocerosate esters (DIMs) are especially important and occur, almost exclusively, in pathogenic mycobacteria. Since *Mycobacterium tuberculosis* alone is responsible for over 2 million deaths per year, there is considerable current interest in DIMs (Onwueme et al., 2005).

The cytosol of Gram-negative bacteria is surrounded by a complex cell envelope consisting of, at least, three layers (Figure 2.4). The cytoplasmic membrane is composed of the usual phosphoglyceride bilayer with integral and peripheral proteins. Then comes a peptidoglycan layer, which is separated from the outer membrane by a periplasmic space. The outer membrane is extremely asymmetric, but contains lipopolysaccharide and enterobacterial-common antigen in its outer leaflet. Phospholipid molecules are missing from the outer membrane in many Gram-negative bacteria, but are present in some species. When phospholipids are present in the outer membrane, phosphatidylethanolamine is, by far, the most common.

The lipopolysaccharide, which is involved in several aspects of pathogenicity, is a complex polymer with four parts (Figure 2.4 and Figure 2.5). Outermost is a carbohydrate chain of variable length (called the *O*-antigen), which is attached to a core polysaccharide divided into the outer core and the backbone. These two parts vary between bacteria. Finally, the backbone is attached to a glycolipid called lipid A (Figure 2.5). The link between lipid A and the rest of the molecule is usually through a number or 3-deoxy-D-manno-octulosonic acid (KDO) molecules. The presence of KDO is often used as a marker for lipopolysaccharide (or outer membrane) even though it is not present in all bacterial lipopolysaccharides. A very comprehensive review on bacterial lipopolysaccharides is that by Wilkinson (1996), and Raetz and Whitfield (2002) have dealt with these molecules in relation to their endotoxin activity.

Lipid A is composed of a disaccharide of glucosamines. The amino groups are substituted with 3-hydroxymyristate, while the hydroxyl groups contain saturated (12 to 16 carbons) acids and 3-myristoxymyristate. Phosphate and KDO groups are also substituted (Figure 2.5). The fatty acids are sometimes referred to as "bound fatty

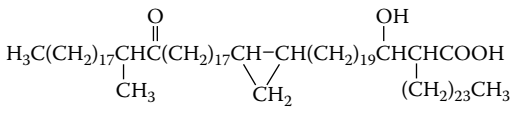
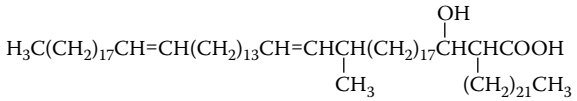
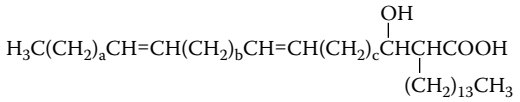
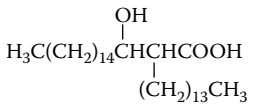
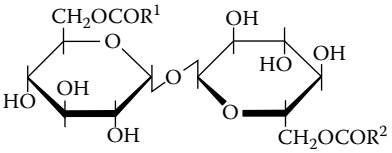
Name	*Formula* (*example of producing organism*)
β-Mycolic acid	 (*Mycobacterium tuberculosis*)
α-Smegmamycolic acid	 (*Mycobacterium smegmatis*)
Nocardomycolic acid	 $a + b + c$ = 32 or 34 (*Nocardia spp.*)
Corynomycolic acid	 (*Clostridium diphtheriae*)

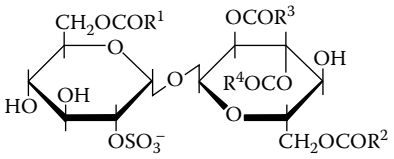
FIGURE 2.2 Some major mycolic acids found in mycobacteria and related actinomycetes. (From Harwood, J.L. and Russell, N.J. (1984) *Lipids in Plants and Microbes.* Allen and Unwin, Hemel Hempstead, U.K. With permission.)

(a) *Trehalose mycolate*



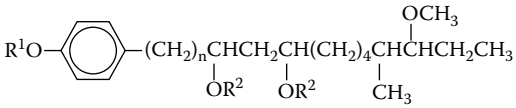
R^1OH and R^2OH are mycolic acids

(b) *Sulfolipid*



R^1OH–R^4OH are palmitic acid or very-long-chain branched fatty acids

(c) *Mycosides A and B*



R^1OH is a mono- or trisaccharide

R^2OH is a C_{12}–C_{18} saturated fatty acyl or a mycocerosic acyl, e.g., $H_3C(CH_2)_{21}\left(\underset{CH_3}{CHCH_2}\right)_2\underset{CH_3}{CHCO}$

(d) *Mycoside C*

RO-Phe-alloThr-Ala-alaninol-sugar
|
O-sugar

ROH is a long-chain (C_{28}–C_{34}) methoxy fatty acid, e.g., $H_3C(CH_2)_{22}CH{=}CH\underset{OCH_3}{CH}CH_2COOH$

Phe, phenylalanine; alloThr, allo-threonine; Ala, alanine

FIGURE 2.3 Structures of mycobacterial lipids. (From Harwood, J.L. and Russell, N.J. (1984) *Lipids in Plants and Microbes.* Allen and Unwin, Hemel Hempstead, U.K. With permission.)

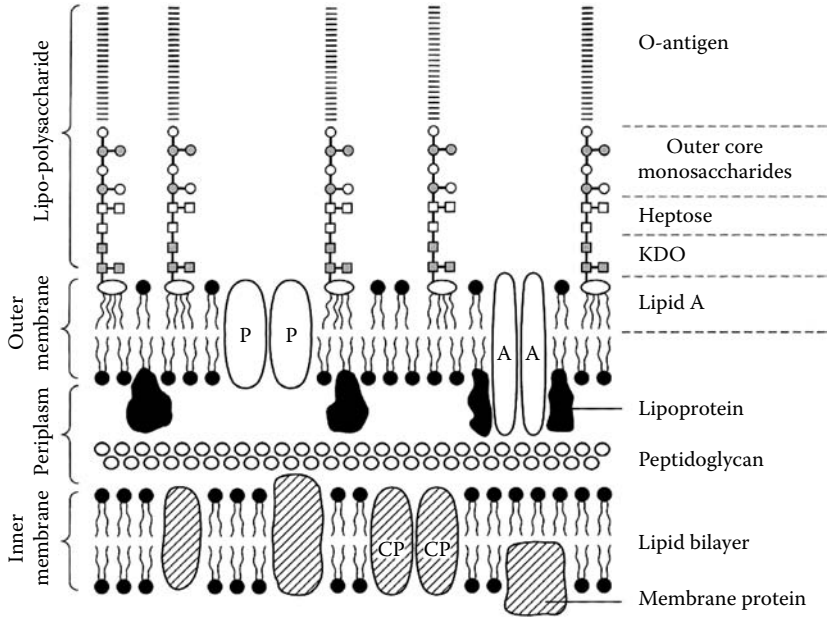


FIGURE 2.4 Organization of the membrane system of a Gram-negative bacterium. P = porin; A = transmembrane peptidoglycan-associated protein; CP = carrier protein. (Adapted from information in Wilkinson, 1996).

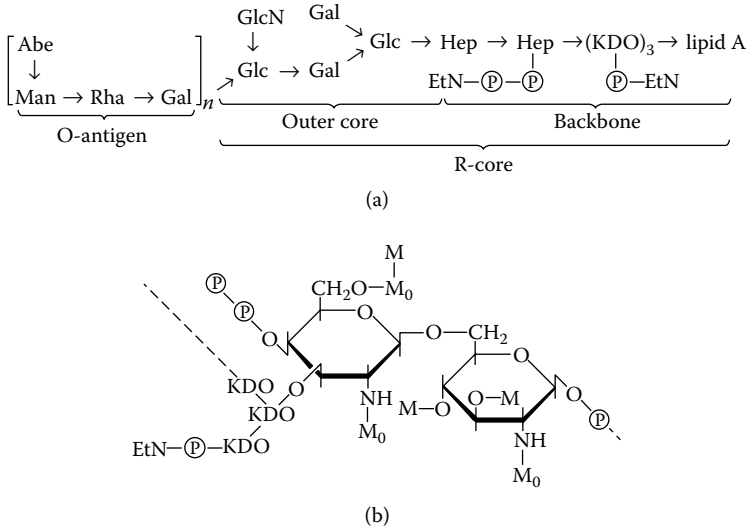


FIGURE 2.5 Generalized structures of (a) lipopolysaccharide and (b) lipid A showing the KDO link region. Abe, abequose; Man, mannose; Rha, rhamnose; Gal, galactose; Glc, glucose; Hep, heptose; KDO, deoxy-D-manno-octulosonic acid; (P), phosphate; EtN, ethanolamine; M, myristate; M_0, β-hydroxymyristate; --- represents the connection to the rest of the molecule (see (a)). (From Harwood, J.L. and Russell, N.J. (1984) *Lipids in Plants and Microbes.* Allen and Unwin, Hemel Hempstead, U.K. With permission.)

acids" while lipopolysaccharide is "bound lipid." This is because common lipid extraction procedures do not solubilize them and they are only released after hot phenol/water treatment. Lipopolysaccharide not only contains hydroxyl fatty acids, but also lacks unsaturated or cyclopropane fatty acids, which are characteristic of other lipids in Gram-negative bacteria (Goldfine, 1982).

Gram-positive bacteria contain teichoic acid and other highly anionic polymers in their cell walls and membranes. Some of the membrane teichoic acids are covalently linked to glycolipids, such as diacylglycoglycerol, to give a lipoteichoic acid (Figure 1.20). The amounts of esterification and glycosylation and the type of sugars or glycolipid vary amongst bacteria.

2.13.4 Other lipid types

Several other types of lipids have been reported in bacteria. Anaerobic bacteria, including those of the rumen, contain large quantities of plasmalogens with both ether and ester linkages. In contrast, plasmalogens are found in very few aerobic or facultative anaerobes.

Archaebacteria contain two types of phytanylglycerol ethers that lack ester bonds (Tornabene et al., 1979; Bayley and Morton, 1978). Extreme halophiles contain diglycosyldiphytanylglycerol diether analogues of phosphatidylglycerol, phosphatidylglycerophosphate, and phosphatidylglycerol sulfate. Acidophilic thermophiles contain diethers and tetraethers of diglyceroldiphytanyldiglycerol. In methanogenic bacteria, both types of ether are found.

Other lipid types that have been found in bacteria include wax esters, hydrocarbons, sphingolipids, steroids and terpenoids. These occur sporadically, however, and are seldom major components. An obvious exception is the presence of carotenoids and the ornithine-containing lipid of purple photosynthetic bacteria. Further details of minor lipid components of bacteria can be found in Razin and Rotten (1982), Shaw (1975), and Goldfine (1972).

2.13.5 Archaebacteria and other specialized bacteria

For further information on these bacteria, refer to Smith (1988) and De Rosa and Gambacorta (1988).

Archaebacteria have distinctive features that allow them to be classified within a separate kingdom from other prokaryotes. These features include the presence of large amounts of ether-linked lipids (Section 1.2.2.3). They often occupy extreme environments and include methanogens, the extreme halophiles, and most of the thermoacidophiles.

Neutral lipids are a significant fraction of the total, with isoprenoid hydrocarbons making up 35 to 95% of the apolar lipids. The complex lipids of archaebacteria are unique in that they are based upon alkyl ethers of glycerol or some other polyol. The diether has the structure 2,3-di-*O*-phytanyl-*sn*-glycerol. The major phospholipid of halophiles is the diethyl analogue of phosphatidylglycerophosphate (i.e., 2,3-di-*O*-phytanyl-*sn*-glycerol-1′-phosphoryl-3′-*sn*-glycerol-1-phosphate). Various unidentified polar lipids are present in *Thermoplasma*, of which the major compound is a phosphoglycolipid.

Photosynthetic prokaryotes include those which carry out anoxygenic photosynthesis (green and purple bacteria) and those that utilize oxygenic photosynthesis (cyanobacteria and prochlorophyta). Of course, these organisms contain

photosynthetic as well as cytoplasmic membranes — both with a distinct lipid composition. The photosynthetic pigments (chlorophylls, carotenoids. etc.) are confined to the photosynthetic membranes. Glycosyldiacylglycerols are major components in green bacteria and cyanobacteria, but are generally absent from purple bacteria, apart from small amounts of sulfoquinovosyldiacylglycerol. The purple bacteria have phosphatidylglycerol as their major phospholipids, but also possess significant amounts of phosphatidylethanolamine. Phosphatidylglycerol is also the major phospholipid in green bacteria and is the only significant phospholipid of cyanobacteria. An ornithine lipid is a significant component of purple bacteria.

The mollicutes (e.g., *Acholeplasma laidlawii*) are unique among prokaryotes in lacking an outer cell wall. Thus, they are very useful organisms for biochemical experiments and have been extensively studied. Glycolipids are usually major components with three types (glycosylacylglycerols, polyterpenol glycosides, and acylated sugars) occurring. About 50% of the lipids of mollicutes are polar, mainly phosphoglycerides. Phosphatidylglycerol and diphosphatidylglycerol are always found and aminoacyl derivatives of the former are present in some species. Phosphatidylethanolamine is only found in *Ureaplasma.* Unique phosphoglycolipids containing glyceraldehyde and phosphotriester groupings occur in some *Acholeplasma* spp.

References

Barry, C.E. III et al. (1988) Mycolic acids: structure, biosynthesis and physiological functions. *Prog. Lipid. Res.,* **37**, 143–179.

Bayley, S.T. and Morton, R.A. (1978) Recent developments in the molecular biology of extremely halophilic bacteria. *CRC Crit. Rev. Microbiol.,* **6**, 151–205.

Brennan, P.J. (1988) Mycobacterium and other actinomycetes. In *Microbial Lipids,* Vol. 1, Eds. C. Ratledge and S.G. Wilkinson, Academic Press, London, pp. 203–298.

Cronan, J.E. (1978). Molecular biology of bacterial membrane lipids. *Annu. Rev. Biochem.,* **47**, 163–189.

De Rosa, M. and Gambacorta, A. (1988) The lipids of archaebacteria. *Prog. Lipid Res.,* **27**, 153–175.

De Smet, M.J. et al. (1983) Characterization of intracellular inclusions formed by *Pseudomans oleovorans* during growth on octane. *J. Bacteriol.,* **154**, 870–878.

Fautz, E. et al. (1979) Iso-branched 2- and 3-hydroxy fatty acids as characteristic lipid constituents of some gliding bacteria. *J. Bacteriol.,* **140**, 852–858.

Finnerty, W.R (1978) Physiology and biochemistry of bacterial phospholipid metabolism. *Adv. Microb. Physiol.,* **18**, 177–233.

Goldfine, H. (1972) Comparative aspects of bacterial lipids. *Adv, Microb. Physiol.,* **8**, 1–58.

Goldfine, H. (1982) Lipids of prokaryotes-structure and distribution. *Curr. Top. Membr. Transp.,* **17**, 1–43.

Harwood, J.L. and Russell, N.J. (1984) *Lipids in Plants and Microbes.* Allen and Unwin, Hemel Hempstead, U.K.

Heath, R.J. et al. (2001) Lipid biosynthesis as a target for antibacterial agents. *Prog. Lipid Res.,* **40**, 467–497.

Intriago, P. and Floodgate, G.D. (1991) Fatty acid composition of the estuarine *Flexibacter* sp. strain Inp: effect of salinity, temperature and carbon source for growth. *J. Gen. Microbiol.,* **137**, 1503–1509.

Lee, M. et al. (2005) Synthesis of mycolic acid biosurfactants and their physical and surface-active properties. *J. Am. Oil Chem. Soc.,* **82**, 181–188.

Matsui, Y. et al. (1991) Phospholipid and fatty acid compositions of *Alteromonas putrefaciens* and *A. haloplanktis. Lett. Appl. Microbiol.,* **12**, 51–53.

Minnikin, D.E. Ed. (1982) Mycobacterial lipids: complex lipids, their chemistry, biosynthesis and roles. In *The Biology of the Mycobacteria, Physiology, Identification and Classification,* Vol. 1, Academic Press, New York, pp. 95–184.

O'Leary, W.W. and Wilkinson, S.G. (1988) Gram-positive bacteria. In *Microbial Lipids,* Vol. 1, Eds. C. Ratledge and S.G. Wilkinson, Academic Press, London, pp. 117–201.

Onwueme, K.C. et al. (2005) The dimycocerosate ester polyketide virulence factors of mycobacteria. *Prog. Lipid. Res.,* **44**, 259–302.

Pommier, M.T. and Michel, G. (1981) Structure of 2′, 3′-di-*O*-acyl-alpha-D-glucopyranosyl-(1→2)-D-glyceric acid, a new glycolipid from *Nocardia caviae. Eur. J. Biochem.,* **118,** 329–333.

Raetz, C.R.H. (1978) Enzymology, genetics, and regulation of membrane phospholipid synthesis in *Escherichia coli. Microbiol. Rev.,* **42**, 614–659.

Raetz, C.R.H. and Whitfield, C. (2002) Lipopolysacharide endotoxins. *Annu. Rev. Biochem.,* **71**, 635–700.

Ratledge, C. (1989) Biotechnology of oils and fats. In *Microbial Lipids.* Vol. 2, Eds. C. Ratledge and S.G. Wilkinson, Academic Press, London, pp. 567–668.

Ratledge, C. and Wilkinson, S. G. Eds. (1988) *Microbial Lipids*, Vol. 1, Academic Press, London.

Ratledge, C. and Wilkinson, S. G. Eds. (1989) *Microbial Lipids*, Vol. 2, Academic Press, London.

Razin, S. and Rottem, S. Eds. (1982) *Membrane Lipid of Prokaryotes.* Academic Press, London.

Schweizer, E. (1989) Biochemistry of lipids. In *Microbial Lipids,* Vol. 2, Eds. C. Ratledge and S.G. Wilkinson, Academic Press, London, pp. 3–50.

Scott, C.C.L. and Finnerty, W.R. (1976) Characterization of intracytoplasmic hydrocarbon inclusions from the hydrocarbon-oxidizing *Acinetobacter* species HO1-N. *J. Bacteriol.,* **127**, 481–489.

Shaw, N. (1975) Bacterial glycolipids and glycophospholipids. *Adv. Microb. Physiol.,* **12**, 141–167.

Smith, P.F. (1988). Archaebacteria and other specialized bacteria. In *Microbial Lipids,* Vol. 1, Eds. C. Ratledge and S.G. Wilkinson, Academic Press, London, pp. 489–553.

Tornabene, T.G. et al. (1979) Squalenes, phytanes and other isoprenoids as major neutral lipids of methanogenic and thermoacidophilic archaebacteria. *J. Mol. Evol.,* **13**, 73–83.

Wilkinson, S.G. (1988) Gram-negative bacteria. In *Microbial Lipids,* Vol. 1, Eds. C. Ratledge and S.G. Wilkinson, Academic Press, London, pp. 299–488.

Wilkinson, S.G. (1996) Bacterial lipoplysaccharides — themes and variations. *Prog. Lipid Res.,* **35**, 283–343.

Zepke, H.D. et al. (1978) Combination and positional distribution of fatty acids in lipids from blue-green algae. *Arch. Microbiol.,* **119,** 157–162.

TABLE 2.133 Phospholipid composition of viruses

	Percentage of phospholipids				
	PC	**PE**	**Sph**	**PS**	**PI**
RNA viruses					
Paramyxoviridae, e.g., Sendai, measles	8–53	10–41	12–30	2–17	0–11
Rhabdoviridae, e.g., rabies	16–38	20–34	16–31	7–20	0–10
Orthomyxoviridae, e.g., influenza	10–39	12–45	16–23	7–22	0–8
Togaviridae, e.g., rubella	21–49	19–35	7–29	9–21	0–9
Retroviridae, e.g., Rose's sarcoma	16–30	26–42	22–33	12–17	0–4
DNA viruses					
Herpetoviridae, e.g*,* herpes simplex	51–57	21–25	4–21	4–13	1–13
Poxviridae, e.g., vaccinia	34–49	12–14	2–4	0–5	12–19

Note: PC = phosphatidylcholine; PE = phosphatidylethanolamine; Sph = sphingomyelin; PS = phosphatidylserine; PE = phosphatidylinositol.
Source: Steiner and Steiner (1988).

2.14 Lipids of viruses

Lipids are found in a wide range of both RNA and DNA viruses. Usually the lipids are present in an external membrane, the viral envelope, for those viruses which infect eukaryotic cells. For other viruses, such as bacteriophages, the lipid is in internal membranes. A comprehensive summary of viral lipids is available (Steiner and Steiner, 1988).

Phospholipids account for 50 to 75% of the total viral lipid in most cases. Examples of the phospholipid composition are shown in Table 2.133 for both RNA and DNA viruses. The major components are phosphatidylcholine, phosphatidylethanolamine, sphingomyelin and phosphatidylserine. Phosphatidylinositol is frequently absent in the RNA viruses. Percentages of the three zwitterionic phospholipids are somewhat variable (Table 2.133). As a generalization, high proportions of phosphatidylcholine are accompanied by relatively lower percentages of both phosphatidylethanolamine and sphingomyelin and vice versa. Sphingomyelin is much less important in the DNA viruses — only *Pseudorabies* virus having more than 4%.

As far as fatty acid composition is concerned, comparisons have usually been made between viral composition and the host cell. The viruses often contain lowered amounts of polyenoic acids and may accumulate large proportions of acids that are only trace components in the host. For example, the *Rubella* virus contains about 25% odd-carbon fatty acids (C_{15}, C_{17}, C_{19}) (Voiland and Bardeletti, 1980). A considerable proportion of the total fatty acids may be acylated to protein (see Steiner and Steiner, 1988).

Viruses that bud from the plasma membrane often have quite a high ratio of cholesterol to phospholipid. Thus, for the simian virus 5, the ratio is 0.84 and is about 0.75 for the host MDBK cells (Klenk and Choppin, 1969) (see remarks about HIV below). For plant viruses, sterols, such as stigmasterol, β-sitosterol, and campesterol will be found.

Because of its medical importance, the human immunodeficiency virus (HIV) has been well studied recently. One interesting fact is that the retroviral envelope contains a higher cholesterol/phospholipid ratio (>1) than either the plasma membrane or other membranes of the host cell. It seems that when HIV-1 buds, the host cell membrane rafts become the viral coat (Raulin, 2002). HIV-1 infection also induces alterations in T-cell lipids such as to cause a shift from phospholipid synthesis to neutral lipids with viral load. The success of highly active anti-retroviral therapy (HAART) in reducing morbidity has its downsides with many side effects. In the long term, HAART induces dyslipidaemia, insulin resistance, diabetes and hypertriglyceridaemia. Moreover, peripheral lipoatrophy and central fat accumulation (lipodystrophy or LD syndrome) complicate HIV therapy (Raulin, 2002). The molecular basis of these complications, some of which originate from a build-up of the nuclear form of sterol regulatory element binding proteins (nSREBP), are discussed by Hui (2003).

For further details of other viral lipids and their metabolism and function, the reader is referred to Steiner and Steiner (1988).

References

Klenk, H.-D. and Choppin, P.W. (1969) Lipids of plasma membranes of monkey and hamster kidney cells and of parainfluenza virus grown in these cells. *Virology,* **38**, 255–268.

Hui, D.Y. (2003) Effects of HIV protease inhibitor therapy on lipid metabolism. *Prog. Lipid. Res.,* **43**, 81–92.

Raulin, J. (2002) Human immunodeficiency virus and host cell lipids: interesting pathways in research for a new HIV therapy. *Prog. Lipid. Res.,* **41**, 27–65.

Steiner, M. and Steiner, S. (1988) Viral lipids. In *Microbial Lipids,* Vol. 1, Eds. C. Ratledge and S.G. Wilkinson, Academic Press, London, pp. 83–116.

Voiland, A. and Bardeletti, G. (1980) Fatty acid composition of rubella virus and BHK21/13S infected cells. *Arch. Virol.,* **64,** 319–328.

3

PRODUCTION AND REFINING OF OILS AND FATS

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3.1 Introduction

Edible oils and fats have been produced and used since time immemorial. They served as food, were used in food preparation, and were also used as a source of light in oil lamps or as candles. Olive oil even served as lubricant for Greek wrestlers. The first fats were probably obtained by rendering since this is a relatively easy operation that only involves simmering fatty animal tissue (Harrison, 1986). Besides, the fat that was released could also be used for preserving the meat (Dijkstra, 2004).

Crushing nuts and oilseeds is a more sophisticated way of producing edible oils, but according to Blank (1942), sesame seeds and linseed were already pressed in Egypt around 259 BCE and some 75 years later, screw and wedge presses were used in ancient Rome for the production of quite a variety of nut and seed oils and of course olive oil. It was only in 1900 that Anderson introduced the mechanical screw press (Hastert, 1998).

Trade in oils and fats was minimal and people consumed what they produced locally. Consequently, local produce governed the local cuisine, which then created a demand for that type of products. Greek cuisine is still very much based on olive oil, while the German housewife tends to use shortening because her style of cooking is traditionally based upon lard (Dijkstra, 2000).

In ancient Greece, oils were not refined and nobody bothered about refining for the next 2000 years. It is only in 1842 that Schmersal patented the refining of cottonseed oil with caustic soda and it took another 16 years before Bareswil actually deacidified cottonseed oil with a 30% caustic solution. In 1891, a steam deodorisation process operating at atmospheric pressure was developed by Eckstein; shortly afterward, Bataille in France and Wesson in the U.S. introduced improved vacuum deodorisation processes (Blank, 1942). Refining edible oils (neutralisation, bleaching, and deodorisation), therefore, has only been practised for just over a century, but it has had a great impact on eating habits. It makes seed oils obtained by extraction palatable for human consumption. The cultivation of oilseed crops also makes meal available for pig and poultry farming and this has greatly reduced the cost of meat in the diet.

Farming cattle for its meat generates not only meat as the primary product, but also tallow and hides. The tallow can be used for both edible and inedible purposes and the hides go to the leather industry. The price of this total package of products can be worked out, but how should each product within this package be priced?[1] This is a question facing many industries and becomes especially important when substitute products become available as a result of trade; the whaling industry is an early example.

The former gas works that pyrolysed coal to produce coke and town gas had to decide how to price their co-products.[2] In the chemical industry, the chlor-alkali

[1] The chemical industry has been quite successful in developing substitutes for scarce natural products. Synthetic fibres are a prime example of this. When the chemical industry realised that farmers would not breed more cattle to combat a leather shortage, should one arise, it developed a leather substitute, which it called "poromerics." Technically, the product was a success. but commercially, the product was a total failure since it could not compete with leather. Since leather is a co-product without an independent price of its own, its price could be dropped at will. Besides, there was no leather shortage since cutting a hide more efficiently (with less wastage) markedly increased the leather supply.

[2] The manufacturer of wooden toilet seats, who sold the "holes" as breadboards, encountered a similar problem.

industry faces a similar situation because producing 1 tonne of chlorine by electrolysing salt automatically leads to the production of 1.13 tonnes of sodium hydroxide (100%). How should the cost of producing these two products be shared between them?

In this example, alternative routes provide the answer to this question. Sodium hydroxide can also be produced by the so-called caustification process that involves allowing soda ash (sodium carbonate, as produced by the Solvay process from salt and chalk) to react with slaked lime (calcium hydroxide). Soda ash has a cost price, lime has a cost price, and the caustification process has a cost price. Accordingly, a cost price can be calculated for caustic soda produced by this process, and this price is independent of the electrolysis process. This means that, ultimately, the cost price of the sodium hydroxide obtained by the caustification process determines the selling price of chlorine and that the demand for chlorine determines how much caustic soda will be produced by salt electrolysis and, thus, how much soda ash has to be caustified to meet the total demand for caustic soda.

Not surprisingly, the situation is actually less straightforward in that elemental chlorine can also be produced by the electrolysis of hydrogen chloride, which does not lead to the co-production of sodium hydroxide, and that there are more balancing mechanisms in operation, but the possibility of caustification is the main one and it is effective. Besides, the system is self-regulatory. If demand for chlorine was to fall causing the availability of caustic soda to drop as well, industries for which caustic soda is mandatory, like the alkali refining of edible oils, will be willing to pay more for their caustic soda[3] and, thus, encourage the production of this essential product by caustification, which then becomes a profitable exercise.

Another example of an alternative process setting the price of a co-product is, or rather was, glycerol. Glycerol is a by-product of hydrolysing triglyceride oils, but it can also be made synthetically from propylene. So, in a balanced environment, the manufacturing cost of synthetic glycerol should govern the selling price of the glycerol that is co-produced when hydrolysing fats, and the availability of this co-product and the total demand for glycerol should govern how much glycerol is produced synthetically. However, with the advent of biodiesel, more glycerol emerges as co-product than the market can absorb. Consequently, it is no longer produced synthetically and, therefore, no longer has an independent price of its own.

Tallow is a co-product of beef, just like lard is a co-product of pork, and mutton tallow is a co-product of "lamb." Being co-products, these animal fats have no price of their own. Similarly, meal, as for instance soya bean meal, can be a co-product of vegetable oils, just as fishmeal is a co-product of fish oil. Cottonseed can even be regarded as a co-product of cotton fibre. Here again, we come up against the question of how these co-products are to be priced.

Just as in the chlor-alkali example, alternative and independent routes should provide the answer to this question. Palm oil has hardly any co-products. Its production also generates some palm kernel oil and palm kernel meal, but the volume of these products is much smaller than that of the palm oil and, therefore, they can be disregarded for argument's sake. Consequently, palm oil has a cost price of its own and this should set the price of other oils it can replace. It, therefore, should determine the cost price of hydrogenated soya bean oil, which can replace palm oil in many applications. Given the cost price of hydrogenating soya bean oil, this should allow the cost price of crude soya bean oil to be calculated. Given the cost of soya beans and of crushing them, this should determine the soya bean meal price.

Just as the costs of soda ash, caustification and salt electrolysis ultimately govern the price of chlorine, and the demand for chlorine and caustic soda determines how much soda is caustified, so the price of palm oil determines the price of soya bean meal, the demand for meal determines how much soya beans are grown, and the total demand for oil determines how much room is left for palm oil. The situation is somewhat complicated in that palm oil originates from trees that take time to mature and then go on producing for years to come, whereas seed oils originate from annual crops the farmer decides each year to plant or not to plant. Accordingly, annual crops have to ensure that sufficient oil is produced, whereas the tree oils, which in practice means palm oil, have to ensure a proper balance between oil and meal.

Animal oils and fats that were once the major edible oils for cooking and lighting now should be seen as co-products that are sold at prices that guarantee their being sold. Fish oil, being unique in its content of long-chain, highly unsaturated fatty acids, is becoming an exception to this rule since it has no substitute as yet. But, in general terms, seed oils with meal as a co-product are planted in sufficient amounts to satisfy the meal market and palm oil is there to meet the demand for oil that is not met by animal oils and fats and seed oils.

Sadly enough, governments, being what they are, can in their infinite wisdom upset such relationships between co-products and do so in the edible oils and fats industry. Under the guise of encouraging self-sufficiency, governments may introduce import duties and/or subsidize farmers and upset the underlying pricing mechanism. Consequently, Indian consumers use peanut oil for purposes, where more affluent countries use cheaper soya bean oil or canola (Gulati and Phansalkar, 1994).

The EU Chocolate Directive (Berger, 2003; Stewart and Kristott, 2004) provides another example of government interference. Chocolate is made of cocoa liquor (ground

[3] In the long run, they may switch to physical refining and no longer use caustic soda at all.

cocoa beans), cocoa butter and sugar, plus small amounts of vanillin and lecithin. Cocoa butter is still made according to the process invented in 1828 by Van Houten by pressing cocoa liquor. Subsequently, the press cake is ground up to yield cocoa powder that can be used for chocolate-flavoured dairy products (and even soya milk) and imitation chocolate by mixing it with cocoa butter replacers (CBR) and substitutes (CBS). However, the demand for cocoa butter in chocolate manufacturing exceeds the uses and applications that have been developed for cocoa powder.

There is an imbalance between the two co-products, and although the dutching process of cocoa liquor involves an alkali treatment, it does not generate the product that is in short supply as the caustification process does for caustic soda, as mentioned above. The only way to resolve the imbalance and arrive at logical pricing is by allowing cocoa butter equivalents (CBE) to be used in chocolate. The Directive has done so, but half-heartedly, by limiting them to 5% of the chocolate and their origin to 6 specified sources. Fortunately, the U.S. and Japan allow more vegetable fat in chocolate and, thus, can aim at redressing the present imbalance.

A further complication is that oils are different and certain oils are needed for particular applications. Fish oil for instance is used more and more as an ingredient in feed used in fish farms (Barlow, 2004). Lauric oils are another example. Originally, coconuts were almost the only source of lauric oil and its price fluctuated heavily, especially after a hurricane hit the Philippines. Then, the availability of palm kernel oil grew with the expansion of palm oil plantations, so that the availability of lauric oils, which tended to command a premium over nonlauric oils, now exceeds the demand for those applications specifically requiring lauric oils. Consequently, their price has moved closer to the price of the common seed oils. This may have contributed to the fate of the genetically modified high-laurate canola, Laurical®. This was developed as a domestic annual crop and hurricane-insensitive source of lauric oil. Agronomically, it was a success (Del Vecchio, 1996), but in practice, it turned out to be yet another example of biotechnology not living up to expectations as the entire stock of Laurical® was sold as fuel (Anon., 2001).

Whereas the refining processes (neutralisation, bleaching, and deodorisation) have increased the availability of sufficiently palatable oils, the oil modification processes (hydrogenation, interesterification, and fractionation) have increased the usefulness of edible oils by increasing their interchangeability. These processes, therefore, have a great economic significance.

According to O'Brien and Wan (2001), fractionation was practiced in France for the illumination industry in the early 1800s and in 1869, Mège-Mouriès[4] described tallow fractionation leading to olein to be used in his "oleomargarine." Hydrogenation dates from 1903 (Normann, 1903) and another major oil modification process, interesterification, was also invented by Normann, although it took several decades before the process was exploited industrially.

What will happen to these refining and modification processes during the 21st century? Will they be developed further or will some of them have become obsolete by the end of this century? What is certain is that the edible oils and fats industry will expand, perhaps some threefold in this century (Fry, 2001), but what about the individual processes? How long will the present vilification of *trans* isomers last? How long will it take before the present controversy on blood cholesterol levels (Ravnskov, 2000) is resolved? Will an attempt to cash in on the presumed chemophobia of the general public by offering cold-pressed oils of doubtful quality and keepability at greatly increased price be a permanent fixture? After all, even our own trade journals and representatives from our own industry claim benefits for expeller-pressed, physically refined oil (Cheng, 2004) and of avoiding chemical processes by opting for physical refining in general (Ten Brink and Van Duijn, 2003).

With respect to oils produced in large tonnages, most consumers and, thus, producers will continue to regard product price and safety as the most important criteria. On the other hand, it is highly probable that people will go on using what they have been used to for generations; in Greece, olive oil will remain important despite the fact that much cheaper oils are also available (Dijkstra, 2000). There will also be a niche market for gourmet oils such as walnut oil (Dijkstra, 2003). And on the fringe, customers who are fashion conscious and can afford it, may well go for whatever is presented as the latest fad; what this will entail is impossible to predict since it will hardly be science-based.

What will happen to the various processes? On a technological level, it looks as if there is scope for improvement and development. Take degumming, for instance. Seed oils as presently produced by expelling and solvent extraction tend to contain nonhydratable phosphatides (NHP). It has been shown that the concentration in crude oil of phosphatides in general and NHP in particular can be made to vary by choice of solvent (Desnuelle et al., 1951) and pretreatment of the oilseed (Kock, 1983; Dahlén, 1998). Accordingly, producing crude oils with a negligible NHP content may be a possibility. However, the mechanism underlying NHP formation has hardly been investigated, let alone elucidated for a number of reasons.

The first reason could be that crushing oil seeds requires reliable and specialised equipment, so that the sector is more oriented toward mechanical engineering as a discipline than toward botany or chemistry. The second reason is that the profitability of crushing oil seeds is strongly affected by trading seeds, oil and meal on the futures

[4] The second part of the name Mège-Mouriès must have been added at a later stage because the patent itself only mentions Mège.

market. This calls for a mentality that thrives in making snap decisions with an immediate impact on profitability, which is not the mentality that fosters opting for the elucidation of what happens on a molecular level during the crushing operation.

The third reason is concerned with the mentality within the industry comprising oil milling, feed compounding, oil refining, and downstream activities, which is one of perpetual distrust. Whereas, in a cooperative situation a specification would describe an agreed compromise between what the customer wants and what the supplier can produce, the main concern of meal and oil specifications looks like preventing fraud. Hence, the inclusion of penalties? The reason that soya bean meal must contain 48% protein is not so much that layers will stop laying eggs if this specification is not met, but to prevent the oil miller from putting all kinds of waste products into the meal. Consequently, the miller will put in just so much waste to ensure that he is not outside specification and if the "waste" contains valuable ingredients like oil, this is not taken into account. Similarly, treating canola with an enzyme and pressing it in an expeller so that 90 to 93% of the oil is recovered and the meal only contains some 7% (Sosulski and Sosulski, 1993) has not become an industrial process because there is no specification for this meal.

Also because traders seldom have a technical background, these specifications have become very rigid. From a technical point of view, it would make sense to make an article of trade of acid-refined oil having such a low residual phosphatide content that it only requires bleaching and physical refining before yielding fully refined oil, thereby commanding a premium over just crude oil (Dijkstra, 1999); it has hardly happened. Instead, crude oil has a single specification that limits the phosphorus content, the FFA, and the "mandi" (moisture and impurities), and that permits an oil miller to purchase acid oils from the refiner to whom he sold his crude oil, and blend as much of these acid oils into his crude oil as the crude oil specification permits and sell these acid oils back to the refiner at greatly increased price. To a technologist, this doesn't make sense, but to a trader, it makes money, at least for one of the parties involved. That the other party loses more money this way than the trader makes for his employer is of no concern since everything was within specification.

The likelihood that vertically integrated companies waste money this way, or in one of the many other ways permitted by the specifications in force, is smaller but by no means excluded. After all, these companies may be divided into business units that operate as profit centres and, thereby, may be prone to the misconception that each party striving for its own optimal situation will automatically lead to an overall optimum; it doesn't and never will. Nevertheless, these vertically integrated companies represent the best chance of the stalemate holding back technological development and improvement being broken.

References

Anon. (2001), High-laurate canola oil sold for use as boiler fuel, *inform*, **12**, 1019–1020.

Barlow, S. (2004), Raw material supply issues within the fish oil industry, *Paper presented at the 3rd EuroFedLipid Congress, Edinburgh*, Abstracts: page 5.

Berger, K.G. (2003), EU Chocolate Directive discussed in May, *inform*, **14**, 511.

Blank, E.W. (1942), Chronological list of important dates in the history of the fats and waxes, *Oil & Soap*, **19**, 110–113.

Brink, H.B. ten and Duijn, G. van (2003), Refined seed oil production: a milder future? *inform*, **14**, 274–275.

Cheng, V. (2004), Frying leap pays off, *Oil Mill Gazetteer*, **110**, 16–17.

Dahlén, J.Å.H. (1998), The Super Expro process, *Oléagineux Corps Gras Lipides/OCL*, **5**, 360–363.

Del Vecchio, A.J. (1996), High-laurate canola, *inform*, **7**, 230–243.

Desnuelle, P. et al. (1951), Traitement thermique des graines oléagineuses et teneur en lécithine des huiles, *Oléagineux*, **6**, 264–267.

Dijkstra, A.J. (1999), Trends in edible oil processing, *Lipid Techn.*, **11**, 53–56.

Dijkstra, A.J. (2000), Edible oils in Europe, *inform*, **11**, 386–394.

Dijkstra, A.J. (2003), My visit to a walnut oil mill, *inform*, **14**, 755–756.

Dijkstra, A.J. (2004), Coin-coin, *inform*, **15**, 336–337.

Fry, J. (2001), The world's oil and fat needs in the 21st century: lessons from the 20th century, *Paper presented at the 50th Anniversary Meeting of the SCI Oils & Fats Group, Hull*.

Gulati, V.P. and Phansalkar, S.J. (1994), *Oilseeds and Edible Oil Economy of India*, Vikas Publishing House PVT LTD, New Delhi.

Harrison, R. (1986), Traditional aspects of animal fat production and use, in *Symposium Proceedings No. 33, Fish Oils and Animal Fats*, Leatherhead Food RA, 3–13.

Hastert, R.C. (1998), Oilseed processing: past present and future, *inform*, **9**, 1140–1144.

Kock, M. (1983), Oilseed pretreatment in connection with physical refining, *J. Am. Oil Chem. Soc.*, **60**, 150A–154A.

Mège, H. (1869), Production de certains corps gras d'origine animale, French Patent 86 480.

Normann, W. (1903), Process for converting unsaturated fatty acids or their glycerides into saturated compounds, British Patent 1 515, assigned to Herforder Maschinenfett-und Ölfabrik Leprince und Siveke.

O'Brien, R.D. and Wan, P.J. (2001), Cottonseed oil: processing and utilization, in *Proceedings of the World Conference on Oilseed Processing and Utilization*, Wilson, R.F., Ed., AOCS Press, Champaign IL, 90–144.

Ravnskov, U. (2000), *The Cholesterol Myths Exposing the Fallacy that Saturated Fat and Cholesterol Cause Heart Disease*, New Trends Publishing, Inc., Washington, D.C.

Sosulski, K. and Sosulski, F.W. (1993), Enzyme-aided *vs* two-stage processing of canola: technology, product quality and cost evaluation, *J. Am. Oil Chem. Soc.*, **70**, 825–829.

Stewart, I. and Kristott, J. (2004), European Union Chocolate Directive defines vegetable fats for chocolate, *Lipid Techn.*, **16**, 11–14.

3.2 Production of animal oils and fats

Did man, when still hunting and gathering, also produce animal fats by rendering his catch? Probably (Boekenoogen, 1948), but then only on a small scale because hunting was piecework and the pieces concerned were quite perishable so there would never be much to render at any one time. Domesticating cattle and slaughtering a number of them in the autumn provides a sudden supply of raw material for rendering. It also provided the farmer with a supply of meat (and fat) to see him and his family through the winter, and reduced the number of cattle he had to feed in the winter when feed was scarce. Very convenient indeed, at least for the farmer.

When cattle began to be farmed on a larger scale, this led to specialisation and a separate meat packing industry. This also allowed animal fat to be rendered on an industrial scale so that nowadays, rendering companies buy their raw material from slaughterhouses or freezing works, as they are called in New Zealand, and may also run a collection system serving butchers and meat processors to collect their fatty trimmings in a hygienic manner. Another industry that aimed at providing mankind with triglyceride oil and light in a dark world was the whaling industry. This industry caught and processed whales and sold whale oil as lamp oil; in New England, it started in 1690 (Blank, 1942). It was only after the invention of the hydrogenation process by Normann (1903) that whale oil was also used for edible purposes in the form of a partially hydrogenated hardstock for margarine and shortenings. Early whaling vessels relied on wind power, but nevertheless were quite effective. As mentioned by Blank (1942), the exports of whale oil from Boston to England in 1768 amounted to 4000 tons. Whalers subsequently went to the South Pacific where whales were then still plentiful. This illustrates how valuable whale oil (and oil, in general) must have been at that time, in view of the hard labour involved in conditions of extreme cold. Whaling was apparently worth it.

More modern whaling vessels were motor driven and eventually so efficient that they became a threat to the survival of the whale population; world whale oil production in 1954 had already reached 325,000 tonnes when it was limited to 16,000 blue whales per annum (Heinz, 1956). Whaling has now been banned except for scientific purposes and whale oil is no longer an article of trade.

Naturally, the early production of whale oil was a rather crude undertaking. After the animal had been caught and killed, it was cut up and the resulting blubber pieces were fed into cookers where they were treated with live steam. Separation of the oil from the cooking water was by gravity or centrifugal separator. The residues could be processed into a valuable feed meal (Heinz, 1956) and even whale blood was processed (Fauth, 1940). Whale meat is still considered to be a delicacy in Japan.

The fish oil industry is a more recent development and differs from whaling in that it regarded fishmeal as the main product. Before producing fish oil became an industry, it had been carried out on a small scale for thousands of years (Bimbo, 1998) and the use of cod liver oil as a source of vitamins A and D was clinically investigated between 1752 and 1783 (Hjaltason, 1992). Fishmeal has a high protein content and is a valuable component of poultry feed. The oil was a by-product and sold to the edible oil industry at a price that guaranteed that the fishmeal industry could get rid of its oil. Oil quality control was minimal, which meant that deposits could build up in fish oil storage tanks and that hydrogenation catalyst usage could be high and unpredictable because of variable types and concentrations of catalyst poisons (Baltes, 1967; Mørk, 1972; Mørk and Norgård, 1976).

All of this has changed now that fish feed has become the major outlet for fish oil (Barlow, 2004). Fish is considered to be healthy because it contains ω-3 fatty acids and, consequently, the consumer expects farmed fish to contain these acids as well. The most expedient way to ensure this is by incorporating them as fish oil in the feed used in aquaculture.

Anhydrous milk fat is produced industrially nowadays, but it has been produced on a small scale for thousands of years. Making butter and using it as such is a perfectly adequate way of utilising milk fat in countries enjoying a moderate climate. In hotter climates, butter melts, so that the emulsion breaks and a continuous aqueous phase is formed. This is an ideal culture medium for almost every spoilage organism, so in hot climates, butter has to be dehydrated and processed into ghee. There are as many different types of ghee as there are lactating animals being milked by man: cow ghee, camel ghee, buffalo ghee, etc. In addition, and to meet the demand for ghee when this outgrew the potential supply, there is a vegetable substitute for ghee that is commonly referred to as vanaspati (Podmore, 2002).

Vegetable substitutes are a common phenomenon anyway since animal fats are a by-product of the meat industry and their availability is determined by the demand for and supply of meat. Over the years, this availability has increased, but the production of vegetable oils and fats has increased more; accordingly, the relative importance of animal fats is declining (Rossell, 2001). Shortenings are a vegetable substitute for lard and to some extent for tallow, and margarine is the vegetable substitute for butter. It, therefore, is interesting to note that cookbooks in countries that traditionally used lard, now prescribe shortening in their recipes and use lard or shortening for shallow frying, whereas "dairy countries" fry in butter or margarine (Dijkstra, 2000).

3.2.1 Carcass fats

The main carcass fats produced industrially are tallow and lard, but they also comprise, for instance, mutton tallow and poultry fats, such as those derived from chickens, ducks, geese, and turkeys. All carcass fats are produced by the rendering process. These are relatively simple processes because cell walls and membranes in animal tissue rupture on cooking when the intercellular fat expands; they can also be ruptured mechanically as in the Supraton® process[5] (Zucker, 1968). A 1.75% caustic solution can also be used to rupture the cells and if soap is formed, it is not by saponification, but by prior enzymatic hydrolysis (Deatherage, 1946). Cell walls in seeds and nuts, on the other hand, require more drastic treatment to be opened, but were later also processed in the Supraton process (Coenen et al., 1989). They need to be ground in a mill, hence "oil milling" as opposed to the rendering plant, which several non-English languages describe as just "fat melting works."

Various rendering processes are currently in use. In their review that also covers separation equipment, Düpjohann and Hemfort (1975) describe several rendering processes including wet rendering and dry rendering and mention that dry rendering has become less important. Much later, when describing the production and applications of tallow in Argentine, Levembach (2001) only mentions the dry rendering process. During a symposium on animal fats held in Chicago in 1983, only dry rendering systems were presented (Prokop, 1985), both for inedible and edible tallow. When describing recovery of fats from animal sources, Johnson (2000) describes both processes, but limits the dry rendering process to the high temperature process where cooking is carried out at 115 to 120°C. However, low-temperature, dry rendering processes have also been described (Schaefer et al., 1998); the particular process referred to aims not only at rendering, but also at providing a protein fraction that can be used in meat products for human consumption. The protein fraction resulting from high temperature rendering processes can only be used in animal feed (Prokop, 1985).

Quite appropriately, Johnson (2000) likens the dry rendering process to frying streaky bacon, just as the preparation of *confit de canard* has been likened to the wet rendering process (Dijkstra, 2004). Accordingly, the protein fraction resulting from the wet rendering process is collected as a water-wet cake that has to be dried, whereas in the dry rendering process this fraction has to be separated from oil. Potential oil yield, therefore, is higher in the wet rendering process than in the dry process and fat quality is also reported to be better with the wet rendering process (Düpjohann and Hemfort, 1975; see also Søbstad, 1990a).

[5] This piece of equipment has also been used for the comminution of corn germs (Anon., 1974).

Recently, the European animal by-products regulation (ABPR) 1774/2002/EC (available from http://europa.eu.int/eur-lex/pri/en/pj/dat/2002/l_273/l_27320021010en00010095.pdf), first of all, classified these by-products according to:

- Category 1 material. Whole animals or by-products from animals suspected of being infected by a TSE (transmissible spongiform encephalopathy). This highest risk material must be destroyed, for instance, by incineration.
- Category 2 material. Waste products, such as intestines, screenings, trap grease, material containing veterinary drugs or growth hormones, imported material without veterinary certificate, dead animals, etc.
- Category 3 material. Parts of slaughtered animals that themselves are fit for human consumption, but those parts are not intended for this purpose. Apart from fat trimmings, this category also includes hides, hooves, etc.

For rendering, five permitted methods have been specified in the above ABPR that are reasonably in line with established procedures. They comprise various combinations of continuous and batch operations, atmospheric and superatmospheric processing, recycling of molten fat, and reprocessing of preprocessed material (Woodgate and Van der Veen, 2004).

The rendering process starts with the reception and refrigerated storage of the raw materials. If these come from a slaughterhouse and have been classified as edible by a veterinarian inspector, they can be combined with the trimmings collected from butcheries for the production of edible tallow or lard. If the rendering plant processes both of these animal fats, some form of separate processing will be necessary for the edible tallow to be kosher or halal. Since the raw material is highly perishable, refrigeration during transport and intermediate storage is common, and storage time is kept as short as possible; the material is preferably processed the same day.

As indicated in Table 3.1, the composition of the raw material can vary between rather wide limits. However, the values listed in Table 3.1 are literature data. Within a rendering plant relying on the same suppliers, a raw material with an average fat content of 55% by weight and an average water content of 30% by weight is not uncommon. In continuous operations, rather constant values will be aimed for by selecting the order in which the bins are to be processed because the performance of, for example decanters, depends upon the feed characteristics.

The first step in all rendering processes is some form of comminution by mechanical means. Smaller pieces can be more easily transported or agitated and, moreover, heat transfer and oil removal are also facilitated by comminution. According to the ABPR, the permitted maximum

TABLE 3.1 Overall compositions of raw materials for rendering

Reference	Origin	Type	Fat (%)	Protein (%)	Water (%)
(Fernando and Dunn, 1990)	Example 1	Beef/mutton	16	16	68
(Fernando and Dunn, 1990)	Example 2	Beef trimmings/mutton soft offal	35	9	56
(Levembach, 2001)	Slaughterhouses	Beef	50	15	35
(Levembach, 2001)	Butcheries	Beef	60	15	25
(Filstrup, 1980)	Slaughterhouse	Bones	15		39
(Prokop, 1985)	Meat industry	Beef trimmings	12	20	63

size is method dependent, and ranges from 20 mm for Method 5 to 150 mm for Method 2. Although preheating before comminution has been described (Blumberg and Gould, 1965), it is more common to cut the material when cold or at ambient temperature (Prokop, 1985).

Because the raw material to be cut up can vary in size, a two-stage operation can be advantageous. In a first cutter, pieces of about 5 cm are then cut to pieces of 2 cm (Keith, 1968a) or smaller in the next cutter. This can, for instance, be a plate grinder with a hole size of ¼ in. to 1 in. (6 to 25 mm) (Gruver and Lyon, 1970). According to Prokop (1985), pieces of 1 to 2 in. ensure efficient cooking in a batch cooker, but for a continuous system, he describes a Reitz disintegrator for further size reduction to some 10 mm. An even smaller particle size is recommended by Aikins (1965). Care should be taken to avoid the formation of fines since, after rendering, they tend to end up in the fat stream and may be difficult to remove.

In the wet rendering process, the comminuted raw material is heated with live steam whereby process water may be recycled into the raw material stream to increase its fluidity and heat transfer. This mixture is then fed into an agitated, plug-flow cooker feeding a centrifugal separator. This is commonly a decanter with two exit conduits (Little, 1964): one for the solids phase, which is connected to a drier, and another for the fat/water mixture that is connected to a second centrifugal separator to isolate the molten animal fat (Filstrup, 1980).

A three-phase decanter can also be used in the wet rendering process to isolate product and waste streams (Sifferd et al., 1956). According to the examples given in the patent (Fernando and Dunn, 1990), the solids stream contained 65 to 69% water and only 5 to 7% fat on dry basis. The aqueous stream contained 2.3 to 2.8% solids with low fat content and the fatty stream contained 2 to 5% water and a small amount of fat-free solids; it required further purification by water washing in a centrifugal separator. The aqueous stream is preferably concentrated by multistage evaporation so that the concentrate can be combined with the decanter solids and dried at the same time. Effluent streams can also be purified before being discharged by adding an aluminate as flocculating agent (Tarbet et al., 2002).

Emulsions impair the performance of the centrifugal separators used in the wet rendering process. Acidifying the material to be separated so that the stickwater has a pH of about 4.1 to 5.8 is reported to minimise emulsion formation (Gruver and Lyon, 1970). Another additive that has been recommended is finely dispersed hydrated silica (Libby and Henderson, 1948).[6] Even small amounts cause the fat to have a better colour and lower peroxide value.

In the continuous dry rendering process (Keith, 1968a; 1968b), the fluidity of the comminuted raw material can be ensured by recirculating part of the fatty phase; this aspect has been included in the ABPR Method 3. The fluidity is necessary to ensure proper heat transfer by the indirect heating system of the cooker. Preheating the recirculating fat to a temperature of 70 to 120°C also helps to heat the material to be rendered (Klubien, 1967). The cookers or digesters in the dry rendering process can operate at atmospheric pressure, but the elimination of water by evaporation can also be expedited by applying a slight vacuum, but according to Woodgate and Van der Veen (2004), stand-alone vacuum systems are now obsolete. Operating under vacuum has the advantage of maintaining a lower temperature, which prevents the collagen in the raw material being hydrolysed and converted to gelatine (Downing, 1958). This gelatine would then be extracted and impair subsequent separation (Filstrup, 1980).

Like the wet rendering process, the dry rendering process also comprises a separation stage where the products are isolated by centrifuge. Instead of being wet and having to be dried, the greaves resulting from the dry rendering process will contain a fair amount of oil. Accordingly, they can be fed to a screw press or expeller to reduce their oil content. The expeller oil may well contain fines and is preferably recycled in the cooker.

The greaves or cracklings generally find their way to the feed industry, but following the outbreak of BSE (bovine spongiform encephalopathy) in the UK, which has also affected beef consumption and, hence, tallow availability (Watkins, 2001), its use in feed is now subject to the ABPR. Finally, a low temperature, wet rendering process has also been described (Schaefer et al., 1997), whereby the solid protein fraction is combined with the protein dissolved in the stickwater to form a meat product.

[6] Patents assigned to Unilever subsidiaries like Lever Brothers Limited, Lever Brothers Company, Lever Brothers & Unilever, Unilever Ltd., Octropa or N.V., Van den Bergh's, etc. all mention "Unilever" as assignee.

3.2.2 Fish oil

Fish oil and fishmeal are produced only by the wet rendering process (Papenfuß, 1967). As shown in Figure 3.1, representing this process, whole fish, such as anchovy, menhaden, sardine, etc., are first of all minced and then indirectly heated in a cooker to ensure cell rupturing and liberation of the oil. The cooker normally consists of a long, steam-jacketed cylinder, through which the fish are moved by a screw conveyor that can itself be steam heated (Barlow, 1986). Cooking like this takes about 20 minutes, during which time the temperature of the fish is raised to 100°C. The Condec process (Søbstad, 1990b) uses a scraped surface heat exchanger for this stage and, thereby, reduces the cooking time to less than 2 minutes. Then the product stream is directed to a continuous screw press, normally of twin-screw design, to separate the solids from the liquid. A liquid content of 50% in the press cake should be possible. In the Alfa-Laval "Centrifish" process, a decanter is used instead of a screw press (Søbstad, 1990b). The wet cake is broken up and dried in a steam-heated drier to a water content of 8 to 10% and finally ground to a fine powder.

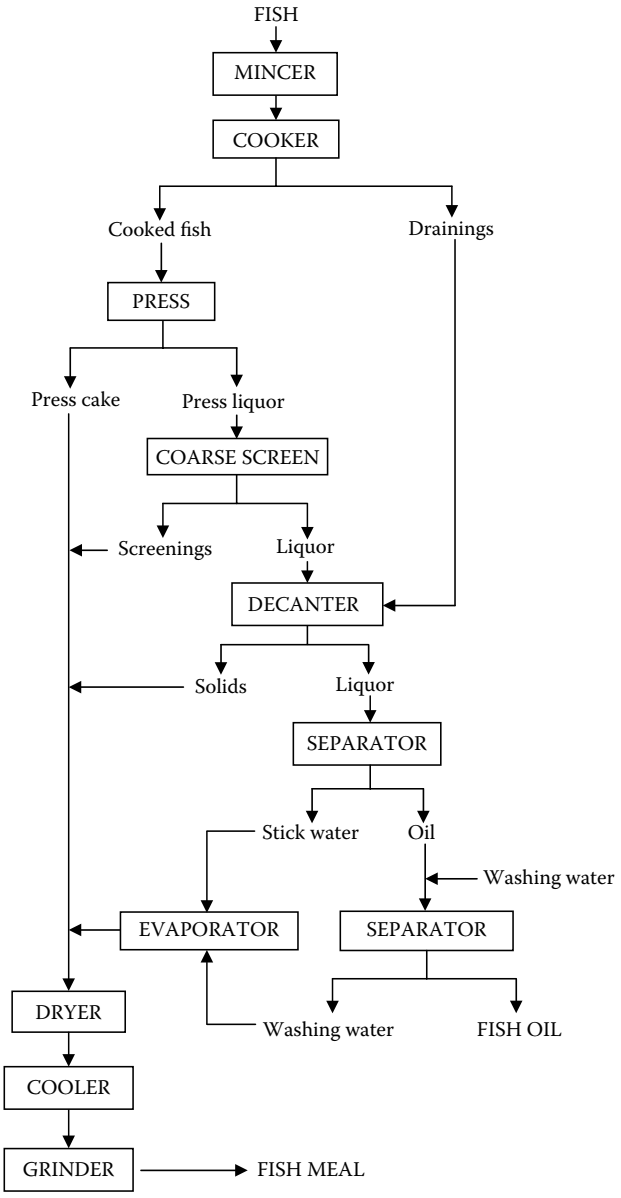


FIGURE 3.1 Fishmeal production flow sheet. (From Bimbo, A.P. (1998), Guidelines for characterizing food-grade fish oil, *inform*, **9**, 473–483. With permission.)

The liquid stream emerging from the screw press or decanter is first screened and then separated into an oil stream and the stickwater. The oil stream is washed with water, dried, and stored as crude fish oil, whereas the screenings and the stickwater concentrate are both combined with the press cake. In this way, a maximum yield is achieved. The oil yield amounts to some 90% of theory and the theoretical amount depends very much upon the fish species, the age of the fish, and the season of the catch (Bimbo, 1998). In Japan, where pilchard is the main fish processed, an amount of 2.27 tonnes of fishmeal is produced per tonne of fish oil, whereas in Chile, this is almost 6 tonnes of meal (see Padley, 1994, Table 3.212).

A totally different process to isolate fish oil is the Friolex® process (Hruschka and Frische, 1998). In this process (Best et al., 1999), the oil-bearing material is homogenised and mixed with aqueous alcohol; both ethanol and isopropanol, being food grade, can be used in amounts leading to solutions with 15 to 50% by weight of alcohol. On centrifugation, an oily layer containing about 10% of water and alcohol will form, which can be collected separately. The process is very suitable for isolating fish oil from the residues resulting from fish filleting. By immersing these into the aqueous alcohol as soon they become available, all enzymes present are denatured so that enzymatic breakdown of oil and proteins and the concomitant development of a fishy smell is effectively prohibited. Microbial spoilage of the residues is also prevented in the sterile alcoholic environment. Consequently, the resulting fish oil is remarkably bland. For cod liver oil, this would be a great improvement. Isopropanol was also used by Drozdowski (1969) when preparing fish protein concentrates on a laboratory scale.

Fish oil can also be processed into pharmaceutical-grade fractions by converting the oil to fatty acid ethyl esters and removing the saturated esters by urea inclusion (Hwang and Liang, 2001); this technique can also be used for fish FFA (Zuta et al., 2003). Preferably, vacuum distilled or deodorised oil (Hjaltason, 2002) is used as starting material for the interesterification to ensure the absence of chlorinated hydrocarbons, FFA, and cholesterol (Bimbo, 1998). Care should be taken not to overheat the oil during the distillation step since this will lead to isomerisation of the polyunsaturated fatty acids and thus diminish their pharmaceutical usefulness.

3.2.3 Anhydrous milk fat

The fatty matter present in milk is isolated as anhydrous fat in several stages: First of all, raw milk containing 3 to 4% milk fat is heated to 40 to 50°C and then sent to a centrifugal separator and split into a skimmed milk stream containing 0.05% fat and cream containing 30 to 40% milk fat.

This cream is then pasteurised (82°C/20s to 100°C/1s (Poot and Biernoth, 1994) or 95°C/15s (Robinson and Rajah, 2002)) to destroy any microbial contamination and denature any enzymes present before being quickly cooled to below the melting point of milk fat so that the fat globules present in the cream will partially crystallise. Since each fat globule must be nucleated, this so-called physical ripening stage takes several hours with a minimum of 2 hours at 8°C. It can be combined with the bacteriological ripening of the cream whereby a starter culture containing bacteria including *Lactobacillus cremoris* is mixed into the cooled cream, converting lactose into lactic acid and also generating flavouring compounds such as diacetyl.

The next stage is the churning process whereby air is beaten into the cream so that foam is formed. The partially crystallised milk fat globules gather at the air interface and because their membranes have been inactivated by the aeration, they can coalesce causing a phase inversion to a water-in-oil emulsion. However, the situation is more complicated in that the butter will also contain some oil-in water droplets arising from the original milk fat globules.

Churning is continued until pea-sized butter granules emerge and the buttermilk can be easily drained away. The granules can then be washed with water before the butter is kneaded to remove excess water. Finally, the water and salt content of the butter is adjusted to specification levels. Added solutions are worked into the butter using perforated plates and revolving knives (Poot and Biernoth, 1994).

Butter leaving the continuous butter-making machine is immediately packed in grease-proof wrappers. Only individual portions are packed in plastic mini-tubs. Common consumer package sizes are 125, 250, and 500 g and, for industrial customers or long-term storage in freezer rooms, larger sizes are used. These large blocks are the starting material for the production of anhydrous milk fat.

This starts with allowing the blocks to thaw if they originate from a freezer. Then, they are unwrapped manually and melted. This melting is done quite gently, for instance on a grid through which warm water is circulated. Molten butter is then collected at the bottom of the melting vessel at a temperature of some 40 to 50°C by a pump that sends the molten butter through a heat exchanger that raises the temperature to 75 to 80°C. Molten butter at this temperature is then forwarded through a holding tube before it is fed to the first centrifugal separator. In the case of sweet butter, the pH is adjusted to 4.5 to facilitate separation and give a high yield of clear oil.

The fatty phase leaving this first separator has a fat content of about 95%, which means that a second separation is required. Prior to this second separation, the fatty stream is further heated to 90 to 95°C. The fatty phase leaving the second separator has a fat content of 99.5%. It may still contain some milk solids so that a washing stage can be included and, afterwards, the fat is dried under vacuum before being cooled in the heat exchanger where it will heat ingoing product, and being sent to storage.

Although ghee can be described as anhydrous milk fat, a different production process is used in order to generate the typical ghee flavour (Podmore, 2002). Instead of removing the aqueous phase by centrifugal separation, it is removed by evaporation at elevated temperature. At this temperature, there is an interaction between the fat and the fermented residue of the nonfat solids and this leads to the formation of the characteristic ghee flavour.

3.2.4 Wool grease and derivatives

Wool grease is the fatty product secreted by the sebaceous glands of sheep to protect its wool and the animal against wetting. Chemically, the material is an extremely complicated mixture of esters of wool wax acids (Motiuk, 1979a; Wolf, 1999) (C_{10} to C_{30} fatty acids; C_9 to C_{29} (ω–1) monomethyl fatty acids also referred to as "iso acids;" C_8 to C_{30} (ω–2) monomethyl fatty acids, also called "anteiso acids;" C_{13} to C_{24} nonsubstituted α-hydroxy fatty acids; C_{13}–C_{24} (ω–1) monomethyl α-hydroxy fatty acids; C_{12} to C_{24} (ω–2) monomethyl α-hydroxy fatty acids; C_{26} to C_{32} ω-hydroxy fatty acids and various minor fatty acid derivatives) and wool wax alcohols (Knol, 1954; Motiuk, 1979b) (unbranched fatty alcohols, (ω–1) and (ω–2) monomethyl fatty alcohols, unbranched and (ω–1) and (ω–2) monomethyl 1,2-diols, sterols and triterpenoid alcohols). This leads to a number of lipid classes as shown in Table 3.2 (Jacob, 1999). Wool wax hydrocarbons have been reviewed by Motiuk (1980).

Wool grease is isolated by scouring (washing) raw wool with an aqueous detergent solution or by solvent extraction using a hydrocarbon or alcohol. However, according to Koubík et al. (1969), "solvent extracted wool is of considerably poorer quality than similar wool that has been washed with an alkaline aqueous soap or detergent-containing solution without the addition of a solvent." According to Warth (1956), solvent extraction "leaves the fiber in a much better condition" and according to Clark (1999), solvent extraction "did not improve the quality of the wool wax, but rather impaired it." So although solvent extraction has been completely abolished in several countries like Australia, it is still included in Figure 3.2 showing

TABLE 3.2 Lipid classes occurring in wool wax

Lipid Class	(Fawaz et al., 1973)	(Chemtob et al., 1975)
Alkanes	0.6	0.4
Sterol and triterpene alcohol esters	35.4	28.9
Mono- and diesters of aliphatic alcohols	23.7	
Monoesters of aliphatic alcohols		13.9
Monohydroxyesters of aliphatic alcohols	5.3	
Mono- and polyhydroxy esters		25.7
Monohydroxy esters of sterols and triterpene alcohols	14.7	
Free fatty acids	0.5	1.0
Free alcohols	6.5	20.2
Free sterols	4.1	5.3
Free dihydroxy esters and diols	7.8	
Others	1.4	4.6

Source: Jacob, J. (1999), in *The Lanolin Book*, Hoppe, U., Ed., Beiersdorf AG, Hamburg, 53–84.

wool grease processing. Subsequently, wool grease can be purified to provide lanolin (the major use and outlet for wool grease), which can be saponified to yield wool fatty acids and wool alcohols that may serve as a source for cholesterol that can be used as a raw material for vitamin D production[7].

Raw wool contains many constituents considered to be contaminants by wool processors who insist on their removal. Their amounts and type vary according to breed, nutrition, environment, and position of the wool on the animal, but unwashed wool contains only 45 to 62% clean wool (Warth, 1956). These constituents are mainly the wool grease, water-soluble perspiration salts (suint), and particulate matter such as dirt and vegetable matter. They are preferably removed from the wool fibres by washing in the scouring process.

In this process, as described in a technical report available from http://www.canesis.com/home.shtm, raw wool is first of all "opened" and "dusted" to facilitate the subsequent scouring treatment, to reduce the effluent load and remove vegetable matter; these are dry processes. Subsequently, the wool is treated countercurrently with an aqueous detergent solution that may contain some soda ash. In a first series of bowls, the wool may be treated at 60 to 70°C and then it may be subjected to a number of cold rinses before being rinsed at some 55°C; this hot rinse may involve some peroxide bleaching of the wool. The water emerging from the hot rinse bypasses the cold rinse bowls and is sent immediately to the first series of bowls. For the removal of the particulate dirt, a settling tank can be used, but Adams (1929; 1933) developed a special nozzle centrifuge, which would be called a clarifier today. The wool grease emulsion is very stable. Accordingly, it requires breaking and a strong acid like sulfuric acid has been used to this end. Another additive is "copperas" (ferrous sulfate) that has been oxidised by passing elemental chlorine through a solution. Using this additive (0.2 to 1.5%) and passing air through the reaction mixture before adding the sulfuric acid leads to a good separation (Clark and Kitchen, 1966). Wood (1981) discovered that maintaining the suint concentration in the scouring liquor above 6% by weight greatly improves the grease recovery to >85%. This is an improvement on the process that relied on acidification of the scour liquor to break the emulsion followed by extraction with an apolar solvent; recycling the aqueous phase leaving the grease recovery unit is, therefore, standard practice in New Zealand. Improved separation also results from the addition of a soluble silicate (Masri et al., 1982). A grease-recovery system using small amounts of an alcohol, such as *n*-pentanol, and an electrolyte, such as acrylamide, has also been described (Smith and McLachlan, 1977).

As is only to be expected, this isolated grease is a dirty product with a distinct billy goat smell and most unlike the lanolin for which the national pharmacopoeias provide strict specifications. It may still contain detergent residues, which can be removed by solvent extraction (Anderson and Warner, 1983). It may also contain traces of pesticide residues since sheep are treated with certain pesticides by dipping in order to prevent the animals suffering from sheep scab, blow fly infestation, and other unpleasant conditions. Persistent organo-chlorine pesticide such as DDT, Aldrin, and Dieldrin are no longer used, but traces can often still be found in wool wax because of their persistency.

They are effectively removed by modern refining methods to below their detection limit. The 5th Supplement to the USP 22 (1992) specifies limits for 34 named pesticides and a limit of 40 ppm is set for lanolin for general use with no individual pesticide residue exceeding 10 ppm. For modified lanolin, intended for more exacting applications, the limit for total pesticides has been reduced to 3 ppm (Clark, 1999).

Neutralisation of the grease with caustic soda is difficult because the grease tends to form strong emulsions. Accordingly, Handy and Isham (1916) introduced a miscella neutralisation by dissolving the grease in petroleum ether and neutralising this miscella with

[7] This vitamin D production is by a photochemical process as developed by E.H. Reerink and A. van Wijk of the Natuurkundig Laboratorium of Philips, Eindhoven, The Netherlands. In 1930, Philips founded a joint venture with the chocolate producer Van Houten called Philips-Duphar (Dutch Pharmaceuticals) to produce vitamin D in Weesp. Now Philips-Duphar is part of Solvay Pharmaceuticals, subsidiary of the Belgian Solvay Group. Philips-Duphar obtained its cholesterol from another Dutch firm (Van Schuppen), which was originally active in wool and textiles and later also de-oiled lecithin. Van Schuppen was acquired by Philips Duphar in 1969 and also became part of Solvay Duphar (now Solvay Pharmaceuticals) in 1980.

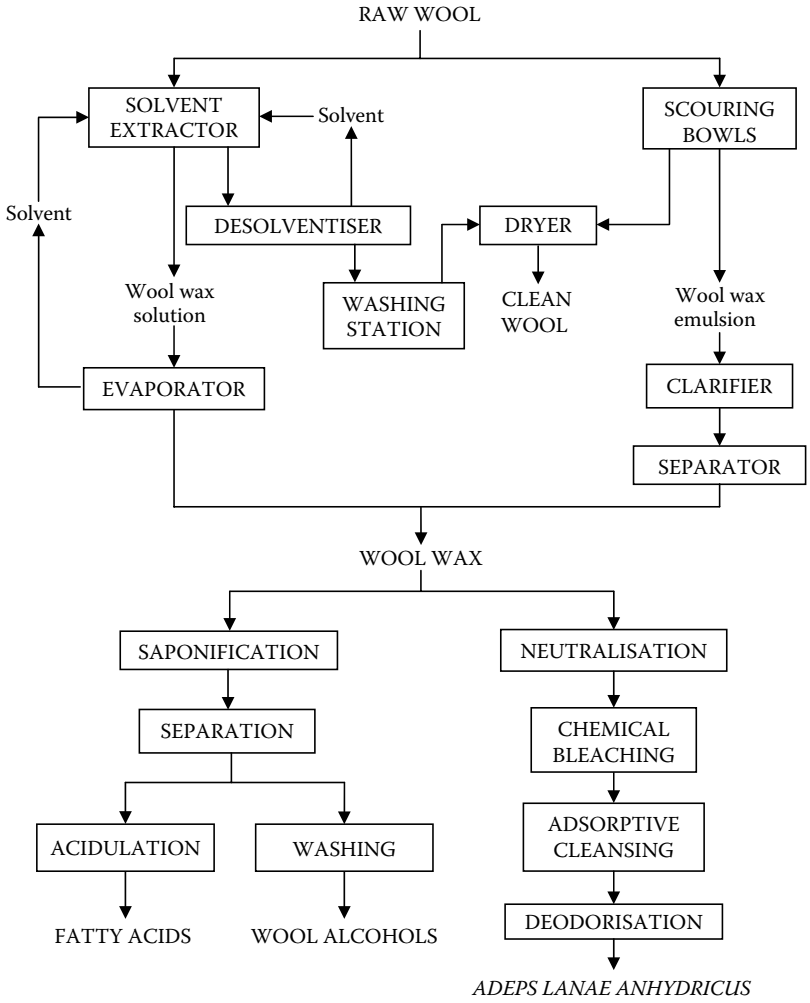


FIGURE 3.2 Wool grease processing.

methanolic ammonia. Subsequently, hexane was used in conjunction with an aqueous-alcoholic alkali solution (Arutjunyan et al., 1981). However, the use of an apolar solvent is not necessary since wool grease can also be neutralised by using methanolic sodium or potassium hydroxide (Chaikin and McCracken, 1980). The neutralisation processes also have a bleaching effect, but further bleaching can be achieved by using hydrogen peroxide (Lower, 1954; Anderson and Wood, 1963) or organic peroxides, bleaching earth, and/or activated carbon. Lanolin (*Adeps lanae anhydricus*) is finally produced by the deodorisation of the neutralised, bleached wool wax, which may also cause some heat bleaching.

As shown in Figure 3.2, wool alcohols result from the saponification of wool grease or wool wax with caustic soda at high (130 to 180°C) temperatures and, thus, elevated pressures (Senda et al., 1977). The soaps and the alcohols thus obtained can be separated by using a dual solvent system: a water-immiscible solvent such as toluene or hexane to extract the wool alcohols and a water-miscible solvent such as a lower alcohol to dissolve the fatty acid soaps. After acidulation, these soaps can be fractionated into hydroxy acids and acids without a hydroxyl group by extracting the latter from a solution of these acids in a polar organic solvent, such as ethyl acetate, acetone, or lower alcohols with carbon dioxide (Heidlas et al., 1996). This leaves the wool alcohols. Since their isolation and purification is similar to the isolation of sterols from vegetable sources, the processes concerned will be discussed in Section 3.6.2 (Sterol recuperation processes).

References

Adams, J.W. (1929), Centrifugal liquid purifier, US Patent 1,738,553, assigned to Preston Street Combing Company.

Adams, J.W. (1933), Verfahren und Vorrichtung zum Reinigen von Schmutz oder andere feste Bestandteile enthaltenden fetthaltigen Wollwaschwässern, German Patent 578 856, assigned to Preston Street Combing Company.

Aikins, G.A. (1965), Rendering process, US Patent 3,171,847, assigned to Armour and Company.

Anderson, C.A. and Warner, J.J. (1983), The dual-solvent extraction of wool grease with hexane and aqueous isopropanol, *J. Am. Oil Chem. Soc.*, **60**, 1997–2001.

Anderson, C.A. and Wood, G.F. (1963), Bleaching of wool grease, *J. Am. Oil Chem. Soc.*, **40**, 333–336.

Anon. (1974), *Procédé d'obtention de l'huile de matières premières végétales*, French Patent 2.196.648, assigned to Corn Products Company.

Arutjunyan, N.S. et al. (1981), Process for purification of wool grease, US Patent 4,288,377.

Baltes, J. (1967), Zur Kenntnis schwefelhaltiger Fette und ihres Härtungsverhaltens, *Fette Seifen Anstrichm.*, **69**, 512–514.

Barlow, S. (1986), Fish oils in new markets, new uses, new geographical areas, in *Symposium Proceedings No. 33, Fish Oils and Animal Fats*, Leatherhead Food RA, 14–21.

Barlow, S. (2004), Raw material supply issues within the fish oil industry, *Paper presented at the 3rd EuroFedLipid Congress, Edinburgh*, Abstracts page 5.

Best, B. et al. (1999), Process for extracting native products which are not water-soluble from native substance mixtures by centrifugal force, US Patent 5,928,696, assigned to Dr. Frische GmbH.

Bimbo, A.P. (1998), Guidelines for characterizing food-grade fish oil, *inform*, **9**, 473–483.

Blank, E.W. (1942), Chronological list of important dates in the history of the fats and waxes, *Oil & Soap*, **19**, 110–113.

Blumberg, M.E. and Gould, G.J. (1965), Process for rendering animal fat, US Patent 3,222,384, assigned to Pennsalt Chemicals Corporation.

Boekenoogen, H.A. (1948), *De Scheikunde der Oliën en Vetten*, A. Oosthoek's Uitgevers Mij., Utrecht.

Chaikin, M. and McCracken, J.R. (1980), Process for separation of wool wax from fats in wool grease or mixtures containing wool grease, US Patent 4,207,244, assigned to Unisearch Limited.

Chemtob, C. et al. (1975), Analyse des pommades, des huiles et des cires. XIX. Étude de la composition chimique d'un échantillon de lanoline cireuse. I. Fractionnement et étude des acides, *Ann. pharm. franç.*, **33**, 109–118.

Clark, E.W. (1999), The history and evolution of lanolin, in *The Lanolin Book*, Hoppe, U., Ed., Beiersdorf AG, Hamburg, 15–52.

Clark, E.W. and Kitchen, G.F. (1966), Improvements in the treatment of liquors from the scouring of raw wool, British Patent 1,029,114, assigned to Woolcombers Limited.

Coenen, H. et al. (1989), Erste Ergebnisse zu einem neuartigen Ölextraktionsverfahren, *Fat Sci. Technol.*, **91**, 583–586.

Deatherage, F.E. (1946), An alkali rendering process for lard and other animal fats, *Oil & Soap*, **23**, 327–331.

Dijkstra, A.J. (2000), Edible oils in Europe, *inform*, **11**, 386–394.

Dijkstra, A.J. (2004), Coin-coin, *inform*, **15**, 336–337.

Downing, F.P. (1958), Process for rendering animal fat, US Patent 2,823,215, assigned to The Sharples Corporation.

Drozdowski, B. (1969), Isopropyl alcohol extraction of oil and lipids in the production of fish protein concentrate from herring, *J. Am. Oil Chem. Soc.*, **46**, 371–376.

Düpjohann, J. and Hemfort Jr., H. (1975), Verfahren zur Verarbeitung von tierischen Rohfetten unter besonderer Berücksichtigunng zentrifugaler Klär- und Trennvorgänge, *Fette Seifen Anstrichm.*, **77**, 81–93.

Fauth (1940), Verfahren zur Gewinnung und Verarbeitung von Walblut, *German Patent 688 451,* assigned to *Gesellschaft zur Verwertung Fauth'scher Patente* m.b.H.

Fawaz, F. et al. (1973), Analyse des pommades, des huiles et des cires. XII. Étude de la composition de la lanoline. I. Examen préliminaire et fractionnement de l'échantillon étudié, *Ann. pharm. franç.*, **31**, 63–73.

Fernando, T. and Dunn, S.E. (1990), Rendering process, US Patent 4,966,733, assigned to The Meat Industry Research Institute of New Zealand.

Filstrup, P. (1980), A method for separating animal raw material, British Patent 1 564 277, assigned to Alfa-Laval AB.

Gruver, M.E. and Lyon, L.R. (1970), Fat separation process, US Patent 3,519,662, assigned to Sybron Corporation and Lycoil, Inc.

Handy, J.O. and Isham, R.M. (1916), Method of refining wool-grease, US Patent 1,194,013, assigned to Pittsburgh Testing Laboratory.

Heidlas, J. et al. (1996), Verfahren zur Fraktionierung von Wollwachssäuregemischen, European Patent Application 0 732 393 A1, assigned to SKW Trostberg AG.

Heinz, H.-J. (1956), *Fette und Fetsäuren*, in *Ullmanns Encyklopädie der technischen Chemie*, Foerst, W., Ed., Urban & Schwarzenberg, München-Berlin, 454–552.

Hjaltason, B. (1992), Fish oils as vitamin sources, in *AOCS Short Course Manual on Modern Applications of Marine Oils*, Toronto, Canada.

Hjaltason, B. (2002), Marine oils with reduced levels of contaminants, WO Patent 02/06430, assigned to Lysi HF.

Hruschka, S. and Frische, R. (1998), A new oil extraction process: FRIOLEX®, *Oléagineux Corps Gras Lipides/OCL*, **5**, 356–360.

Hwang, L.S. and Liang, J.H. (2001), Fractionation of urea-pretreated squid visceral oil ethyl esters, *J. Am. Oil Chem. Soc.*, **78**, 473–476.

Jacob, J. (1999), The chemical composition of wool wax, in *The Lanolin Book*, Hoppe, U., Ed., Beiersdorf AG, Hamburg, 53–84.

Johnson, L.A. (2000), Recovery of fats and oils from plant and animal sources, in *Introduction to Fats and Oils Technology*, O'Brien, R.D., Farr, W.E., and Wan, P.J., Eds., AOCS Press, Champaign, IL, 108–135.

Keith, J.G. (1968a), Continuous rendering process, British Patent 1,126,723, assigned to Duke, Inc.

Keith, J.G. (1968b), Simplified continuous rendering system, British Patent 1,126,724, assigned to Duke, Inc.

Klubien, O. (1967), Method of continuously recovering protein from fatty animal material by employing directly admixed fat at a temperature of from 70°C–120°C, US Patent 3,345,353, assigned to Thrige-Titan A/S.

Knol, H.W. (1954), The alphatic wool wax alcohols. A review, *J. Am. Oil Chem. Soc.*, **31**, 59–62.

Koubík, M. et al. (1969), Method of treating spent wool scouring liquors, US Patent 3,458,543, assigned to Mosilana.

Levembach, M. (2001), Refined beef tallow in Argentina: production and applications, in *Proceedings of the World Conference on Oilseed Processing and Utilization*, Wilson, R.F., Ed., AOCS Press, Champaign IL, 64–66.

Libby, L.H. and Henderson, L.F. (1948), Process for rendering oleaginous tissue material, US Patent 2,455,374, assigned to Unilever.

Little, T.H. (1964), Animal fatty tissue defatting process, US Patent 3,141,774, assigned to Pennsalt Chemicals Corporation.

Lower, E.S. (1954), Improvements in woolgrease and woolgrease fatty acids, fatty alcohols and soaps, British Patent 706,422, assigned to Croda Limited.

Masri, M.S. et al. (1982), Wool scouring, US Patent 4,349,347, assigned to Wool Development International Limited.

Motiuk, K. (1979a), Wool wax acids: A review, *J. Am. Oil Chem. Soc.*, **56**, 91–97.

Motiuk, K. (1979b), Wool wax alcohols: A review, *J. Am. Oil Chem. Soc.*, **56**, 651–658.

Motiuk, K. (1980), Wool wax hydrocarbons: A review, *J. Am. Oil Chem. Soc.*, **57**, 145–146.

Mørk, P.C. (1972), Heterogeneous hydrogenation of fish oils: kinetic determination of catalyst poisoning, *J. Am. Oil Chem. Soc.*, **49**, 426–432.

Mørk, P.C. and Norgård, D. (1976), Nickel-catalyzed hydrogenation: a study of the poisoning effect of halogen-containing compounds, *J. Am. Oil Chem. Soc.*, **53**, 506–510.

Normann, W. (1903), Process for converting unsaturated fatty acids or their glycerides into saturated compounds, British Patent 1 515, assigned to Herforder Maschinenfett- und Ölfabrik Leprince und Siveke.

Padley, F.B. (1994), Marine oils: fish and whale oils, in *The Lipid Handbook*, Gunstone, F.D., Harwood, J.L., and Padley, F.B., Eds., Chapman & Hall, London, 167–183.

Papenfuß, H.-J. (1967), *Fischnebenprodukte*, in *Industrielle Fischverarbeitung*, Brandenburg, W. and Krämer, H., Eds., VEB Fachbuchverlag Leipzig, Leipzig, 475–514.

Podmore, J. (2002), Culinary fats: solid and liquid frying oils and speciality oils, in *Fats in Food Technology*, Rajah, K.K., Ed., Sheffield Academic Press, Sheffield, 333–359.

Poot, C. and Biernoth, G. (1994), Margarine and butter production, in *The Lipid Handbook*, Gunstone, F.D., Harwood, J.L., and Padley, F.B., Eds., Chapman & Hall, London, 288–295.

Prokop, W.H. (1985), Rendering systems for processing animal by-product materials, *J. Am. Oil Chem. Soc.*, **62**, 805–811.

Robinson, D.J. and Rajah, K.K. (2002), Spreadable products, in *Fats in Food Technology*, Rajah, K.K., Ed., Sheffield Academic Press, Sheffield, 192–227.

Rossell, J.B. (2001), Origins and chemical properties, in *Animal Carcass Fats*, Rossell, J.B., Ed., LFRA Ltd., Leatherhead, U.K., 1–31.

Schaefer, D.L. et al. (1997), Low temperature rendering process, and meat product, PCT Patent Application 97/01963, assigned to Cargill Incorporated.

Schaefer, D.L. et al. (1998), Low temperature rendering process, US Patent 5,725,897, assigned to Cargill Incorporated.

Senda, H. et al. (1977), Separation of wool fatty acids from wool alcohol or wool wax, British Patent 1 477 860, assigned to Dai-Ichi Kogyo Seiyaku Co. Ltd.

Sifferd, R.H. et al. (1956), Rendering process for recovery of fat and gelatin, US Patent 2,748,152, assigned to Armour and Company.

Smith, D.K. and McLachlan, C.N.S. (1977), Methods for recovering wool grease from spent wool scouring liquor, US Patent 4,007,117, assigned to New Zealand Inventions Development Authority.

Søbstad, G.E. (1990a), Animal fats: the effect of process technology on fat quality, in *Edible Fats and Oils Processing, Basic Principles and Modern Practices*, Erickson, D.R., Ed., AOCS Press, Champaign IL, 31–36.

Søbstad, G.E. (1990b), Marine oils: The technology of separation and purification of marine oils, in *Edible Fats and Oils Processing, Basic Principles and Modern Practices*, Erickson, D.R., Ed., AOCS Press, Champaign IL, 37–42.

Tarbet, B.J. et al. (2002), Fat and protein removal from process streams, US Patent 6,372,145 B1, assigned to Power Engineering Company.

Warth, A.H. (1956), The natural waxes, in *The Chemistry And Technology of Waxes*, Reinhold Publishing Company, New York.

Watkins, C. (2001), Tallow, *inform*, **12**, 580–588.

Wolf, F. (1999), Antimicrobial properties of wool wax acids, in *The Lanolin Book*, Hoppe, U., Ed., Beiersdorf AG, Hamburg, 237–251.

Wood, G.F. (1981), Process for the purification and re-use of wool-scouring liquors, British Patent 1 598 818, assigned to Commonwealth Scientific and Industrial Research Organization.

Woodgate, S. and Veen, J. van der (2004), The role of fat processing and rendering in the European Union animal production industry, *Biotechnol. Agron. Soc. Environ.*, **8**, 283–294.

Zucker, F.J. (1968), Anlage zum kontinuierlichen Aufschliessen von Fettrohstoffen, *Fette Seifen Anstrichm.*, **70**, 529–531.

Zuta, C.P. et al. (2003), Concentrating PUFA from mackerel processing waste, *J. Am. Oil Chem. Soc.*, **80**, 933–936.

3.3 Production of vegetable oils and fats

Vegetable oils can be (and still are) produced in primitive ways that nevertheless reflect the essential elements also present in the more sophisticated and large-scale production plants that now dominate the edible oil industry. Take shea nut butter, for instance. In countries like Ghana and Mali, the nuts from the shea tree *(Butyrospermum parkii,* later called *Butyrospermum paradoxum* and finally *Vitellaria paradoxa)* have been used for a long time as a source of fat (Lovett, 2005). Consumption of this fat was just local until it was discovered that shea butter had a high content of symmetrical monounsaturated triglycerides and, thus, forms a suitable raw material for the production of cocoa butter equivalents. According to the EU Chocolate Directive (Berger, 2003), these equivalent may contain shea butter.

The tree is not cultivated but grows wild in West and Central Africa. Women collect the nuts and isolate shea butter from these nuts by allowing the fruit surrounding the nuts to rot away and then crushing the nut into grits. These may then be heated or dried and then they are stamped to open the cells. Because the fat content of the shea kernel is some 50 to 55%, stamping results in a paste, which is then boiled with water and left to cool and settle. The shea butter floating on top is collected as such, but

the yield is low. The use of enzymes can increase the yield (Tano-Debrah and Ohta, 1994; 1995), but a much higher yield is attained when using screw presses and solvent extraction (Lanzani et al., 1984). However, their use may reduce the quality of the butter and the use of wooden utensils only has been advocated (Marteau and Marteau-Danvin, 1975).

This artisanal production process has in common with modern industrial processes that the raw material is conditioned to facilitate cell opening and that subsequently the cells are opened mechanically, whereupon the oil/fat is isolated by making use of its (in)solubility characteristics. Because stamping is less effective than grinding, the shea butter yield is lower than in the case of artisanal walnut oil production (Dijkstra, 2003). In the latter, the shelled nuts are ground between granite millstones leading to almost complete cell rupture, but the hydraulic pressing of the paste resulting from the grinding operation is an inefficient way of isolating the liberated oil and the residual oil content in the press cake is still quite high.

In the case of artisanal olive oil milling, which the author was fortunate to witness in the communal oil mill in Montauroux near Cannes, granite millstones were also used and the resulting paste was also pressed hydraulically. However, the residual oil content of the press cake was far lower because of the vegetable water present. This water wets the pomace, which then has less affinity for the olive oil, and oil retained by the mats separating the layers of paste only affects the yield of the first pressing.

The butter and oils mentioned above were not refined before being sold to the consumer and, in fact, they still aren't. There are people who actually claim to like the taste of unrefined oil and are willing to pay such a premium for it that inherently expensive oils, such as olive oil, continue to be produced. However, refining became really necessary when oil started to be obtained by solvent extraction, as for instance cottonseed oil in the U.S. (O'Brien and Wan, 2001). Palm oil as originally produced in West Africa by traditional methods had such a high FFA content (>>10%) that it could only be used to manufacture soap and candles (Boekenoogen, 1948), but subsequent production on Sumatra and, in what later became Malaysia, yielded oil with lower FFA. However, it was darkly coloured and needed refining for that reason.

3.3.1 Fruit oils

Fruit oils have in common that their production does not lead to the simultaneous production of meal. The residues are commonly burned to provide steam used in oil production. Fruit oils, therefore, are suited to play a role in balancing demand and supply of oil and meal, although seed oils result from an annual crop whereas fruit oils result from trees that take some time to mature; their production cannot be just switched on or off. There are two main fruit oils: olive oil and palm oil, which of late has become a major vegetable oil with expanding production in Malaya, Indonesia, and Papua New Guinea. Annual production at the time of writing is just over 30 million tonnes *per annum*. It will rise and is expected to replace soya bean oil as the No. 1 oil.

3.3.1.1 Olive oil

According to Boskou (1996), the origin of the olive tree (*Olea Europæa*) is not clear, but what is clear is that it has been cultivated for several thousand years as a source of vegetable oil. It takes a long time (some 8 years) to mature and start bearing fruit, but then it can go on producing fruit for well over 100 years. Villemur and Dosba (1997) show a picture of a Corsican tree that is over a thousand years old. It is grown mainly around the Mediterranean, with Spain, Italy and Greece being the main growers, but Australia and the U.S. are gaining in importance. Annual production, being weather-dependent, can vary widely, but annual consumption is more steady. Ninety percent of the world's olive oil production is consumed in the producer countries themselves (Luchetti, 1997) with Greece having the highest per capita consumption of olive oil (Dijkstra, 2000).

Ripe olives contain on average some 48% water and 21% oil, the remainder being carbohydrates like mono- and disaccharides at 3%, and polysaccharides (cellulose, hemicellulose, and pectins) at 27% and some protein (Bianchi, 1999). They are harvested in late autumn and there are several ways of harvesting (Di Giovacchino, 1996). Smallholders and farmers with orchards that are not accessible to mechanical harvesting equipment may have to hand pick or rake the branches while on ladders. This is a labour-intensive and, therefore, an expensive way of harvesting. Gathering fallen olives from the ground is not much better in this respect and also yields lower quality oil (Di Giovacchino, 1997). Therefore, farmers aim at using mechanical shakers that grip and shake the tree so that the ripe olives drop on nets that have been spread underneath. A mechanical harvesting machine used in Australia is shown in Figure 3.3. The olives harvested are stored in plastic crates to prevent them from crushing each other and are processed in the oil mill as soon as possible.

This processing involves three steps: crushing, malaxation, and separation. Some authors like Finch et al. (1983; 1985) advocate pitting the olives before crushing, but others (Patumi et al., 2003) show that stoning has no effect upon oil quality. During the crushing step, the olives are ground into a fine paste whereby cells are opened and the oil contained in these cells is freed. Originally, granite millstones were used for this purpose and although they are still used in small oil mills, continuous hammer mills are gradually replacing them.

The next step is the malaxation during which the paste resulting from the crushing step is slowly agitated; it may

FIGURE 3.3 Olive harvesting machinery.

possibly be heated at the same time. The purpose of the malaxation step is to cause small oil droplets to coalesce into larger ones and thus facilitate the subsequent separation. According to Bianchi (1999), paste resulting from a hammer mill requires a longer malaxation time than paste resulting from a more slowly moving stone mill.

After the malaxation, olive oil can be recovered from the paste. Traditionally, this is achieved by pumping the paste from the malaxation reservoir and spreading it on annular synthetic fibre mats encircling the hollow shaft of a hydraulic press. Metal spacers are placed over the shaft after every four mats bearing paste, to ensure even distribution of the pressure. The must obtained by pressing is a mixture of the vegetation water and olive oil. It can easily be separated into both constituents by centrifugal separator (Pieralisi, 1986). If need be, the oil can be washed, but washing is not common since it lowers the polyphenol content of the oil. The oil is dried and possibly filtered before being sent to intermediate storage. The residual oil content in the press cake (pomace) is some 6%, which corresponds to some 1.8% of the olives having been processed. Normally, the pomace is not reprocessed to extract further oil since this would be considerably lower in quality (Di Giovacchino, 1996).

Another way of separating the oil from the olive paste is by using decanters. Because the solid fraction leaving a decanter contains more moisture than a press cake, water must be added to the paste before it is fed to the decanter. This can be fresh water but then, the oil mill will have more aqueous effluent and the olive oil will have a lower polyphenol content. Accordingly, it is not uncommon to recycle the vegetation water by mixing it with the paste.

The decanter most used in Spain (Martinez Suarez and Alba Mendoza, 1986) is a two-phase decanter providing a wet (65 to 72% moisture) pomace stream and a liquid stream that is separated into oil and vegetation water streams in a downstream centrifugal separator. In Italy, the commonly used separator is a three-phase decanter yielding a pomace of 55 to 60% moisture and separate vegetation water and oil streams (Di Giovacchino et al., 2002).

To improve the consistency and porosity of the paste, micronised natural talcum, a hydrated magnesium silicate ($Mg_3(Si_4O_{10})(OH)_2$), may be added to the paste in amounts of up to 4% by weight (Ranalli, 1997); it ends up in the pomace. Similarly, enzyme preparations containing pectolytic and (hemi)cellulolytic enzymes can be used to increase oil yield without affecting oil properties and quality. An inferior grade of olive oil, referred to as sulfur olive oil,[8] is produced by solvent extraction of olive stones or husks.

3.3.1.2 Palm oil

As its Latin name reveals, the oil palm (*Elaeis guineensis*) was first noted in West Africa and palm oil has been found in Egyptian tombs dating from 3000 BCE (Hiscocks and Raymond, 1964). It was not until 1848 that four seeds were brought to the Far East (Law and Thiagarajan, 1990). It takes an oil palm 3 years to mature and start bearing fruit and the economic life span of the tree is about 25 years (Johnson, 2000). It fruits all the year round so that palm oil mills can enjoy a good capacity utilisation. The bunches weigh between 5 and 30 kg depending on the age of the plantation and the climatic conditions and contain 800 to 4000 fruits with an average weight of some 5 g. Because the oil present in the ripe fruit is prone to lipase catalysed hydrolysis after a bunch of fruit has been cut off the palm tree, these bunches have to be processed almost immediately. This lipase is not endogenous to the palm fruit, but originates from yeast cells (Oo, 1981).

Accordingly, as shown in Figure 3.4, freshly cut bunches are transported to the oil mill where they are sterilised to inactivate the lipolytic enzymes, loosen the fruit on the bunch, soften the fruit, condition the kernels, and cause protein to coagulate. The sterilisation process uses live steam at 3 bar and involves the elimination of air from the sterilizer vessel by alternating pressurisation and depressurisation of this vessel (see Fonade, 1979, Figure 3). The final pressurisation step at 3 bar lasts about 20 to 30 minutes and the cycle takes 80 to 90 minutes including loading and unloading.

Stripping the fruit from the sterilised bunches is carried out in a rotating cage with bars that allow the fruits to pass through, but retain the empty bunches. The loosened fruit is collected by a screw conveyor below the cage and the empty bunches emerge at the end of the cage; they serve as fuel for steam generation, but can also be returned to the land as a mulch.

At this stage, the fruit has been softened, but it is still mostly intact. It is then fed to a digester, which is a cylindrical, steam-jacketed vessel kept at 90 to 100°C by the injection of live steam and the addition of hot water. It is

[8] Probably the sulfur refers to the solvent carbon disulfide that has been used as extraction solvent in the past.

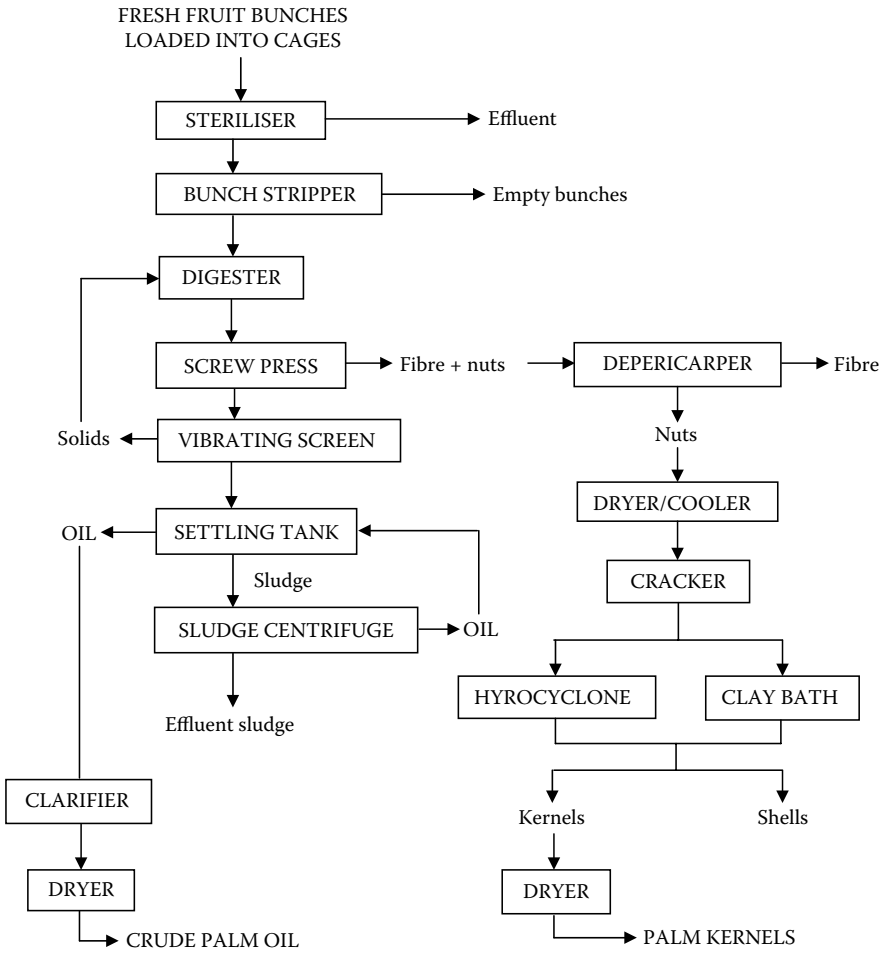


FIGURE 3.4 Palm oil mill flow sheet. (From Berger, K.G. (1983), Production of palm oil from fruit, *J. Am. Oil Chem. Soc.*, **60**, 206–210. With permission.)

fitted with beater arms that break up the fruit and liberate the oil; the average residence time in the digester is some 20 minutes.

The digester contents are then fed continuously to a screw press that produces a liquid stream consisting of some 53% oil, 7% fines, and 40% aqueous phase (Berger, 1983) or 65% oil, 10% fines, and only 25% water (Johnson, 2000), and a press cake containing the fruit fibre residue and, of course, the palm kernels. The liquid stream is passed to a settling tank via a vibrating screen that returns what it retains to the digester. The oil recuperated from the settling tank is first of all passed through a clarifier and then dried under vacuum before being sent to storage. The sludge collecting in the settling tank is passed to a decanter that separates this sludge into a heavy effluent phase and a light, oily phase that is returned to the settling tank.

The press cake emerging from the screw press is first of all broken up to separate the fibres from the nuts, then dried, and finally separated pneumatically. Since the nuts are generally processed in separate oil mills employing an extraction solvent, they have to be prepared for transport and storage at the palm oil mill itself. This involves drying the kernels in a silo with heated air to a residual moisture content of some 11% over a period of some 15 hours. This causes the shell of the nuts to become brittle so that it will crack on impact in the centrifugal nutcracker and also causes the kernel to detach itself from its surrounding shell. Nuts and shell fragments can be separated pneumatically by winnowing, in a hydrocyclone or a clay bath. Since the kernels and shells differ in specific gravity (1.07 vs. 1.17), adding them to a mixture of water and clay having a specific gravity of 1.12 causes the kernels to float and the shell fragments to sink (Johnson, 2000). After the hydrocyclone or clay bath, the kernels should be promptly dried to suppress lipolytic activity. The shell fragments can be burned.

3.3.2 Seed oils

Oilseeds like linseed have been milled for a long time, but it is only fairly recently, with the increase in soya bean production, that seed oils have become the major group of edible oils. Given the growing demand for meal, their relative importance is likely to increase even further. This

growth in the demand for meal has meant that there has been a steady investment in oil mills, which has also led to some research and development in support of the seed oil milling processes, whereby a distinction must be made between seeds like soya beans with a low (<20%) oil content, and soft seeds such as rapeseed and sunflower seed containing >40% oil.

Since pressing or expelling unavoidably leaves some oil in the cake, which residual oil content is relatively high for oil seeds with a low oil content, it has become customary to extract oil seeds with low oil content with a solvent. This led to extraction plants being built and from there it was only a small step to regard the press cake resulting from soft seeds as raw material for the extraction plant. Moreover, the electrical energy required for expelling additional oil increases and the press capacity decreases with decreasing residual oil content. Accordingly, with prepress/solvent extraction, the optimum level of expelling can be calculated as the point where leaving more residual oil in the expeller cake would save less in electrical energy than the additional variable cost of solvent extracting more oil. The position of this optimum depends, of course, on many factors and can, thus, vary widely.

Almost fat-free meal is an important feed ingredient providing protein and essential amino acids. However, animal feed must provide more nutrients than just protein, so that fat is often added as a relatively cheap source of energy. This raises the question of what is cheaper: leaving some oil in the meal and utilising this residual oil instead of added fat, or aiming for the lowest possible residual oil content in the meal and adding fat as a separate feed ingredient. After all, there has been an evolution and at one stage, an "increasing trend among oilseed processors towards more exhaustive extraction" was observed (D'Aquin et al., 1961).

No general answer can be given to this question, but when people are willing to pay for physically refined, expeller-pressed soya bean oil (Cheng, 2004), a market for expeller cakes as feed ingredients may emerge. This would be in line with the trend to feed white cottonseed directly to livestock (O'Brien and Wan, 2001). At the time of writing, there is hardly any market for expeller cake. Quite the opposite, minimum values for protein content (48 and 44%) have been specified for soya bean meal so as to prevent the meal being used as a dump for gums, soapstock, spent bleaching earth, etc.

These minimum values, of course, preclude partial extraction. This causes extraction plants to be, perhaps, unnecessarily large since most oil is extracted in the very early stages of the extraction process. Wiese and Snyder (1987) report that 80% of the oil was extracted at 2 minutes and 95% at 4 minutes. "Scraping the barrel" may also cause the oil to be of inferior quality since Bull and Hopper (1941) noted that the oil quality deteriorates on successive extractions (see also Nash and Frankel, 1986).

Nieh and Snyder (1991), noting "the high quality of crude oil from soya bean flour extraction raise a new thought about the soya bean oil solvent extraction. Since it is possible to minimise the extraction of the phospholipids, free fatty acids and pigments, and to obtain a thorough extraction of triglycerides at the same time, it follows that these compounds are not mixed with triglycerides in the lipid bodies before solvent extraction. The high phosphatide content is not inherent in soya bean oil extraction. Therefore, modification of the soya bean preparation procedures may be a key to producing high quality oil" (Snyder et al., 1992).

Figure 3.5 represents a flow diagram including all the various industrial routes that start with oilseeds and arrive at meal and oil. It includes crushing processes that only involve pressing, processes that only involve extraction as well as prepress/solvent extraction processes. It shows at what stages expanding can be incorporated into the chain of events, how the Alcon® process (Kock, 1978; 1981b) fits into this chain, and what alternatives can be used to prepare a proper extractor feed. Several of the unit operations will be discussed in more detail below, but for the sake of clarity, the extraction mechanism will be treated first.

3.3.2.1 Extraction mechanism

When discussing the theory of solvent extraction, Karnofsky (1949b) noted that it is complicated by the fact that what is extracted is not a single compound, but changes in composition during the course of the extraction from nearly pure triglycerides to mixtures containing increasing amounts of slowly dissolving, nonglyceride material. Moreover, the total amount of extractible material also depends upon what solvent is used for the extraction. Desnuelle et al. report for example (1951) that the Bollmann solvent (equal parts of petroleum ether, benzene, and alcohol) extracts far more phospholipids than hexane on its own.

By observing that the rate of extraction of soya bean flakes is determined by flake thickness, temperature, type of solvent and flake size, Karnofsky concludes that the extraction mechanism could well be a combination of diffusion, dialysis, and the dissolution of slowly dissolving material. Subsequently, it was noted that this slowly dissolving material could also be made to dissolve by soaking it in miscella. This led to the theory that oilseed flakes contain two kinds of oil, one of which dissolves as soon as it is brought in contact with the solvent, whereas the other dissolves much more slowly (Coats and Karnofsky, 1950). Once this latter material has dissolved, it stays in solution so that "reconstituted" flakes (extracted flakes to which the extract has been returned) extract much more rapidly than they did the first time (Karnofsky, 1986).

Subsequently, Smith (1952) concluded that diffusion apparently controls the entire process in the solvent

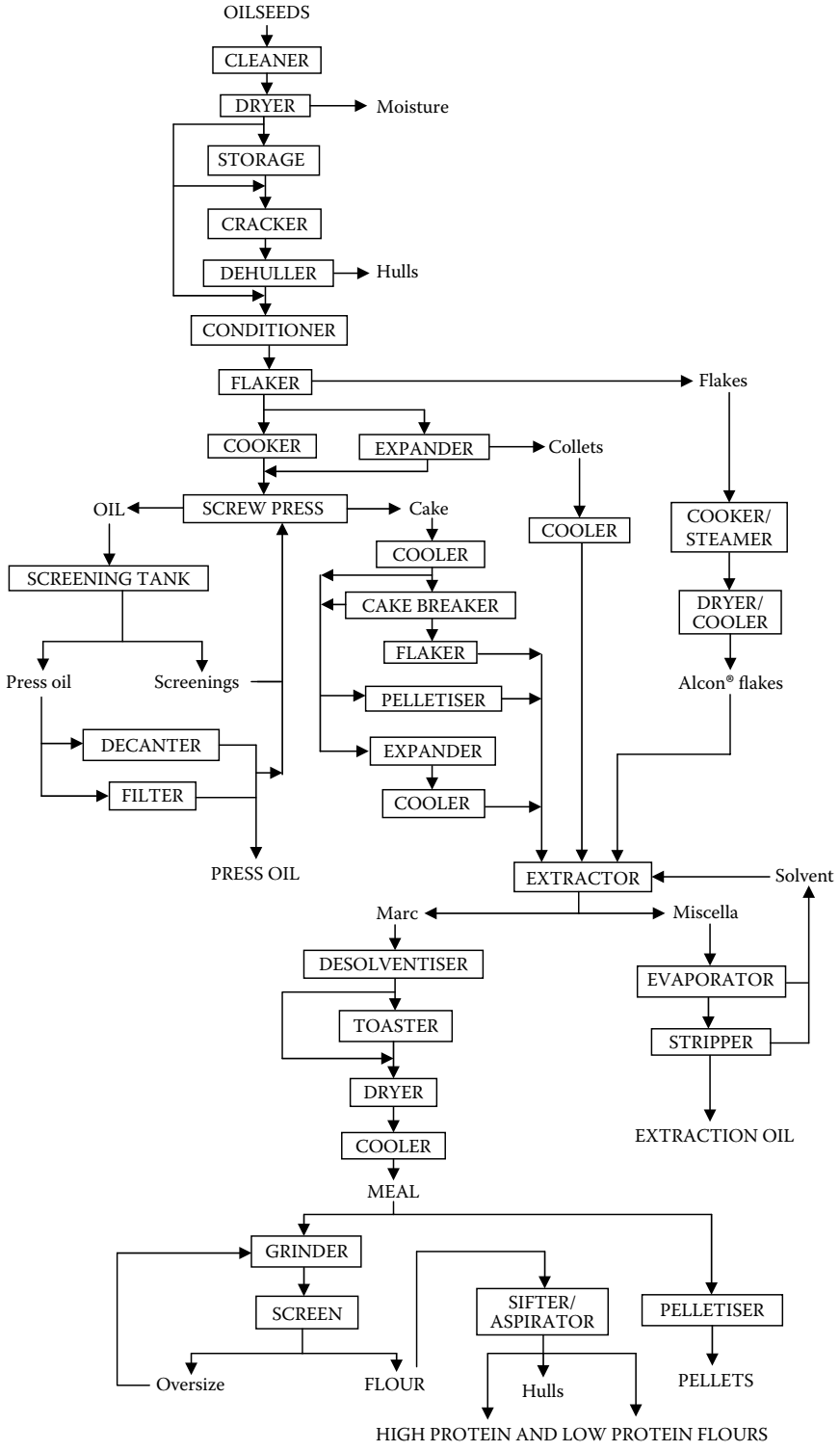


FIGURE 3.5 Seed crushing flow sheet.

extraction of commercial oil-bearing materials. This conclusion did not prevent Othmer and Jaatinen (1959) from arriving at a different conclusion, *viz.* that the penetration of the miscella into the capillary structure of the flakes is the only factor responsible for all phenomena observed. A different approach is taken by Rac (1967), who correlates the rate of oil extraction with cell dimensions. He notes a slow extraction from small cells and since these are less likely to have been opened, he, in fact, concludes that unopened cells are responsible for slow extraction. In 1979, Patricelli et al. studied the extraction kinetics of sunflower seed flakes of various

thicknesses and concluded that this can be expressed as a sum of a washing term and a diffusion term, whereby the diffusion term becomes less important when the flakes are thinner.

Whereas Karnofsky (1949) assumed that the easily extracted fraction is apparently characteristic of unruptured cells and that diffusion through the cell walls is rapid in comparison with the rate of dissolution of the oil, Schneider reached the opposite conclusion (see also Fehsenfeld et al., 1979). When studying the behaviour of soya beans he submersed in hexane (1980), he noted that they increase in size and alter in shape. He explains this by assuming that hexane diffuses into closed cells and builds up an osmotic pressure. This pressure stretches the cell walls, which thereby allow oil/miscella to pass through (Schneider and Rütte, 1991). Accordingly, the authors correlate the low rate of extraction of the "slowly dissolving material" to oil present in unruptured cells. In doing so, they explain why thick flakes extract more slowly — they contain more unopened cells.

They also explain (Schneider and Rütte, 1984) why rapeseed meal may continue to release hexane after it has been toasted. The cells in the aleuron layer of rapeseed have relatively thick walls and, therefore, are difficult to rupture. During the extraction process, hexane diffuses into these closed cells and the toasting process is insufficiently long for the hexane to completely diffuse out again. So when the meal enters the silo, some hexane is still present in closed cells and this hexane is then slowly liberated (J. De Kock, personal communication). Karnofsky (1985) on the other hand, persists in his own approach and "accounts for high residuals by assuming that the dimension of void spaces in flakes after extraction of oil is about 10^{-6} cm, with consequent substantial reduction in vapour pressure." Somewhat later, he again lists the arguments in favour of his assumption (Karnofsky, 1987) and does not mention unruptured cells at all.

The realisation that unruptured cells are the cause of slow and incomplete oil extraction and may also cause high residual hexane levels after toasting, has provided further justification to, for example, the pelletizing or expanding of broken press cake before the extraction. Not only do these operations lead to an increase in bulk density and improved drainage and, thus, to an increased extractor capacity, they also cause unruptured cells to be opened. This increases the oil yield and avoids high residual hexane levels. Cells have to be opened to yield oil and "no measurable quantity of oil could be recovered from whole, intact seeds" when extracting them with liquefied carbon dioxide (Fattori et al., 1988).

What mechanism determines how much and which phospholipids are extracted is far from clear. During industrial extractions using hexane, only a small fraction of the phospholipids present in the oilseed is extracted together with the oil (Desnuelle et al., 1951). Giving the oilseeds a thermal pretreatment (expander, Alcon® or Exergy®) before extracting them also affects phosphatides extraction. It not only increases the phosphatides fraction in the extraction oil, but it also affects their composition in that there are hardly any nonhydratable phosphatides present. As will be discussed in Section 3.4.1, nonhydratable phosphatides (NHP) consist mainly of calcium and magnesium salts of phosphatidic acid. These salts are oil soluble and form a normal constituent of crude oil. Expeller oil tends to have a lower content of these NHP than extraction oil, but oil extracted from thermally pretreated oilseeds can have a negligible NHP content. Accordingly, Kővári (2004) introduces the temperature range of 40 to 70°C as a critical process zone that has to be passed through as quickly as possible by assuming enzyme inactivation to be the key element of low NHP formation.

These oils may exhibit appreciable contents of phosphatidic acid, so it looks as if the thermal pretreatment prevents the phosphatidic acid already present from combining with calcium and magnesium ions and forming NHP. In this context, the following remark (Letan and Yaron, 1972) is quite relevant: "Industrially extracted degummed soya bean oils contain appreciable amounts of calcium (ca. 100 ppm) and its source was traced to the processed soya beans. Various preextraction treatments of the soya bean industry augment the calcium content of the oils, since crude Soxhlet-extracted soya bean oils contained only 15 ppm."

Of course, the thermal treatment will also inactivate enzymes such as phospholipases, but this inactivation cannot explain the almost total hydratability of the phosphatides present in the crude oil since this oil contains hydrolysis products. There must be another mechanism involved, but since the oil milling literature doesn't even note the fact that this issue is unresolved, it follows that it doesn't put forward a mechanism either.

3.3.2.2 Seed storage

Because oilseeds are harvested at certain times of the year and an oil mill preferably operates throughout the year, it is advantageous to store the oilseeds provided their quality can be more or less maintained during storage. In Table 3.3 some literature values have been listed for the recommended maximum moisture contents of various oleaginous materials.

These values allow the moisture content of the lipid-free, dry matter to be calculated and although the values in the last column of Table 3.3 show a rather wide range, a value of 20% looks appropriate. This compares well with the 15% quoted for the lipid-free, but moist matter (Fils, 2000). At higher values, moulds can develop, causing the oil seed temperature to rise, which in turn causes an increase in FFA-content and damages oil colour and quality. When this process raises the temperature above 60°C, the seeds may catch fire and start to smoulder after spontaneous combustion (Carr, 1993). Accordingly, reliable temperature probes inside the silo, combined with adequate means of ventilation, are essential (Holló, 1987).

TABLE 3.3 Water and oil contents of various oil seeds

Oilseed	Maximum Water Content (wt %) (Jáky et al., 1960)	Oil Content of Oilseed (wt %)	Water Content of Nonlipid Part (wt %)
Linseed	9	37 (Loury and Feng, 1958)	17
Poppy seed	9		
Safflower seed	9	44.2 (Table 3.2 in Smith, 1996)	19
Tobacco seed	10		
Tomato seed	10	22.4 (Padley et al., 1994)	15
Rapeseed	10 or 8–9 (Carr, 1993)	40 (Carr, 1990)	20
Castor seed	10	40–50 (Vernois, 1958)	22
Grape seed	10	13–18	14
Corn germ	10	50 (Leibovitz and Ruckenstein, 1983)	17
Cotton seed	11 or 9 (O'Brien and Wan, 2001)	19 (Vernois, 1958)	16
Mustard seed	11	24–40 (Padley et al., 1994)	
Coriander seed	12		
Hempseed	14	31 (Vernois, 1958)	25
Sunflower seed	14	46 (Veldstra and Klère, 1990)	35
Soybeans	14 or 13 (Woerfel, 1995)	18.3 (Perkins, 1995)	21
Pumpkin seed	14	36 (Padley et al., 1994)	28

Although soya bean crushing plants in Europe can switch from North American beans to South American beans as and when the season changes, the beans processed in Europe are stored for considerable periods of time. They are commonly purchased with a maximum of 14% moisture, but on transport and storage, this level tends to decrease to below 13%, at which moisture level the beans are perfectly stable (Fetzer, 1983).

Prior to storage, the seeds may have to be dried (Hunt Moore, 1983) and they are also preferably sampled and weighed on intake. This protects the interest of both the seller and the buyer and indicates to the buyer whether or not a drying treatment prior to storage is appropriate.

3.3.2.3 Seed preparation

As indicated in Figure 3.5, oilseeds can be cleaned to protect downstream processing equipment. If the seeds have already been precleaned before being stored, the subsequent cleaning can be simple. Both processes may employ vibrating screens, magnets, and/or some form of aspiration (Fils, 2000). Destoning is also important since stones will damage the flaking rolls as well as the screw presses (Bell, 2001).

The decision whether or not to dehull depends on a number of factors, amongst which the meal market demands may be overriding. It may, for instance, ask for a soya bean meal with a minimum protein content ($N \times 6.25$) of 48%; in that case, removing the 8% hulls present in soya beans is imperative. Hopefully, there is also a demand for a meal with a minimum protein content of 44% so that some hulls can be blended in. In the case of sunflower seed, there may be little demand for sunflower seed meal with a highish fibre content, which then necessitates dehulling.

However, dehulling sunflowers generates a large volume of hulls since their bulk density is only 70 to 100 kg/m^3 (Beal, 1981). The hulls can be burned to generate steam, but modern equipment has become so efficient that nowadays, processing sunflower seeds generates more hulls than are needed for steam generation (Ruckenstein, 1981). Accordingly, some European plants process sunflower seeds and then stock the excess hulls to be used when they process for instance rapeseed, and switch back to sunflower seed when they have run out of hulls.

The excess hulls can also be used for making a kind of decorative chipboard (D. Callewaert, personal communication) or as a substrate for growing the oyster mushroom, *Pleurotus ostreatus* (D. De Buyser, personal communication). More recently, sunflower seed hulls were found to contain a food antioxidant comprising chlorogenic acid and its hydrolysis product caffeic acid (De Leonardis et al., 2005).

For soya beans, there are several dehulling processes. There is the process that involves first of all drying the beans to an average residual moisture content of 10% and then allowing them to equilibrate for some 72 hours. Subsequently, the tempered beans can be cleaned and scaled before being cracked into four to six pieces and then treated in the dehulling system where the cracked beans are graded by size and aspirated in multiaspirators to remove the hulls (Hunt Moore, 1983). Drying causes moisture to be removed from the outside of the beans while the inside remains relatively moist. Drying also causes material to shrink so the initial drying causes the hull to shrink away from the meat of the bean. During the equilibration, moisture moves from the bean to the hull, so the bean shrinks and loosens itself further from the hull. Subsequent cracking, therefore, leads to loose hull fragments and meat grits. For their separation, equipment as described by Anderson et al. (1998) can be used.

In another so-called "hot dehulling" process (Florin and Bartesch, 1983), there is no resting/tempering stage. Drying the beans causes the hulls to shrink away from

the bean meats that then split into cotyledons; the hulls are then removed before the beans are cracked (Woerfel, 1995). The beans can also be cracked in superimposed sets of fluted rolls that cause the hulls to separate from the cracked beans. In the so-called tail-end dehulling process, soya beans are cracked but no hulls are removed at this stage. Meats and hulls are extracted, desolventised, toasted, dried and cooled, and ground to yield a soya bean flour. It is this flour that is then subjected to a dehulling treatment, which amounts to a simple sifting process that can be combined with a fractionation into high protein and low protein meal as indicated in Figure 3.5 (Fetzer, 1983).

Whether dehulled or not, all seeds must be flaked to open as many cells in the oilseed meat as possible (Fils, 2000). However, Witte advocates an extraction process (1978) without a flaking stage. For the flaking operation, smooth flaking rolls are used. They are usually hydraulically loaded and are typically 600 to 700 mm in diameter and 1000 to 1500 mm long. Ensuring the proper moisture content of the material to be flaked is important since too low a moisture content will lead to an excessive level of fines. Flaking is a fairly expensive part of the crushing process because of its energy requirement and the maintenance of the flaking rolls.

Before being expelled in a screw press or extracted with a solvent, the flakes thus produced can be fed to an expander. This is nothing more than an extruder that heats the material while its water content is adjusted to some 12% by the addition of water or live steam, and forces it through a die, downstream of which its pressure is released so that it expands, just as corn-based cocktail snacks are manufactured. As explained by Rittner (1984), who was the first to install this kind of equipment in his mill in Brazil, expanding soya bean flakes to produce collets has a number of advantages (see also Buhr, 1990):

- As explained above, the expander opens cells that have not been opened before by "explosive vaporisation of water, added or present," so that the flaking operation becomes less critical; in fact, Rittner omitted flaking and just expanded comminuted soya bean particles.
- The liquid hold-up in the marc is much reduced, from 42.5 parts liquid/100 parts solids in the case of flaked soya beans (which is an above average figure and, therefore, not fully representative), to only 17.2 parts liquid if the solids have been expanded. This not only reduces residual oil, but above all, it can reduce steam consumption during desolventising.
- Because of this reduced liquid hold-up, a more concentrated miscella can be tolerated: 35 to 40 wt % instead of the normal 25 to 28 % (Marchand, 1984).
- The percolation rate is much increased from only 23 m^3/m^2.hour to 70 $m^3/m^2 \cdot$ hour in laboratory experiments, but the data are in line with industrial figures reported by Fils (2000) at 17 and >45, respectively.
- Expanding also increases the bulk density of flakes from 320 kg/m^3 to some 600 kg/m^3 (see Rittner, 1984, Table 1), which greatly increases the extractor capacity.

Given these advantages (Pavlik and Kemper, 1990), it is not surprising that by 1989, 70% of the U.S. soya bean and cottonseed crops was being processed by expanders (Woerfel, 1995). Using a mixture of soya bean flakes and collets is also a possibility and has the advantage that a cost optimum can be arrived at since not all cost elements are proportional to the collet content of the extractor feed (Kemper, 1995). As pointed out somewhat later by Fils (2000), the use of expanders varies considerably from country to country. "In Europe, expanders are generally used to increase the capacity of an existing plant, but are often excluded at the design stage of new plants."

Another seed preparation route that is also depicted in Figure 3.5 is the Alcon®[9] process, which is specific to soya beans (Kock, 1978; 1981a). In this process, soya bean flakes are heated in a steam atmosphere to 95 to 110°C and allowed to equilibrate for some 15 minutes before being dried and cooled. The effect of this treatment shows some similarities to the expander treatment in that the bulk density of the material increases, percolation is improved, and liquid hold-up is decreased, but in addition, it also causes more phosphatides to be extracted which are more readily hydrated, so that on water-degumming a lower (<10 ppm) residual phosphorus content is observed; according to Penk (1981) this allows the water-degummed Alcon®-oil to be physically refined. The observation that more phosphatides are extracted after soya bean flakes have been exposed to moist heat has also been made with respect to expander collets (Lusas and Watkins, 1988), and has been studied in detail by Zhang et al. (1994).

In Europe, the Super ExPro® process (Dahlén, 1996; 1998) has been developed specifically for rapeseed. Originally, an ExPro heat treatment section was positioned between the desolventiser/toaster and the cooler/dryer to produce meal with a low protein dissolution index: so-called "bypass proteins for ruminant feed" (see also www.barr-rosin.com for the Exergy® steam processor). Subsequently, it was discovered that giving rapeseed flakes an ExPro treatment not only had the same desirable effect on meal protein quality, but also improved press oil quality by reducing its nonhydratable phosphatides (NHP) to such an extent that the residual phosphorus content after water-degumming is <10 ppm, so that this water-degummed rapeseed oil can be physically refined after bleaching (Kővári, 2004). Veldsink et al.

[9] The word "Alcon" is derived from **A**kzo N.V. (the mother company of Noury & Van der Lande, owners of the Emmerich plant where *M. Kock* developed the process), Lurgi who bought the process, and the fact that it is a continuous process.

(1999) studied various heat pretreatments of oilseeds and prefer steam. They also note enzyme inactivation and a higher phosphatide content of the rapeseed and sunflower seed oil and an increase in phenolic antioxidants in rapeseed oil.

3.3.2.4 Oil production by pressing

Whereas in the past, wedge presses were used for oilseeds and hydraulic presses for olive oil, since the introduction of the continuous screw press by Andersen in 1902, this has become the standard type of equipment for prepressing oilseeds and other oleaginous materials like copra, groundnuts, etc. The screw press operates by continually applying pressure to these materials by the decreasing volume surrounding the Archimedean screw that rotates within a barrel (the so-called "cage") provided with slits through which the oil can drain away (Bredeson, 1977). To prevent the fatty material being pressed from just rotating with the screw, the worm elements constituting the screw are interrupted at regular intervals and replaced by distance pieces that permit stationary knife bars, also referred to as "breaker bars," to be fitted to the cage. At the discharge end of the screw press, an adjustable cone or choke ensures pressure control within the barrel.

Such screw presses are used for prepressing flakes originating from soft seeds, such as rapeseed and sunflower seed, to a residual oil content of some 15 to 20% and thus prepare these seeds for solvent extraction (Ward, 1976). At a much reduced throughput, they can also be used for full pressing (Buhr, 1990), a process that became obsolete with the introduction of prepressing followed by solvent extraction, and that is only suitable for small-scale operation or people who want to avoid the use of solvents (Tysinger et al., 2004).

They can also be used to produce so-called "cold pressed oil," which commands a premium in the market since it is perceived to be free of chemical intervention. Flakes or even whole seeds are pressed without having undergone a prior heat treatment or cooking and then yield an amount of oil corresponding to a residual oil content of the cake of 10 to 20%. Most of this residual oil can then be recovered by cooking the cake resulting from the cold-pressing operation and giving it a second pressing. The oil resulting from this second pressing certainly requires a full refining treatment before becoming palatable (Fils, 2000), whereas for the cold-pressed oil, clarification and bleaching may already suffice for the market segment eager to pay a premium for non-bland oils.

As indicated in Figure 3.5, the fines contained in the press oil are removed in two stages. The first stage comprises a screening tank where the press oil is allowed to settle. The settled oil is as yet insufficiently clear so that it is then either filtered (small plants) or clarified with a decanter (large plants). The filter cake or the decanter solids are recycled to the screw press just as the screenings from the screening tank. In the press, the fines are compacted so that there is no danger of them building up, since there is no purge.

3.3.2.5 Extraction solvents

In a historical survey on solvent extraction (Hutchins, 1977), the editor has added a note mentioning that the first patent on solvent extraction was issued in France on 13 November 1855 to a Mr. Diess on a process to extract fat from bones and wool using carbon disulfide. The survey itself mentions only "aviation-type gasoline," benzene and "a petroleum of the hexane type" as solvents used industrially in the U.S.

In Europe, people were a bit more adventurous, at least on paper. In 1914 a patent was published (Anon., 1914) that mentions petrol, carbon tetrachloride, and carbon disulfide as extraction solvents and advocates the addition of formaldehyde to improve the separation. Bollmann when describing a process for producing lecithin in 1923 used a mixture of alcohol and benzene, the water containing azeotrope of which was again studied by Kahane et al. (1964). Wilhelm (1932) prefers acetone with less than 2% water but also claims petrol, benzene, chlorinated hydrocarbons, and azeotropic mixtures such as methanol/methyl acetate or methanol/methyl acetate/acetone. Pons Jr. and Eaves, on the other hand, prefer more (i.e., 25 to 30%) water in acetone (1967; 1971). The use of the constant boiling mixture consisting of 53 parts by volume of acetone, 44 part by volume of hexane and 3 parts by volumes of water has also been described (King and Frampton, 1960). When used for the extraction of cottonseed, it has the advantage that it preserves lysine and reduces total gossypol (King et al., 1961). A mixture of methanol and an aliphatic hydrocarbon was shown to have the advantage of denaturing the protein during the extraction (Hutchins and Williamson, 1949); a mixture with a major proportion of hexane and minor proportions of methanol and water can also be used to prepare a protein concentrate from comminuted soya beans (Rauer, 1942).

The chemical industry is not afraid to claim the use of trichloroethylene (Levine and Dent, 1943; Sweeney and Arnold, 1949) and this extraction solvent has in fact been used industrially in the U.K. during WW II to reduce the risk of explosions and fire, the devastating effects of which can be quite spectacular (see Goss, 1946, Figure 6). This trichloroethylene posed some corrosion problems and cattle fed with the resulting meal quickly began to die of "bloody nose disease" (Johnson and Lusas, 1983), but 1,2,2-trifluoro-trichloroethane does not present this drawback. Besides, it has a fairly low boiling point (47.6°C), does not hydrolyse, is thermally stable, is not toxic, does not burn, and also dissolves oils and fats (Kaufmann and Orde, 1955). When compared with hexane, the performance of this solvent was found to depend more heavily on the temperature (Temple and Sullivan, 1978). It was

also compared with a whole range of other chlorofluorohydrocarbons by Kaufmann and vom Orde (1955) and shortly afterwards, other extraction solvents (hexane, cyclohexane, acetone, trichloroethylene, 1,2-dichloroethane, absolute ethanol, the ethanol azeotrope, absolute isopropanol and its azeotrope) were compared (Loury and Feng, 1958). Quite recently, a mixture of hexane and a fluorocarbon or a chlorocarbon has been claimed (Kapila et al., 2004).

Apparently, the choice of extraction solvent was still not that clear. An extensive list of potential solvents and their physical properties has been published by Johnson and Lusas (1983), who also quote an impressive number of literature references (see also Lusas et al., 1990). However, with regulations governing which solvents were allowed in contact with food products, the choice of which solvent to use became more limited and excluded, for instance, aromatics like benzene and toluene. Methylene chloride continues to be permitted in the U.S. for special food products (Lusas et al., 1990). It was studied as extraction solvent for cottonseed and found to generate gossypol-free meal (Johnson et al., 1986).

Given their role in solvent extraction, a list of requirements to be met by those solvents could also be drawn up:

- Oils and fats must dissolve in the extraction solvent.
- This solvent should also penetrate into the oilseed flake or collet and lead to a sufficiently rapid and complete extraction of the oil.
- The solvent should preferably not extract other non-fat constituents from the oilseed.
- Desolventising the resulting marc should lead to low residual solvent levels in the meal and appropriate meal quality.
- The resulting miscella should permit the oil to be isolated.
- Solvent recovery for re-use in the extractor should be possible.
- The solvent should make extraction of prepressed cake a profitable exercise, which means that:
  - The liquid holdup in the marc should be as low as possible.
  - Heat of vaporisation should be as low as possible.
  - The need for rectification should be avoided.

In practice, the above list of requirements has limited research and development on solvent extraction to the use of hexane, ethanol, isopropanol, and mixtures with acetone and acetic acid. An example of such a mixture is the use of hexane containing 2 to 25% acetic acid (Hensarling et al., 1976; Hensarling and Jacks, 1983), which extracts some 6 to 9.5% more oil and much more phosphorus containing compounds than hexane on its own; extraction of membranes is mentioned as a possible cause (Hensarling and Jacks, 1983). Further advantages are the increased filtration rate of the miscella through the marc, which increases plant capacity and energy savings. Potential problems are the corrosive nature of the solvent mixture and a smelly meal.

As pointed out by Beckel et al. in 1948, "alcoholic extraction of vegetable oils has always been intriguing because of the high temperature coefficient of solubility." At high temperatures, alcohols such as ethanol and isopropanol are completely miscible with oil, but at low temperatures, the solubility is much decreased, which means that cooling the alcoholic miscella will lead to the formation of a two-phase system. The oil phase will contain only a little alcohol that can easily be removed by a water wash, and the alcoholic phase will contain hardly any oil.

To attain this miscibility, Sato and Ito (1932) operated the plant in Dairen, Manchuria, above the atmospheric boiling point of ethyl alcohol but below 120°C. Beckel et al. (1948a) operated at atmospheric pressure and cooled the alcoholic miscella to below 20°C and preferably to below 0°C to cause the oil to separate. Figure 3.6 shows the flow sheet of their process (Beckel et al., 1948b). They claim that when soy flakes are dried to below 3% residual moisture, the alcohol no longer extracts water from this material. Accordingly, there is no moisture accumulation in the system and no rectification is required.

In their process, the alcohol will extract sugars and possibly other alcohol-soluble compounds from the soya bean flakes (cf. soy concentrate production). However, Beckel et al. claim that a steady state will be reached whereby the amount extracted will eventually equal the amount dissolved in the miscella contained in the marc. On drying, this amount will end up in the meal, which thereby acts as a purge. Difficulties with the nonoil solids forming an interphase between the lower oily phase and the supernatant alcoholic phase were overcome by heating (Beckel et al., 1950; Hron Sr. and Koltun, 1984).

Subsequently, Rao noted (1954) that cottonseed oil obtained by ethanol extraction had a much lower colour than hexane extracted oil. Hron Sr. et al. (1992) extracted gossypol from cottonseed flakes by acidifying the alcohol, determined what moisture level in the flakes was in equilibrium with which water content in the ethanol (Abraham et al., 1993), and finally concluded that the ethanol extraction process, although technically feasible, was not economical (Hron Sr. et al., 1994).

As pointed out by Singer and Deobald (1945),[10] ethanol has the disadvantage that it is preferably used above its boiling point which necessitates extraction under pressure; this makes it difficult to introduce the material to be extracted. Accordingly, they propose using a mixture of

[10] This patent also contains a most intriguing remark: "We have discovered that through the use of small quantities of a salting out agent, such as calcium chloride, which may be dissolved in the solvent, the phosphatides are precipitated in a flocculent form." No data on residual phosphorus are given, however.

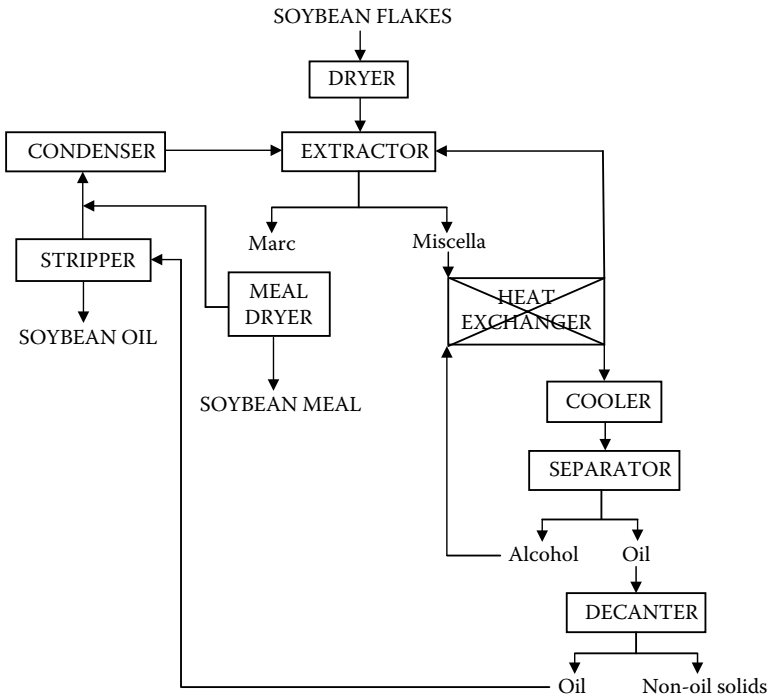


FIGURE 3.6 Flow sheet ethanol extraction.

ethanol with 20 to 30% (v/v) isopropanol and operating at atmospheric pressure. The miscella is cooled to isolate the crude oil and the solvent is recycled to the extractor, except for a bleed of 25%, which is purified by distillation.

When using the isopropanol/water azeotrope to extract cottonseed, Harris et al. first of all (1947; 1949) used hexane to recover the oil from the isopropanol miscella, but subsequently (1950) they recovered the oil by cooling the miscella. However, it took some 30 years before these findings were followed up by what is now Royal Dutch Shell in an attempt to create a market for its isopropanol. In a first patent, part of the upper, isopropanol phase was purified by distillation before being recycled (Youn and Wilpers, 1981). Then fractionation of the desolventiser vapours was introduced (Grimsby, 1984b), presoaking of the seed material was introduced (Grimsby, 1984a), a mechanically aided drainage of the marc was incorporated (Grimsby, 1984c), and finally, the extent of isopropanol recycling was specified by Sullivan (1985).

Previously, Sullivan worked for the U.S. Department of Agriculture on soya bean extraction with isopropanol where he reported (Baker and Sullivan, 1983) that RBD oils and meal from this process compared favourably with their hexane counterparts. As mentioned by Johnson and Lusas (1983), Karnofsky also used isopropanol to obtain a flour (1979) and a concentrate (1980) and finally, aqueous isopropanol can also be used to remove aflatoxins from oilseed meals (Rayner and Dollear, 1968). Isopropanol processes have never been adopted industrially despite the fact that Lusas et al., in their final report (1997), conclude that its use as a replacement solvent for hexane seems feasible, subject to confirmation by sustained trials in a scaled-up pilot plant.

In Canada, a dual solvent system comprising methanol ammonia as the polar phase and hexane as the second phase has been studied with glucosinolate removal in mind (Rubin et al., 1984; Naczk et al., 1988). The use of hydrocyclones in separating the ground rapeseed from the extraction solvents has been studied (Adu-Peasah et al., 1993) and the process also reached pilot-plant stage (Thobani and Diosady, 1997a). Replacing the ammonia with caustic soda leads to a less extensive extraction of glucosinolate and to oil saponification (Thobani and Diosady, 1997b). Given the high solvent to meal ratios involved, the process could be expensive to operate; besides, methanol is not a food-grade solvent. In a move away from hexane, isohexane has been suggested instead (Turner and Venne, 2003).

And then, supercritical gases like ethane, propane, butane, isobutane, carbon dioxide, or dinitrogen oxide were suggested as an extraction solvent for oilseeds (Zosel, 1974; Zosel, 1982) and sulfur hexafluoride, trifluoromethane, trifluorochloromethane, difluorochloromethane 1,2-difluoroethylene, and octofluoroethane were also claimed for the extraction of cocoa butter (Roselius et al., 1975). At that time, liquid carbon dioxide was in use for the extraction of hops and that was found to be very useful since mixing extracts to arrive at a standard product is far easier than mixing hop flowers. Supercritical extraction using wet carbon dioxide was also used for the decaffeination of roasted coffee (Roselius et al., 1974). Accordingly, the industrial process was clearly limited to high value substrates and products.

Then a large number of research groups started to study the use of supercritical carbon dioxide in extraction of various oils, such as evening primrose oil (Favati et al., 1991), *Dimorphoteca pluvialis* seeds (Cuperus et al., 1996), flaxseed oil (Bozan and Temelli, 2002), olive husk oil (de Lucas et al., 2002), walnut oil (Oliveira et al., 2002), and wheat germ oil (Panfili et al., 2003). Reviews and progress reports were published regularly about early progress in Germany (Mangold, 1982), in Canada (Bulley et al., 1984), about oilseeds in general (Eggers and Stein, 1984; Eggers, 1985; Eggers et al., 1986; Lusas et al., 1990; Wilp and Eggers, 1991; Eggers, 1994), and soya beans in particular in the U.S. (Friedrich et al., 1982), which led to a patent (Friedrich, 1984). Carbon dioxide suppliers also published their experiences (Beutler et al., 1988), a Supercritical Fluids Technology Group extracted and fractionated an oil leading to an enrichment in nervonic acid (Santos, 2004) and an entire book was devoted to the subject (King and List, 1996). However, and this is a most serious omission, none of these papers or reviews, nor this book mention that pressure vessels do not profit from an economy of scale.

Whereas most equipment used in the chemical industry and the edible oil industry enjoys an economy of scale, meaning that an apparatus with twice the capacity is not twice as expensive, but only 1.2 to 1.5 times as expensive, this economy of scale does not apply to pressure vessels. For a cylindrical pressure vessel, the tension in the wall is not only proportional to the pressure inside the vessel but also to the diameter of the vessel. The circumference of the vessel is also proportional to the diameter of the vessel so that the cross section of a wall retaining its strength is proportional to the square of the diameter, just like the surface of the cross section through the vessel. In other words, the wall volume or weight of metal required to provide the vessel with sufficient strength to withstand the pressure inside, is proportional to the volume inside the pressure vessel.

This makes any large-scale utilisation of high pressure extraction using supercritical gases and operating at, for instance, pressures in excess of 55 Mpa (550 bar) to improve oil solubility (Friedrich, 1984), prohibitively expensive. Accordingly, the conclusion (Reverchon and Sesti Osséo, 1994) that the operating cost of supercritical extraction falls "in the same range as conventional extraction plants" is positively misleading and the expectation that "the continuous advances in SC-CO_2 technology will reduce the gap" in plant cost is sheer nonsense. Consequently, nearly all the research and development effort that has been spent on supercritical extraction is of academic interest only.

3.3.2.6 Oil production by extraction

A historical review of the solvent extraction of oilseeds (Arbeitskreis "Technologien der industriellen Gewinnung und Verarbeitung von Speisefetten", 1975) also lists extraction equipment and when it was introduced. See also: Karnofsky (1949a), Depmer (1951) and Milligan (1976). One piece of the early equipment, the continuous Bollmann extractor, also referred to as the Hansa-Mühle extractor (Bollmann, 1920), comprises a system of moving buckets similar to a paternoster elevator. Solvent or miscella is sprayed into the buckets at the top of the extractor and drains through the perforated bottoms of the buckets; it is referred to as a percolation-type extractor.

Another type of extractor that also became widely used in Europe and the U.S. is the Hildebrandt extractor (Hildebrandt, 1934). This is an immersion-type extractor in which the material to be extracted is fully immersed in the extraction solvent and transported against the solvent flow. A more recent immersion type of extractor has been invented by Schumacher (1986b), but current industrial extractors tend to be of the percolation-type only. Other types of extractor, such as an English extractor employing a series of screw conveyors (Winters, 1918), the Kennedy extractor (Kennedy, 1927), and the Bonotto extractor (Bonotto, 1937a; 1937b) have been described in the literature, but were not a commercial success.

WW II stopped the Hansa-Mühle and Hildebrandt extractors from being exported (Hutchins, 1977) and caused them to be copied (Robinson, 1940; Beeson, 1950) and also induced independent development. This led not only to the Rotocel-type extractor (Karnofsky, 1950; Beck, 1962) and the stationary basket-type extractor, but also to the belt-type extractor (Van der Voort, 1953; De Smet, 1954) that can have either a shallow bed or a deep bed (Anderson, 1998). After succeeding his father as general manager of a French-owned oil mill in Belgium, De Smet (1975) built an extractor for his own use after the war, there being no German suppliers any more; he only started selling equipment when some of his French colleagues ordered similar extractors.

All equipment described above and in the literature extract their feedstock counter-currently. Accordingly, the feed is mixed with almost full strength miscella and fresh solvent is brought into contact with feedstock that has already been almost depleted. The extractors listed above also have a drainage section that aims at minimising the liquid holdup of the marc in order to minimise the amount of heat needed to dry the marc. Holdup of 30 to 32% is not abnormal (Johnson, 2000), but the use of collets can decrease this figure to 15% by weight (Rittner, 1984); a wider range of 25 to 35% is quoted by Fils (2000).

The solvent to flake (or collet) ratio to be used is a compromise. A low ratio has the advantage that less heat is required in the miscella evaporator, but may cause an unacceptably high residual oil content in the meal. In practice, solvent and miscella are sprayed onto the flakes or collets at a rate that is governed by their drainage characteristics, which means that if they drain well, a higher rate can be maintained that in turn leads to a higher

throughput. A full miscella normally contains 22 to 30% by weight of oil (Johnson, 2000). Given the oil content of the oilseed material to be extracted and the generally very low residual oil content of the marc/meal, this determines the solvent to flake ratio.

3.3.2.7 Marc desolventising

As mentioned above, the marc leaving the extractor may contain some 30% solvent, which in practice is hexane. The marc temperature will be the extraction temperature (55 to 60°C) or just below the hexane boiling point of 65°C. (For hexane properties, see Lusas et al., 1990, p. 69.) The purpose of the marc desolventising process is to remove the hexane to low residual levels and can also be to provide the extraction residue (meal) with a heat treatment referred to as toasting.

This toasting is important for soya bean meal since raw soya beans contain an urease enzyme and protease inhibitors (trypsin and chymotrypsin inhibitors, in particular), which act as antigrowth factors in monogastric animals and that have to be inactivated before the meal can be incorporated into animal feed (Sipos and Witte, 1961). Moisture, heat, and residence time cause inactivation (Becker, 1971) and, thus, improve nutritive value (Kruse and Soldner, 1941), but also cause the soy protein to become less soluble and may decrease the bioavailability of its lysine, an essential amino acid. A toasting time of 15 to 20 minutes is the accepted compromise (Kemper, 2000).

Since the desolventising/toasting operation is a major consumer of energy, the industry has shifted to the most energy efficient equipment as invented by Schumacher (1986a). This equipment is commonly referred to as a DTDC (desolventiser-toaster-dryer-cooler); it has been schematically represented in Figure 3.7. This figure shows a desolventiser with a number of chambers separated by trays. These chambers have been numbered for ease of reference. Trays below chambers 1 and 2 are double to provide indirect steam heating, whereas live steam is injected below chamber 3. An agitator moves the material over the decks with sweep arms (not indicated) and over the gates in the trays below chambers 1 and 2 that allow material to pass to the chamber below.

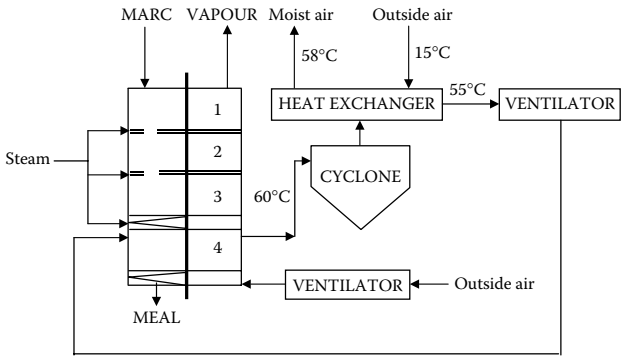


FIGURE 3.7 DTDC.

By heating the material indirectly, hexane will evaporate and as long as there is still hexane present, the temperature will not rise above the boiling point of hexane. By heating the material with live steam, this steam will condense and raise the temperature above the boiling point of hexane as soon as all hexane has been vaporised. Thus, by judicious tuning of the desolventiser/toaster section of the DTDC, it is possible to arrive at toasting temperature in chamber 3, while the temperature of the vapour stream leaving chamber 1 is only 65 to 68°C and contains hardly any water (Schumacher, 1983); this situation corresponds to minimal steam usage.

The atmosphere in chambers 1, 2, and 3 changes from almost pure hexane in chamber 1 to almost pure water vapour in chamber 3; air is excluded. Accordingly, a rotary valve that has been schematically represented by an isosceles triangle below the tray of chamber 3 forwards the material to chamber 4, where it is cooled and dried by air. Heated air is passed over the material as it enters chamber 4 via the rotary valve to dry and cold, outside air is blown through the material to cool it. The drying will also cool the material and the cooling will also dry it.

From a mechanical point of view, hollow stay bolts, holes, and spacers in the deck bottoms have been described in detail (Schumacher, 1983) and a different construction for the decks (Kemper and Farmer, 1999) facilitates vapour passage. Providing an airtight flash chamber at reduced pressure below the direct heating zone will further promote the removal of residual solvent. The pressure can be reduced by using a steam ejector that delivers its compressed vapours to the direct heating zone (Anderson, 2001).

As indicated in Figure 3.5, the meal leaving the cooling section of the DTDC via a rotary valve can be ground and sifted to standard size (Witte, 1995) or pelleted (Robinson, 1977).

3.3.2.8 Miscella evaporation

The overall composition of the various product streams is described in Table 3.4. This assumes the oilseed flakes to contain 10% water and the meal to contain 12%, which means that no water has to be evaporated besides the steam condensed onto the meal in the toaster section. It assumes the marc to contain 30% solvent and the miscella to contain 30% oil, so that with the assumed oil content

TABLE 3.4 Composition of product streams

Product Stream	Solids	Water	Oil	Hexane
Oilseed flakes	72	10	18	–
Marc	72	10	–	35
Meal	72	10		
Miscella			18	42

of 18% in the oilseed flakes, the amount of hexane that has to be evaporated from the miscella exceeds the amount to be removed from the marc by 20%. When collets are used instead of flakes and less solvent is retained in the marc, this figure is even higher.

A number of different ways exist to reduce the amount of energy involved in isolating the crude oil from the miscella. The first way utilises the latent heat contained in the vapours leaving the desolventiser by routing them through the shell side of the first-stage miscella evaporator (also referred to as the economiser). Miscella with an oil content of 25 to 30% and a temperature of some 55°C enters the economiser and leaves it at a concentration of 65 to 75%. This means that about five-sixths of the hexane present in the miscella feed is already evaporated in the economiser without any steam being used.

The second way utilises the sensible heat present in the steam condensate coming from the double trays in the DTDC by heating the miscella leaving the economiser from about 50°C to 75 to 80°C. If this preheated miscella is then exposed to vacuum in the main evaporator, there is a flash evaporation of hexane causing the oil concentration to rise to some 93 to 95%.

The residual hexane is then removed by vacuum stripping, but instead of passing the material to be stripped down a column — and this is the third way — the material is heated and saturated with water by allowing steam to condense in the material. If this solution is then made to enter a flashing vessel, 90 to 95% of the hexane present will evaporate (Schumacher, 1978). By having two such flashing units in series, sufficiently low residual levels are attained. Besides, in the paper concerned (Schumacher, 1984), the temperatures mentioned (80 to 100°C) are much lower than those mentioned in the relevant patents (up to 250°C) so that the steam requirement for the stripping stage has been reduced to only 5 kg per tonne of soya beans.

The fourth way has not yet been industrialized, but could involve the use of membranes. In 1990, commercially available membranes were still insufficiently hexane-resistant (Köseoğlu et al., 1990), but since then membranes have been developed that have been successfully used for the degumming of a soya bean oil miscella (Pagliero et al., 2001; Köseoğlu et al., 2004). Since the present miscella evaporation system is strongly linked to the marc desolventising process and derives nearly all its energy from the latter process, energy saving can only be achieved by using membranes when less energy is available from the marc desolventising process; this could result from the use of collets since they retain less solvent.

Because hexane is quite volatile, extra measures are needed to minimise hexane losses. All vent gases must be cooled in a condenser before being passed to a mineral oil absorption system. This comprises two absorption towers in parallel one of which cleans the vent gases while the other can be regenerated by steam stripping (Woerfel, 1995). Water originating from the hexane/water separator has to be heated to boil off the dissolved hexane before being discharged (Weber, 1972).

3.3.3 Other vegetable oils

The oils discussed in the previous section are primarily soya bean oil, rapeseed oil, and sunflower seed oil because they are the major seed oils. Cottonseed oil has also been mentioned occasionally since, historically, its production sparked off many developments that have subsequently been used in producing other oils (O'Brien and Wan, 2001). Apart from palm oil, which has been discussed in Section 3.3.1.2, the other major (world production >2 million tonnes per annum) vegetable oils are: groundnut oil, the lauric oils (coconut and palm kernel oil), and corn germ oil (Hamm, 2000).

Then there are the minor vegetable oils that may have been cultivated for oil production such as castor oil (Dunning, 1954), sesame oil or safflower oil (Smith, 1996), and cocoa butter, or oils that arise from by-product or waste stream utilisation. Examples of the latter oils are tomato seed oil, grape seed oil, etc. These latter oils have the advantage that their starting material usually has no market value. What else can a tomato canning plant do with all the seeds it accumulates other than produce oil on the side, or a winery for that matter? An early survey (Kester and Van Atta, 1942) with an extensive bibliography, mentions the following raw materials that are being used or could be used for oil production in the U.S.: almonds, apricot pips, avocados, prune pits, peach pits, cherry pits, English walnuts, tomato seeds, citrus seeds from oranges, grapefruits, and also lemons, grape and raisin seeds, squash and pumpkin seeds. It also mentions sunflower seeds, which at that time were used almost entirely in poultry feeds.

3.3.3.1 Grape seed oil

During wine production, fermentation starts in the presence of the skins and pips. Consequently, the filter cake comprising these solids also contains alcohol. This alcohol may be recovered as a special, local spirit by distillation, but this has the disadvantage that the pips may char somewhat. Oil produced from such charred pips may be rather dark and in practice difficult to bleach, and this is why decortication processes have been developed based on abrasion (Jammes and Nugues, 1961), impact (Béchard, 1962), and a specially developed machine, the "Stratoschäler" (Rohne, 1966). Pips originating from a grape jelly plant will not suffer from this colour problem, but then American jelly plants don't process their pips.

3.3.3.2 Cocoa butter

Cocoa butter is a different story not only because of its high value but also because the nonfat solids are used as a highly valued colouring and flavouring ingredient

in products like chocolate milk, chocolate ice cream, etc. Cocoa butter and powder are produced by using a cocoa press developed by Conrad van Houten in 1828 (Padley, 1997; Shukla, 1997); this process starts with dried cocoa beans (*Theobroma cacoa*). At the plantation, it is normal practice to remove the beans with the adhering fruit pulp from the pods and allow this mixture to ferment for a few days in boxes (Padley et al., 1994). After fermentation, the beans are spread out and dried in the sun; when dry (6 to 8% moisture), they are stable and ready for shipment (Kattenberg, 1996). The fermentation process is essential for the development of the cocoa flavour.

When processed, the dried beans are first of all broken so that the shells can be removed from the kernels by winnowing. Then the broken kernels are treated with a concentrated solution of alkali, usually potassium carbonate. This treatment deepens the colour of the cocoa solids and assists in flavour development. The alkalised nibs are then dried, roasted, and ground to yield cocoa liquor that can be used in chocolate manufacture or pressed hydraulically to yield cocoa butter and a press cake that still contains up to 20% butter for drinking chocolate and some 10% for industrial applications. The press cake can also be solvent extracted to yield a lower grade of cocoa butter, and all butters can be deodorised to reduce their free fatty acid content and remove some chlorinated insecticides. The deodorisation temperature is about 160 to 180°C (Shukla, 1995).

3.3.3.3 Gourmet oils

There are some speciality or gourmet oils that are produced in small quantities and sold at a substantial premium; they may also be flavoured (Latta, 2000). Examples are wheat germ oil, walnut oil (Dijkstra, 2003), avocado oil, hazelnut oil (Hansen et al., 2001), almond oil, and pumpkin seed oil, but there are many more, mostly local specialities since most seeds, pips, stones, nuts, etc., contain enough oil to justify its isolation. However, isolation by solvent extraction is usually not justified for these speciality oils, so many of them are produced by hydraulic pressing or by screw expelling (Fitch Haumann, 1997). When this is done at fairly low temperature and not too exhaustively, there is no urgent need to refine the oil and the absence of a treatment involving chemicals is even used as a sales argument.

3.3.3.4 Groundnut oil, etc.

Groundnut oil and minor seed oils are produced very much like rapeseed oil (Canat, 1979); *Crambe abyssinica* seed can be processed in a standard commercial extraction plant (Carlson et al., 1985). The raw material is cleaned, conditioned, flaked, cooked, and expelled, and the scale of operation determines if the expeller cake is extracted by solvent or not (Sanders, 2001). The expeller screw configuration will have to be adapted to the oil content of the material being treated, but otherwise, an oil mill capable of processing rapeseed will also manage groundnuts, safflower seed (Terrones, 2001), palm kernels, etc.

3.3.3.5 Coconut oil

For fresh coconuts, an aqueous process has been developed that starts with grating the meat to form an emulsion. After various treatments, this is then "broken" so that the oil is liberated; this is then is washed and dried (Robledano, 1956; Hagenmaier et al., 1972). However, copra is normally prepared by drying fresh coconut kernels in the sun, in a kiln, or by a combination of the two methods. Drying to 8% moisture in the sun takes some 5 days. Smoke drying can lead to PAH (polycyclic aromatic hydrocarbon) contamination of the copra and the resulting press oil (Swetman et al., 1999). Accordingly, indirect drying with the coconut shell as an energy source is preferred (Graalmann, 1990). In Indonesia, the coconut kernel is dried by frying it in hot coconut oil.

3.3.3.6 Corn germ oil

Oil mills processing soya beans and equipped with flaking rolls can also handle corn germs (Ulrich et al., 2002). Developments in soya bean crushing have been found to be also applicable to, for instance, the production of corn oil from corn germs, despite the fact that the oil content of corn germs (48 to 52%) is much higher than that of soya beans (17 to 19%). Corn oil can be produced by expelling the dried germs and then extracting the expeller cake, but this course of events leads to a large amount of fines. Therefore, Maza (2001) treats hydrated corn germs in an expander and either extracts the resulting collets straight away or includes a prepressing treatment as well. Direct extraction of the flakes germs is also possible in the TOM extractor (Leibovitz and Ruckenstein, 1983) which turns over the material at its half-way point inside the extractor, thereby mixing the material and destroying the impermeable layer formed by the fines.

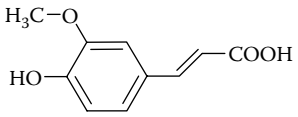
3.3.3.7 Rice bran oil

Similarly, rice bran can be profitably processed in an expander before being extracted with a solvent (Lee, 1991). This not only has the advantage of converting a fine powder with poor drainage characteristics into fast-draining collets, but also inactivates the lipase enzyme that is always present in the bran leaving the rice mill, and that causes the oil present in the bran to acidify/hydrolyse very fast at 4 to 5% FFA formation per day (Loeb et al., 1949). In fact, expanding the rice bran at local rice mills, which is also referred to as extrusion cooking and involves the addition of some water (Sayre et al., 1985), generates a stable product (rice bran collet) that can be stored and transported to a central extraction plant (Williams and Baer, 1965; Baer et al., 1966). Rice

bran can been treated in an expeller, but since the bran contains only about 18% of oil, the oil recovery on expelling is only 50 to 65% (Orthoefer, 2001).

Accordingly, the use of local expanders feeding large extraction plants makes it possible to greatly increase the production of rice bran oil, which at the moment is 0.7 million tonnes (Orthoefer, 2001), i.e., only a fraction of potential production which was estimated at 5 million tonnes per annum (Lee, 1991). Another method of stabilising rice bran by inactivating its lipase is by mixing hydrochloric acid into the bran and lowering the pH to about 4.0 (Prabhakar and Venkatesh, 1986). By comparing various pretreatments, Kim et al. (1987) conclude that extrusion is superior to hot air drying and steam cooking because of increased percolation rate.

Rice bran oil is unique in that the oil has a cholesterol-lowering effect. Crude rice bran oil contains some 2% oryzanol (De and Bhattacharyya, 1998). Oryzanol is the name for a group of esters of ferulic acid (3-(4-hydroxy-3-methoxyphenyl)-*trans*-2-propenoic acid or *p*-hydroxy-*m*-methoxycinnamic acid) as shown below.[11]



This acid can be esterified with cycloartenol, β-sitosterol, 24-methylene cycloartenol, cyclobranol and campesterol (Lloyd et al., 2000).

The wax content of rice bran oil can vary widely (Reddi et al., 1948). Some authors make no mention of any wax associated with rice bran oil, whereas others isolate more than 5%; this variation may stem from extraction conditions. A distinction can be made between "soft" and "hard" wax fractions with the soft fraction comprising esters between saturated C_{24} and C_{30} alcohols and C_{16} and C_{26} fatty acids and *n*-alkanes of C_{21}, C_{29}, and those in between. The hard fraction, on the other hand, comprises wax esters between saturated C_{24}, C_{26} and C_{30} alcohols and saturated C_{22}, C_{24} and C_{26} fatty acids, and C_{29} and C_{31} *n*-alkanes (Yoon and Rhee, 1982). In addition, the tank settlings also comprise aromatic moieties (Belavadi and Bhowmick, 1988).

Crude rice bran oil can be degummed like almost any vegetable oil. Since it contains NHP, the use of a degumming acid is recommended and the lower the degumming temperature, the more waxes are removed at the same time. Miscella dewaxing by using a 45 to 60% oil in hexane miscella and cooling it to 3 to 5°C is another possibility (Bhattacharyya et al., 1983). Even almost immediate lipase inactivation will still result in a crude oil with an appreciable FFA content. As will be pointed out in Section 3.9.4, rice bran oil might be a prime candidate for physical refining, especially since this will retain the oryzanol, and waxes and oryzanol increase the alkali refining losses (Mishra et al., 1988). However, the high temperature, physical refining process may lead to a fixation of colour (Kim et al., 1985).

The reason for this "colour fixation" is not clear. It has also been observed with palm oil by Tan et al. (1985), who noted that this fixation was retarded by oils that had previously been neutralised with sodium hydroxide, and concluded from further analyses that phenolic compounds are responsible for the darkening of palm oil. Since phenols are slightly acidic and, in general, only react as anion, this could imply that avoiding their dissociation by maintaining a certain degree of acidity might also prevent colour fixation. This is hypothetical since Chapman (1995) reports that FFA might be the cause of colour fixation, or at least a nonphenolic form of colour fixation.

During the early part of the physical refining process, the FFA still present will ensure this acidity, but a stage may be reached where they no longer suffice. At that point, an alkaline wash removing the phenolic compounds or the addition of an acid, such as activated bleaching earth, may be necessary to prevent colour fixation. Accordingly, a first flash deacidification followed by alkali neutralisation, bleaching, and deodorisation, looks like a possible process sequence ensuring a good yield and a low colour.

[11] The structural formula is given since a literature reference (Orthoefer, 2001) omitted the double bonds in the benzene ring.

References

Abraham, G. et al. (1993), Water accumulation in the alcohol extraction of cottonseed, *J. Am. Oil Chem. Soc.*, **70**, 207–208.

Adu-Peasah, S.P. et al. (1993), A multistage hydrocyclone/stirred-tank system for countercurrent extraction of canola oil, *J. Am. Oil Chem. Soc.*, **70**, 755–762.

Anderson, G.E. (1998), Self-purging extractor, US Patent 5,770,082, assigned to Crown Iron Works Company.

Anderson, G.E. (2001), Apparatus for enhanced solvent recovery from solvent extracted material, US Patent 6,279,250, assigned to Crown Iron Works Company.

Anderson, G.E. et al. (1998), Treatment for particulate material, US Patent 5,727,689, assigned to Crown Iron Works Company.

Anon. (1914), Verfahren zur Gewinnung des Öles aus Ölsamen u. dgl., German Patent 269 195, assigned to Harburger Oelwerke Brinckman & Mergell.

Arbeitskreis "Technologien der industriellen Gewinnung und Verarbeitung von Speisefetten" (1975), Gemeinschaftsarbeiten der DGF, 63 Mitteilung. Gewinnung von Fetten und Ölen aus pflanzlichen Rohstoffen durch Extraktion I, *Fette Seifen Anstrichm.*, **77**, 373–382.

Baer, S. et al. (1966), Pre-treatment of oleaginous materials, US Patent 3,225,220, assigned to International Basic Economy Corporation.

Baker, E.C. and Sullivan, D.A. (1983), Development of a pilot-plant process for the extraction of soy flakes with aqueous isopropyl alcohol, *J. Am. Oil Chem. Soc.*, **60**, 1271–1277.

Beal, L. (1981), Valorisation des sous-produits de l'industrie des corps gras II - Les coques de tournesol, *Rev. Franç. Corps Gras*, **28**, 165–166.

Béchard, J. (1962), Procédé de fabrication d'huile de pépins de raisin et l'huile conforme à celle obtenue, French Patent 1.281.636.

Beck, F.D. (1962), Solvent extraction apparatus, US Patent 3,062,626, assigned to Blaw-Knox Company.

Beckel, A.C. et al. (1950), Vegetable oil extraction, US Patent 2,505,749, assigned to USA, Secretary of Agriculture.

Beckel, A.C. et al. (1948a), Process of extraction from vegetable materials, US Patent 2,445,931, assigned to USA, Secretary of Agriculture.

Beckel, A.C. et al. (1948b), The nondistillation alcohol extraction process for soybean oil, *J. Am. Oil Chem. Soc.*, **25**, 10–11.

Becker, K.W. (1971), Processing oilseeds to meal and protein flakes, *J. Am. Oil Chem. Soc.*, **48**, 299–304.

Beeson, B.B. (1950), Extraction process and apparatus, US Patent 2,512,710, assigned to The French Oil Mill Machinery Company.

Belavadi, V.K. and Bhowmick, D.N. (1988), An investigation of rice bran oil tank settling, *J. Am. Oil Chem. Soc.*, **65**, 241–245.

Bell, P.M. (2001), Preparation of oilseeds for extraction, in *Proceedings of the World Conference on Oilseed Processing and Utilization*, Wilson, R.F., Ed., AOCS Press, Champaign IL, 8–10.

Berger, K.G. (1983), Production of palm oil from fruit, *J. Am. Oil Chem. Soc.*, **60**, 206–210.

Berger, K.G. (2003), EU Chocolate Directive discussed in May, *inform*, **14**, 511.

Beutler, H.-J. et al. (1988), Erfahrungen mit der CO_2-Hochdruck-extraktion auf dem Gebiet der Fettextraktion, *Fat Sci. Technol.*, **90**, 550–554.

Bhattacharyya, D.K. et al. (1983), A critical study of the refining of rice bran oil, *J. Am. Oil Chem. Soc.*, **60**, 467.

Bianchi, G. (1999), Extraction systems and olive oil, *Oléagineux Corps Gras Lipides/OCL*, **6**, 49–55.

Boekenoogen, H.A. (1948), *De Scheikunde der Oliën en Vetten*, A. Oosthoek's Uitgevers Mij., Utrecht.

Bollmann, H. (1920), Gegenstromverfahren zur abgestuften Auslösung von Fett und Öl aus Rohstoffen, German Patent 322 466.

Bollmann, H. (1923), Verfahren zur Gewinnung des durch Auslaugen von Ölsaaten oder deren Preßkuchen mit einem Gemisch aus Alkohol und Benzol oder Benzin erhaltenen Lecithins, German Patent 382912.

Bonotto, M. (1937a), Apparatus for treatment of soya beans and other material, US Patent 2,086,181, assigned to American Soya Products Corporation.

Bonotto, M. (1937b), Process for removing solvent from solvent-treated material, US Patent 2,086,180, assigned to American Soya Products Corporation.

Boskou, D. (1996), History and characteristics of the olive tree, in *Olive Oil, Chemistry and Technology*, Boskou, D., Ed., AOCS Press, Champaign IL, 1–11.

Bozan, B. and Temelli, F. (2002), Supercritical CO_2 extraction of flax seed, *J. Am. Oil Chem. Soc.*, **79**, 231–235.

Bredeson, D.K. (1977), Mechanical Pressing, *J. Am. Oil Chem. Soc.*, **54**, 489A–490A.

Buhr, N. (1990), Mechanical pressing, in *Edible Fats and Oils Processing: Basic Principles and Modern Practices*, AOCS Press, Campaign IL, 43–48.

Bull, W.C. and Hopper, T.H. (1941), The composition and yield of crude lipids obtained from soybeans by successive solvent extractions, *Oil & Soap*, **18**, 219–222.

Bulley, N.R. et al. (1984), Supercritical fluid extraction of vegetable oil seeds, *J. Am. Oil Chem. Soc.*, **61**, 1362–1365.

Canat, J. (1979), Matériels et technologies des graines oléagineuses tropicales, *Rev. Franç. Corps Gras*, **26**, 63–72.

Carlson, K.D. et al. (1985), Processing of *Crambe abyssinica* seed in commercial extraction facilities, *J. Am. Oil Chem. Soc.*, **62**, 897–905.

Carr, R.A. (1990), Rapeseed/Canola, in *Edible Oils and Fats Processing: Basic Principles and Modern Practices*, Erickson, D.R., Ed., AOCS Press, Campaign IL, 289–298.

Carr, R.A. (1993), Oilseed harvesting, storage and transportation, in *Proceedings of the World Conference on Oilseed Technology and Utilization*, Applewhite, T.H., Ed., AOCS Press, Champaign, IL, 118–125.

Chapman, D.M. (1995), Fatty acids can cause the formation of fixed colors during deodorization, *Paper presented at the 86th AOCS Annual Meeting & Expo, San Antonio, TX*.

Cheng, V. (2004), Frying leap pays off, *Oil Mill Gazetteer*, **110**, 16–17.

Coats, H.B. and Karnofsky, G.B. (1950), Solvent extraction II. The soaking theory of extraction, *J. Am. Oil Chem. Soc.*, **27**, 51–53.

Cuperus, F.P. et al. (1996), Supercritical carbon dioxide extraction of *Dimorphotheca pluvialis* oil seeds, *J. Am. Oil Chem. Soc.*, **73**, 1675–1679.

D'Aquin, E.L. et al. (1961), Improved extraction efficiency by double-soak filtration-extraction process, *J. Am. Oil Chem. Soc.*, **38**, 258–261.

Dahlén, J.Å.H. (1996), A way to produce crude rapeseed oil with very low content of non-hydratable phosphatides, *Poster presented at the World Conference and Exhibition on Oilseeds and Edible Oils Processing,* October 6–10, 1996, Istanbul.

Dahlén, J.Å.H. (1998), The Super Expro process, *Oléagineux Corps Gras Lipides/OCL*, **5**, 360–363.

De, B.K. and Bhattacharyya, D.K. (1998), Physical refining of rice bran oil in relation to degumming and dewaxing, *J. Am. Oil Chem. Soc.*, **75**, 1683–1686.

De Leonardis, A. et al. (2005), A first pilot study to produce a food antioxidant from sunflowerseed shells *(Helianthus annuus)*, *Eur. J. Lipid Sci. Technol.*, **107**, 220–227.

de Lucas, A. et al. (2002), Influence of operating variables on yield and quality parameters of olive husk oil extracted with supercritical carbon dioxide, *J. Am. Oil Chem. Soc.*, **79**, 237–243.

Depmer, W. (1951), Überblick über die kontinuierlichen Extraktionsanlagen, *Fette u. Seifen*, **53**, 699–703.

De Smet, J.A. (1954), Solvent extraction apparatus with automatic controls, US Patent 2,684,288, assigned to Extraction Continue De Smet.

De Smet, J.A. (1975), Cinquante ans d'extraction par solvant, *Rev. Franç. Corps Gras*, **22**, 61–68.

Desnuelle, P. et al. (1951), Traitement thermique des graines oléagineuses et teneur en lécithine des huiles, *Oléagineux*, **6**, 264–267.

Di Giovacchino, L. (1996), Olive harvesting and olive oil extraction, in *Olive Oil, Chemistry and Technology*, Boskou, D., Ed., AOCS Press, Champaign IL, 12–51.

Di Giovacchino, L. (1997), From olive harvesting to virgin olive oil, *Oléagineux Corps Gras Lipides/OCL*, **4**, 359–362.

Di Giovacchino, L. et al. (2002), Influence of olive oil processing on virgin olive oil quality, *Eur. J. Lipid Sci. Technol.*, **104**, 587–601.

Dijkstra, A.J. (2000), Edible oils in Europe, *inform*, **11**, 386–394.

Dijkstra, A.J. (2003), My visit to a walnut oil mill, *inform*, **14**, 755–756.

Dunning, J.W. (1954), Processing of castor beans, *J. Am. Oil Chem. Soc.*, **31**, 290–291.

Eggers, R. (1985), High pressure extraction of oil seed, *J. Am. Oil Chem. Soc.*, **62**, 1222–1230.

Eggers, R. (1994), Extraktion von Fettrohstoffen mit überkritischem CO_2, *Fat Sci. Technol.*, **96**, 513–518.

Eggers, R. et al. (1986), Zum Stand der kontinuierlichen Extraktion von Feststoffen mit überkritischen Gasen, *Fette Seifen Anstrichm.*, **88**, 344–351.

Eggers, R. and Stein, W. (1984), Hochdruck-Extraktion von Ölsaaten, *Fette Seifen Anstrichm.*, **86**, 10–16.

Fattori, M. et al. (1988), Carbon dioxide extraction of canola seed: oil solubility and effect of seed treatment, *J. Am. Oil Chem. Soc.*, **65**, 968–974.

Favati, F. et al. (1991), Supercritical carbon dioxide extraction of evening primrose oil, *J. Am. Oil Chem. Soc.*, **68**, 422–427.

Fehsenfeld, B. et al. (1979), Verfahrenstechnische Grundlagen und mathematische Modellierung der Perkolationsextraktion, *Fette Seifen Anstrichm.*, **81**, 469–473.

Fetzer, W. (1983), Head- and tail-end dehulling of soybeans, *J. Am. Oil Chem. Soc.*, **60**, 203–205.

Fils, J.-M. (2000), The production of oils, in *Edible Oil Processing*, Hamm, W. and Hamilton, R. J., Eds., Sheffield Academic Press, Sheffield, 47–78.

Finch, H.E. and Trapanese, S.P. (1983), Olive oil recovery, US Patent 4,370,274, assigned to FPS Development Partnership.

Finch, H.E. and Trapanese, S.P. (1985), Olive oil recovery, US Patent 4,522,119, assigned to FPS Development Partnership.

Fitch Haumann, B. (1997), Mechanical extraction. Capitalizing on solvent-free processing, *inform*, **8**, 165–174.

Florin, G. and Bartesch, H.R. (1983), Processing of oilseeds using fluidbed technology, *J. Am. Oil Chem. Soc.*, **60**, 193–197.

Fonade, E. (1979), L'extraction industrielle de l'huile de palme, *Rev. Franç. Corps Gras*, **26**, 59–62.

Friedrich, J.P. et al. (1982), Petroleum-free extraction of oil from soybeans with supercritical CO_2, *J. Am. Oil Chem. Soc.*, **59**, 288–292.

Friedrich, J.P. (1984), Supercritical CO_2 extraction of lipids from lipid-containing materials, US Patent 4,466,923, assigned to the USA as represented by the Secretary of Agriculture.

Goss, W.H. (1946), Solvent extraction of oilseeds, *Oil & Soap*, **23**, 348–354.

Graalmann, M. (1990), Laurics (coconut/palm kernel), in *Edible Fats and Oils Processing: Basic Principles and Modern Practices*, Erickson, D.R., Ed., AOCS Press, Champaign IL, 270–274.

Grimsby, F.N. (1984a), Oilseed extraction process, US Patent 4,456,557, assigned to Shell Oil Company.

Grimsby, F.N. (1984b), Oilseed extraction process, US Patent 4,456,556, assigned to Shell Oil Company.

Grimsby, F.N. (1984c), Oilseed extraction process, US Patent 4,457,869, assigned to Shell Oil Company.

Hagenmaier, R. et al. (1972), Critical unit operations of the aqueous processing of fresh coconuts, *J. Am. Oil Chem. Soc.*, **49**, 178–181.

Hamm, W. (2000), Bulk movement of edible oils, in *Edible Oil Processing*, Hamm, W. and Hamilton, R.J., Eds., Sheffield Academic Press, Sheffield, 34–46.

Hansen, M.E.Z. et al. (2001), Canola and Chilean hazelnut products obtained by enzyme-assisted cold-pressed oil extraction, in *Proceedings of the World Conference on Oilseed Processing and Utilization*, Wilson, R.F., Ed., AOCS Press, Champaign IL, 203–209.

Harris, W.D. et al. (1947), Isopropanol as a solvent for extraction of cottonseed oil. I. Preliminary investigations, *J. Am. Oil Chem. Soc.*, **24**, 370–375.

Harris, W.D. and Hayward, J.W. (1950), Isopropanol as a solvent for the extraction of cottonseed oil. III. The use of recycling to effect solvent economy, *J. Am. Oil Chem. Soc.*, **27**, 273–275.

Harris, W.D. et al. (1949), Isopropanol as a solvent for extraction of cottonseed oil. II. Separation of purified oil from miscella, *J. Am. Oil Chem. Soc.*, **26**, 719–723.

Hensarling, T.P. and Jacks, T.J. (1983), Solvent extraction of lipids from soybeans with acidic hexane, *J. Am. Oil Chem. Soc.*, **60**, 783–784.

Hensarling, T.P. et al. (1976), Use of acidic hexane to process oil seeds for protein and oil, US Patent 3,941,764, assigned to USA, Secretary of Agriculture.

Hildebrandt, K. (1934), Apparatus for the continuous extraction of oils and fats, US Patent 1,961,420.

Hiscocks, E.S. and Raymond, W.D. (1964), Some problems in improving tropical materials utilized by the United Kingdom oil milling industry, *J. Am. Oil Chem. Soc.*, **41**, 224–228.

Holló, J. (1987), Trends in der Aufbereitung von Ölsamen, *Fat Sci. Technol.*, **89**, 2–7.

Hron Sr., R.J. et al. (1992), Acidic ethanol extraction of cottonseed, *J. Am. Oil Chem. Soc.*, **69**, 951–953.

Hron Sr., R.J. and Koltun, S.P. (1984), An aqueous ethanol extraction process for cottonseed oil, *J. Am. Oil Chem. Soc.*, **61**, 1457–1460.

Hron Sr., R.J. et al. (1994), Ethanol extraction of oil, gossypol and aflatoxin from cottonseed, *J. Am. Oil Chem. Soc.*, **71**, 417–421.

Hunt Moore, N. (1983), Oilseed handling and preparation prior to solvent extraction, *J. Am. Oil Chem. Soc.*, **60**, 189–192.

Hutchins, R.P. (1977), Continuous solvent extraction, *J. Am. Oil Chem. Soc.*, **54**, 202A–205A.

Hutchins, R.P. and Williamson, W.H. (1949), Production of cottonseed meal, US Patent 2,484,831, assigned to The Procter & Gamble Company.

Jáky, M. et al. (1960), Über die dielektrische Vorbehandlung bei der Verarbeitung von Sonnenblumensaat, *Fette Seifen Anstrichm.*, **62**, 483–486.

Jammes, R. and Nugues, M. (1961), Nouvelle huile comestible et son procédé de fabrication et décortiqueuse pour la mise

en oevre de celui-ci, French Patent 1.257.820, assigned to Centre Biotechnique.

Johnson, L.A. (2000), Recovery of fats and oils from plant and animal sources, in *Introduction to Fats and Oils Technology*, O'Brien, R.D., Farr, W.E., and Wan, P.J., Eds., AOCS Press, Champaign, IL, 108–135.

Johnson, L.A. et al. (1986), Pilot plant studies on extracting cottonseed with methylene chloride, *J. Am. Oil Chem. Soc.*, **63**, 647–652.

Johnson, L.A. and Lusas, E.W. (1983), Comparison of alternative solvents for oils extraction, *J. Am. Oil Chem. Soc.*, **60**, 229–242.

Kahane, E. et al. (1964), Application aux tourteaux d'arachide d'un nouveau procédé d'extraction des lipides, *Rev. Franç. Corps Gras*, **11**, 671–676.

Kapila, S. et al. (2004), Solvent and method for extraction of triglyceride rich oil, US Patent 6,800,318 B2, assigned to University of Missouri Board of Curators.

Karnofsky, G.B. (1949a), The mechanics of solvent extraction, *J. Am. Oil Chem. Soc.*, **26**, 570–574.

Karnofsky, G.B. (1949b), The theory of solvent extraction, *J. Am. Oil Chem. Soc.*, **26**, 564–569.

Karnofsky, G.B. (1950), L'extracteur "Rotocel" Blaw Knox, *Bull. mens. ITERG*, 465–468.

Karnofsky, G.B. (1979), Process for preparing a flour and the product obtained thereby, US Patent 4,144,229, assigned to Dravo Corporation.

Karnofsky, G.B. (1980), Process for preparing a protein concentrate and the product obtained thereby, US Patent 4,219,470, assigned to Dravo Corporation.

Karnofsky, G.B. (1985), Recovering water-immiscible solvents from oilseeds, *J. Am. Oil Chem. Soc.*, **62**, 693–696.

Karnofsky, G.B. (1986), Design of oilseed extractors I. Oil extraction, *J. Am. Oil Chem. Soc.*, **63**, 1011–1014.

Karnofsky, G.B. (1987), Design of oilseed extractors I. Oil extraction (Supplement), *J. Am. Oil Chem. Soc.*, **64**, 1533–1536.

Kattenberg, H.R. (1996), Functional properties of cocoa butter in relation to the origin of the cocoa beans and processing, *Paper presented at the SCI Seminar Production and Application of Confectionary Fats,* 15–16 Oct, London 1996, 1–11.

Kaufmann, H.P. and Orde, H.O. vom (1955), Über die Extraktion von Ölsaaten mit aliphatischen Fluor-Chlor-Kohlenwasserstoffen I. Die Extraktion von Soja- und Baumwollsaat mit 1,2,2-Trifluor-trichlor-äthan, *Fette Seifen Anstrichm.*, **57**, 399–401.

Kemper, T.G. (1995), Guidelines for pellet/flake ratios in soy crushing, *inform*, **6**, 1231–1236.

Kemper, T.G. (2000), Innovations in meal desolventizing, *inform*, **11**, 967–972.

Kemper, T.G. and Farmer, R.D. (1999), Desolventizer, US Patent 5,992,050, assigned to The French Oil Mill Machinery Company.

Kennedy, A.B. (1927), Leaching method and apparatus, US Patent 1,628,787.

Kester, E.B. and Van Atta, G.R. (1942), Minor oil-producing crops of the United States, *Oil & Soap*, **19**, 119–125.

Kim, C.J. et al. (1987), Comparison of solvent extraction characteristics of rice bran pretreated by hot air drying, steam cooking and extrusion, *J. Am. Oil Chem. Soc.*, **64**, 514–416.

Kim, S.K. et al. (1985), Effect of caustic refining, solvent refining and steam refining on the deacidification and color of rice bran oil, *J. Am. Oil Chem. Soc.*, **62**, 1492–1495.

King, J.W. and List, G.R. (1996), *Supercritical Fluid Technology on Oil and Lipid Chemistry*, AOCS Press, Champaign IL.

King, W.H. and Frampton, V.L. (1960), Production of cottonseed meal of improved quality, US Patent 2,950,198, assigned to USA, Secretary of Agriculture.

King, W.H. et al. (1961), Properties of cottonseed meals prepared with acetone-petroleum ether-water azeotrope, *J. Am. Oil Chem. Soc.*, **38**, 19–21.

Kock, M. (1978), Procédé de préparation d'huile alimentaire à partir d'huile de soja brute, French Patent 2 391 272, assigned to Akzo N.V.

Kock, M. (1981a), Practical experience with a process for enzyme deactivation of soybean flakes before extraction and its influence on oil quality, in *Proceedings of the second A.S.A. symposium on soybean processing. Refining of soy oil, caustic or physical?*, Leysen, R.J.F., Ed., American Soybean Association, Brussels, Belgium.

Kock, M. (1981b), Production of an edible oil from crude soy oil, US Patent 4,255,346, assigned to Akzo N.V.

Köseoğlu, S.S. et al. (2004), Degumming of edible oils by membrane filtration, US Patent 6,797,172 B2, assigned to Texas A&M University System.

Köseoğlu, S.S. et al. (1990), Membrane processing of crude vegetable oils: pilot plant scale removal of solvent from oil miscella, *J. Am. Oil Chem. Soc.*, **67** , 315–322.

Kővári, K. (2004), Recent developments, new trends in seed crushing and oil refining, *Chevreul Award Address presented at the 3rd EuroFedLipid Congress, Edinburgh*, page 4 in the abstracts.

Kruse, N.F. and Soldner, W.L. (1941), Process of making soybean products, US Patent 2,260,254, assigned to Central Soya Company.

Lanzani, A. et al. (1984), Technologia integrale di estrazione della sostanza grassa e preparazione di farine dal seme di karite, *Riv. Ital. Sostanze Grasse*, **61**, 375–383.

Latta, S. (2000), Oils to beat the bland, *inform*, **11**, 305–312.

Law, K.S. and Thiagarajan, T. (1990), Palm oil — edible oil of tomorrow, in *Edible Fats and Oils Processing: Basic Principles and Modern Practices*, Erickson, D.R., Ed., AOCS Press, Champaign IL, 260–269.

Lee, T.-C. (1991), Process for the recovery of edible oil from cereal products, US Patent 5,047,254.

Leibovitz, Z. and Ruckenstein, C. (1983), Our experiences in processing maize (corn) germ oil, *J. Am. Oil Chem. Soc.*, **60**, 395–399.

Letan, A. and Yaron, A. (1972), Some practical aspects connected with the presence of calcium in soybeans, *J. Am. Oil Chem. Soc.*, **49**, 702.

Levine, A.A. and Dent, R.J. (1943), Apparatus for extraction, US Patent 2,321,923, assigned to E.I. du Pont de Nemours and Company.

Lloyd, B.J. et al. (2000), Effects of commercial processing on antioxidants in rice bran, *Cereal Chem.*, **77**, 551–555.

Loeb, J.R. et al. (1949), Rice bran oil, IV. Storage of the bran as it affects hydrolysis of the oil, *J. Am. Oil Chem. Soc.*, **26**, 738–743.

Loury, M. and Feng, H. (1958), Étude comparative de divers solvants pour l'extraction des graines de colza et de lin, *Rev. Franç. Corps Gras*, **5**, 83–90.

Lovett, P.N. (2005), Shea butter industry expanding in West Africa, *inform*, **16**, 273–275.

Luchetti, F. (1997), Situation et perspectives du marché international de l'huile d'olives, *Oléagineux Corps Gras Lipides/OCL*, **4**, 336–337.

Lusas, E.W. and Watkins, L.R. (1988), Oilseeds: extrusion for solvent extraction, *J. Am. Oil Chem. Soc.*, **65**, 1109–1114.

Lusas, E.W. et al. (1997), Final report: IPA as an extraction solvent, *inform*, **8**, 290–306.

Lusas, E.W. et al. (1990), Separation of fats and oils by solvent extraction: non-traditional methods, in *Edible Fats and Oils Processing: Basic Principles and Modern Practices*, Erickson, D.R., Ed., AOCS Press, Champaign IL, 56–78.

Mangold, H.K. (1982), Extraction with supercritical fluids: A progress report from Germany, *J. Am. Oil Chem. Soc.*, **59**, 673A–674A.

Marchand, D.E. (1984), Utilisation des machines type "Expander" en trituration: Intérêt et perspectives, *Rev. Franç. Corps Gras*, **31**, 13–17.

Marteau, R. and Marteau-Danvin, A. (1975), Process for the production of karite nut butter, US Patent 3,880,899.

Martinez Suarez, J.M. and Alba Mendoza, J. (1986), Olive oil processing and related aspects. Extraction and refining: general aspects, in *World Conference on Emerging Technologies in the Fats and Oils Industry*, Baldwin, A.R., Ed., AOCS, Champaign IL, 299–305.

Maza, A. (2001), Process for recovery of corn oil from corn germ, US Patent 6,201,142, assigned to Bestfoods.

Milligan, E.D. (1976), Survey of current solvent extraction equipment, *J. Am. Oil Chem. Soc.*, **53**, 286–290.

Mishra, A. et al. (1988), Factors affecting refining losses in rice (*Oryza sativa L.*) bran oil, *J. Am. Oil Chem. Soc.*, **65**, 1605–1609.

Naczk, M. et al. (1988), A new extraction process for rapeseed: a review, *J. Am. Oil Chem. Soc.*, **65**, 3–6.

Nash, A.M. and Frankel, E.N. (1986), Limited extraction of soybeans with hexane, *J. Am. Oil Chem. Soc.*, **63**, 244–246.

Nieh, C.D. and Snyder, H.E. (1991), Solvent extraction of oil from soybean flour I - Extraction rate, a countercurrent system, and oil quality, *J. Am. Oil Chem. Soc.*, **68**, 246–249.

O'Brien, R.D. and Wan, P.J. (2001), Cottonseed oil: processing and utilization, in *Proceedings of the World Conference on Oilseed Processing and Utilization*, Wilson, R.F., Ed., AOCS Press, Champaign IL, 90–144.

Oliveira, R. et al. (2002), Characterization and supercritical carbon dioxide extraction of walnut oil, *J. Am. Oil Chem. Soc.*, **79**, 225–230.

Oo, K.C. (1981), The absence of lipase activity in mesocarp of the palm fruit, *Oléagineux*, **36**, 613–616.

Orthoefer, F.T. (2001), Rice bran oil: composition, production, nutrition and utilization, in *Proceedings of the World Conference on Oilseed Processing and Utilization*, Wilson, R.F., Ed., AOCS, Campaign Il, 151–158.

Othmer, D.F. and Jaatinen, W.A. (1959), Étude du mécanisme de l'extraction du soja par différents solvants, *Rev. Franç. Corps Gras*, **6**, 408–415, see also *Ind. Eng. Chem.,* **51,** 543–546, 1959.

Padley, F.B. (1997), Chocolate and confectionary fats, in *Lipid Technologies and Applications*, Gunstone, F.D. and Padley, F.B., Eds., Marcel Dekker, New York, 391–432.

Padley, F.B. et al. Eds. (1994), Occurrence and characteristics of oils and fats, 3.3 Major vegetable fats, in *The Lipid Handbook*, 2nd ed., Chapman and Hall, London, 57–60.

Pagliero, C. et al. (2001), Degumming of crude soybean oil by ultrafiltration using polymeric membranes, *J. Am. Oil Chem. Soc.*, **78**, 793–796.

Panfili, G. et al. (2003), Extraction of wheat germ oil by supercritical CO_2: oil and defatted cake characteristics, *J. Am. Oil Chem. Soc.*, **80**, 157–161.

Patricelli, A. et al. (1979), Fattori che influenzano l'estrazione dei lipidi da semi decorticati di girasole, *Riv. Ital. Sostanze Grasse*, **56**, 136–141.

Patumi, M. et al. (2003), Effect of fruit stoning on olive oil quality, *J. Am. Oil Chem. Soc.*, **80**, 249–255.

Pavlik, R.P. and Kemper, T.G. (1990), Benefits in utilizing the extruder, *inform*, **1**, 200–205.

Penk, G. (1981), Sojaflocken-Konditionierung. Verfahrenstechnik und wirtschaftliche Aspekte, *Fette Seifen Anstrichm.*, **83**, 558–563.

Perkins, E.G. (1995), Composition of soybeans and soybean products, in *Practical Handbook of Soybean Processing and Utilization*, Erickson, D.R., Ed., AOCS Press and United Soybean Board, Champaign, IL and St. Louis, MO, 9–28.

Pieralisi, G. (1986), Extraction technology, in *World Conference on Emerging Technologies in the Fats and Oils Industry*, Baldwin, A.R., Ed., AOCS, Champaign IL, 306–307.

Pons Jr., W.A. and Eaves, P.H. (1967), Aqueous acetone extraction of cottonseed, *J. Am. Oil Chem. Soc.*, **44**, 460–464.

Pons Jr., W.A., and Eaves, P.H. (1971), Aqueous acetone extraction of cottonseed, US Patent 3,557,168, assigned to USA, Secretary of Agriculture.

Prabhakar, J.V. and Venkatesh, K.V.L. (1986), A simple method for stabilization of rice bran, *J. Am. Oil Chem. Soc.*, **63**, 644–646.

Rac, M. (1967), Influence de la structure cellulaire sur quelques propriétés technologiques de certaines matières premières oléagineuses, *Rev. Franç. Corps Gras*, **14**, 223–228.

Ragminatha Rao, Y.K. (1954), *l'Extraction par l'alcool de l'huile de coton*, *Bull. mens. ITERG*, 27–28.

Ranalli, A. (1997), Processing aids, *Oléagineux Corps Gras Lipides/OCL*, **4**, 362.

Rauer, P. (1942), Process of preparing protein material, US Patent 2,278,670.

Rayner, E.T. and Dollear, F.G. (1968), Removal of aflatoxins from oilseed meals by extraction with aqueous isopropanol, *J. Am. Oil Chem. Soc.*, **45**, 622–624.

Reddi, P.B.V. et al. (1948), Rice bran oil I. Oil obtained by solvent extraction, *J. Am. Oil Chem. Soc.*, **25**, 206–213.

Reverchon, E. and Sesti Osséo, L. (1994), Comparison of processes for the supercritical carbon dioxide extraction of oil from soybean seeds, *J. Am. Oil Chem. Soc.*, **71**, 1007–1012.

Rittner, H. (1984), Conditioning of oil-bearing materials for solvent extraction by extrusion, *J. Am. Oil Chem. Soc.*, **31**, 1200–1203.

Robinson, H.S. (1940), Solvent extraction apparatus, US Patent 2,225,799, assigned to The French Oil Mill Machinery Company.

Robinson, R.A. (1977), Meal pelleting and cooling, *J. Am. Oil Chem. Soc.*, **54**, 495A–499A.

Robledano, P. (1956), Method of extracting oil from mature fresh coconut meats, US Patent 2,742,487, assigned to The Coconut Processes Inc.

Rohne, G. (1966), Neuzeitliche Gewinnung von Traubenkernöl, *Fette Seifen Anstrichm.*, **68**, 805–808.

Roselius, W. et al. (1974), Method for the production of caffeine-free coffee extract, US Patent 3,843,824, assigned to HAG Aktiengesellschaft.

Roselius, W. et al. (1975), Methods of producing cocoa butter, US Patent 3,923,847, assigned to Studiengesellschaft Kohle m.b.H.

Rubin, L.J. et al. (1984), Solvent extraction of oil-bearing seeds, US Patent 4,460,504, assigned to University of Toronto Innovations Foundation.

Ruckenstein, C. (1981), La technologie moderne de l'huile brute de tournesol, *Rev. Franç. Corps Gras*, **28**, 161–164.

Sanders, T.H. (2001), Individual oils: peanut oil, in *Proceedings of the World Conference on Oilseed Processing and Utilization*, Wilson, R.F., Ed., AOCS Press, Champaign IL, 141–144.

Santos, R. (2004), Seed oil extraction using compressed carbon dioxide, *Lipid Techn.*, **16**, 153–157.

Sato, M. and Ito, C. (1932), Method of extracting fatty oil, US Patent 1,892,366, assigned to Minami Manshu Tetsudo Kabushiki Kaisha.

Sayre, R.N. et al. (1985), Extraction and refining of edible oil from extrusion stabilized rice bran, *J. Am. Oil Chem. Soc.*, **62**, 1040–1043.

Schneider, F.H. (1980), Zur extraktiven Lipid-Freisetzung aus pflanzlichen Zellen, *Fette Seifen Anstrichm.*, **82**, 16–23.

Schneider, F.H. and Rütte, U. (1984), Zur Bindung des Resthexans in Raps-Extraktionsschroten, *Fette Seifen Anstrichm.*, **86**, 331–339.

Schneider, F.H. and Rütte, U. (1991), Resthexan in Raps-Schroten I: Zur Entstehung einer intrazellularen Miszella, *Fat Sci. Technol.*, **93**, 319–328.

Schumacher, H.O. (1978), Method and device for spontaneously distilling off secondary substances especially from oily liquids, US Patent 4,088,542.

Schumacher, H.O. (1983), Verfahren und Vorrichtung zum Entbenzinieren von bei der Extraktion mit organischen Lösemitteln anfallendem flockigem oder körnigem pflanzlichem Rückstandsmaterial, European Patent 0077 436.

Schumacher, H.O. (1984), Die Anwendung des Flash-Destillationsverfahren in der Miscella-Destillation von Extraktionsanlagen, *Fette Seifen Anstrichm.*, **86**, 236–237.

Schumacher, H.O. (1986a), Apparatus for desolventizing and drying solvent-containing residue meal, US Patent 4,622,760.

Schumacher, H.O. (1986b), System for continuous solid/liquid extraction, US Patent 4,617,177.

Shukla, V.K.S. (1995), Cocoa butter properties and quality, *Lipid Techn.*, **7**, 54–57.

Shukla, V.K.S. (1997), Chocolate — the chemistry of pleasure, *inform*, **8**, 152–162.

Singer, P.A. and Deobald, H.J. (1945), Method of solvent extraction of oil from seeds, US Patent 2,377,975, assigned to Allied Mills Inc.

Sipos, E. and Witte, N.H. (1961), The desolventizer-toaster process for soybean oil meal, *J. Am. Oil Chem. Soc.*, **38**, 11–19.

Smith, A.S. (1952), Evaluation of extraction rate measurements, *J. Am. Oil Chem. Soc.*, **29**, 421–425.

Smith, J.R. (1996), *Safflower*, AOCS Press, Champaign IL.

Snyder, H.E. et al. (1992), Process for efficiently extracting high quality oil from soybeans and other oil bearing seeds, US Patent 5,085,808, assigned to The Board of Trustees of the University of Arkansas.

Sullivan, D.A. (1985), Oilseed extraction process, US Patent 4,515,726, assigned to Shell Oil Company.

Sweeney, O.R. and Arnold, L.K. (1949), A new solvent extraction process for soybean oil, *J. Am. Oil Chem. Soc.*, **26**, 697–700.

Swetman, T. et al. (1999), Contamination of coconut oil by PAH, *inform*, **10**, 706–712.

Tan, Y.A. et al. (1985), A study of the cause of rapid color development of heated refined palm oil, *J. Am. Oil Chem. Soc.*, **62**, 999.

Tano-Debrah, K. and Ohta, Y. (1994), Enzyme-assisted aqueous extraction of fat from kernels of the shea tree, *Butyrospermum parkii*, *J. Am. Oil Chem. Soc.*, **71**, 979–983.

Tano-Debrah, K. and Ohta, Y. (1995), Enzyme-assisted aqueous extraction of shea fat: a rural approach, *J. Am. Oil Chem. Soc.*, **72**, 251–256.

Temple, S. and Sullivan, R.E. (1978), 1,1,2-Trichloro-1,2,2-trifluoroethane for oilseed extractions: demonstration of an viscosity effect, *J. Am. Oil Chem. Soc.*, **55**, 587–590.

Terrones, A. (2001), Safflower: a speciality oil in the world marker, in *Proceedings of the World Conference on Oilseed Processing and Utilization*, Wilson, R.F., Ed., AOCS Press, Champaign IL, 145–150.

Thobani, M. and Diosady, L.L. (1997a), Pilot-scale two-phase extraction of Canola, *J. Am. Oil Chem. Soc.*, **74**, 201–206.

Thobani, M. and Diosady, L.L. (1997b), Two-phase solvent extraction of Canola, *J. Am. Oil Chem. Soc.*, **74**, 207–214.

Turner, M.S. and Venne, L. (2003), Process for oil extraction, US Patent Application 2003/0134018 A1, assigned to Cargill Incorporated.

Tysinger, J.E. et al. (2004), Soybean oil process, US Patent 6,753,029 B1, assigned to Carolina Soy Products, Inc.

Ulrich, J.F. et al. (2002), High oil corn processing, US Patent 6,388,110 B1, assigned to Cargill Incorporated.

Veldsink, J.W. et al. (1999), Heat pretreatment of oilseeds: effect on oil quality, *Fett/Lipid*, **101**, 244–248.

Veldstra, J. and Klère, J. (1990), Sunflower seed oil, in *Edible Oils and Fats Processing: Basic Principles and Modern Practices*, Erickson, D.R., Ed., AOCS Press, Campaign IL, 284–288.

Vernois, G. (1958), Kontinuierliche Verfahren der Ölsaaten-Verarbeitung, *Fette Seifen Anstrichm.*, **60**, 1087–1092.

Villemur, P. and Dosba, F. (1997), *Oléiculture: évolution variétale et acquisition de la maîtrise des pratiques culturales*, *Oléagineux Corps Gras Lipides/OCL*, **4**, 351–355.

Voort, P. van der (1953), Das De Smet-Verfahren zur kontinuierlichen Lösungsmittel-Extraktion von Ölsaaten, *Fette u. Seifen*, **55**, 361–365.

Ward, J.A. (1976), Processing high oil content seeds in continuous screw presses, *J. Am. Oil Chem. Soc.*, **53**, 261–264.

Weber, K. (1972), Rückgewinnung von Lösungsmitteln aus der Abluft, *Fette Seifen Anstrichm.*, **74**, 605–608.
Wiese, K.L. and Snyder, H.E. (1987), Analysis of the oil extraction process in soybeans: a new continuous procedure, *J. Am. Oil Chem. Soc.*, **64**, 402–406.
Wilhelm, K.F. (1932), Verfahren zur Gewinnung von hellen säurearmen Ölen und Fetten aus Fischen, Fleisch und deren Abfällen sowie zur Herstellung von hellem Fisch- und Fleischmehl, German Patent 551 101.
Williams, M. and Baer, S. (1965), The expansion and extraction of rice bran, *J. Am. Oil Chem. Soc.*, **42**, 151–155.
Wilp, Ch. and Eggers, R. (1991), Hochdruckextraktion mit mehrstufiger fraktionierender Separation zur schonenden Gewinnung von Keimölen mit hochverdichtetem Kohlendioxid, *Fat Sci. Technol.*, **93**, 348–354.
Winters, A.M. (1918), Improvements in apparatus for extracting oil from seeds, beans, nuts, offal or the like, British Patent 120,156.
Witte, J.F. (1978), Process for extracting oils from oil containing raw materials, US Patent 4,122,104, assigned to Stork Amsterdam N.V.
Witte, N.H. (1995), Soybean meal processing and utilization, in *Practical Handbook of Soybean Processing and Utilization*, Erickson, D.R., Ed., AOCS Press and United Soybean Board, Champaign, IL and St. Louis, MO, 93–116.
Woerfel, J.B. (1995), Harvest, storage, handling and trading of soybeans, in *Practical Handbook of Soybean Processing and Utilization*, Erickson, D.R., Ed., AOCS Press and United Soybean Board, Champaign, IL and St. Louis, MO, 39–55.
Yoon, S.H. and Rhee, J.S. (1982), Composition of waxes from crude rice bran oil, *J. Am. Oil Chem. Soc.*, **59**, 561–563.
Youn, K.C. and Wilpers, D.J. (1981), Process for oilseed extraction with an isopropanol-based solvent, US Patent 4,298,540, assigned to Shell Oil Company.
Zhang, F. et al. (1994), Effects of expander process on the phospholipids in soybean oil, *J. Am. Oil Chem. Soc.*, **71**, 1145–1148.
Zosel, K. (1974), Procédé d'obtention de graisses et d'huiles à partir de produits végétaux et animaux, French Patent 2.211.528, assigned to Studiengesellschaft Kohle m.b.H.
Zosel, K. (1982), Production of fats and oils from vegetable and animal products, US Patent 4,331,695, assigned to Studiengesellschaft Kohle m.b.H.

3.4 Degumming of oils and fats

Crude oils and fats, and especially those obtained by solvent extraction, tend to be unpalatable and, therefore, need to be purified before they can be incorporated in food products and/or sold to consumers. In Europe, the various steps involved in this purification are referred to as "refining", but in the U.S., the term "refining" is limited to the removal of free fatty acids.

The entire series of steps that lead to a fully refined or RBD (refined, bleached and deodorised) oil, comprises:

- *Removal of gums.* As illustrated in Figure 3.8 (Dijkstra, 1993), there are many ways to get from crude oil to a fully refined oil, and the route to select depends upon factors to be discussed below. It, however, should be noted that all routes depicted in Figure 3.8 comprise one or more degumming steps because if gums are present, their removal is imperative for both subsequent processing and final product quality.

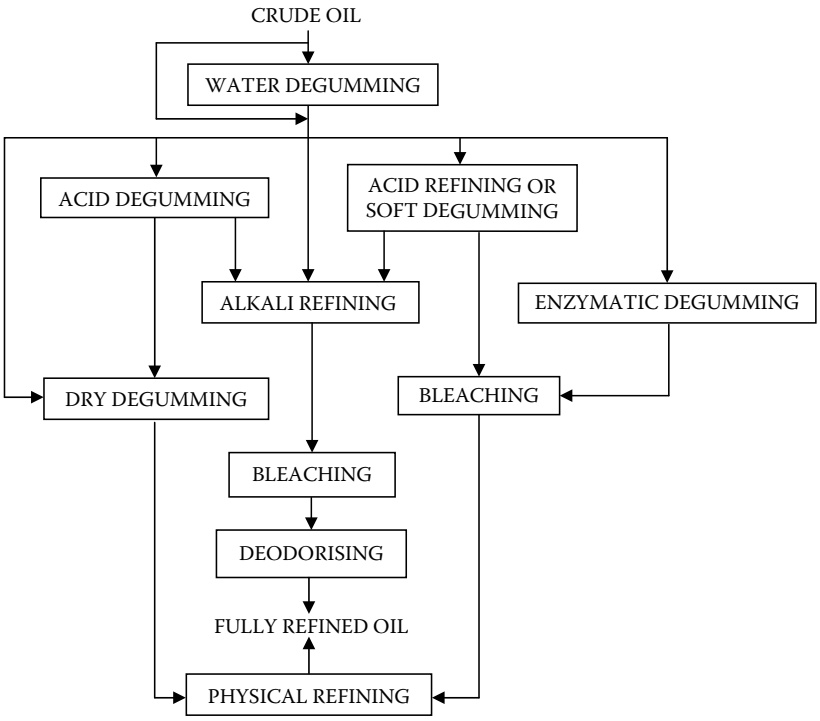


FIGURE 3.8 From crude to RBD.

- *Removal of free fatty acids.* They can be removed chemically by allowing these acids to react with an alkali to form soaps and separating the soaps from the oil; this is called "alkali refining' in Figure 3.8. They can also be removed physically by volatilisation in what is called "physical refining" in Figure 3.8. The physical refining process is also called "steam refining" since the volatilisation of the free fatty acids is achieved by a steam stripping process at reduced pressure and elevated temperature; it, therefore, must be preceded by an adsorptive treatment.
- *Removal of adsorbable compounds.* This step is commonly referred to as "bleaching," but it does more than just decrease the absorbance of the oil. When acid-activated bleaching earth is used, this adsorbent also catalyses the decomposition of peroxides and removes all kinds of polar compounds. Other adsorbents, such as activated carbon, remove some polyaromatic hydrocarbons from, for instance, crude coconut oil.
- *Removal of waxes.* Because of their high melting point, waxes tend to crystallise when present and at refrigerator temperature. This is regarded as unsightly, so they must also be removed by a process of fractional crystallisation called "winterisation" in Europe. In the U.S., "winterization" also refers to a fractionation process, so from now on the term "dewaxing" will be used for wax removal processes.
- *Removal of malodorous compounds.* These compounds are removed by steam stripping, the process that is also used in steam refining. However, the free fatty acids removed during steam refining are already present in the feedstock, whereas the malodorous compounds removed during deodorisation are also generated as a direct result of the deodorisation treatment and its elevated temperature in particular.

The above steps, being concerned with the removal of crude oil constituents, may have a somewhat negative connotation, but as will be discussed in the present and subsequent sections, these steps also aim to retain constituents, such as tocopherols, that are considered to contribute to oil quality, and avoid the formation of compounds, such as *trans* isomers, that some people currently consider to be less desirable.

3.4.1 Hydratability of phosphatides

Phosphatides (also referred to as phospholipids) are major components of cell and organelle membranes, blood lipoproteins, and lung surfactants. The interaction of phosphatides with water is critical to the formation and function of these biological complexes and, consequently, the hydration of phosphatides has been studied with these aspects in mind (McIntosh and Magid, 1993). However, the environment pertaining to these complexes is quite different from industrial degumming process conditions. Accordingly, the conclusions drawn for biological complexes have only limited applicability to degumming processes.

Nevertheless, hydration remains of prime importance. For this reason, a distinction is made (Nielsen, 1960) between hydratable phosphatides, which constitute the commercial product lecithin and nonhydratable phosphatides (NHP).[12] Nielsen also concluded that the NHP consist chiefly of a mixture of phosphatidic acids (PA) and lysophosphatidic acids (LPA). His colleague Hvolby (1971) showed that the atomic ratio of the sum of the calcium and magnesium contents of water degummed oil and its phosphorus content is always less than unity. He concluded that the NHP consist mainly of calcium and magnesium phosphatidates. Finally, Dijkstra (1993) showed that NHP can also contain up to 15% of phosphatidylethanolamine (PE) and, thus, explained why the aforementioned atomic ratio is nearly always significantly less than unity by assuming the PE to be free rather than a calcium salt as claimed in the literature (Segers, 1990).

It should, however, be noted that not all PA and PE present in crude oil are nonhydratable since the gums obtained by water degumming contain appreciable amounts of both PA and PE. Moreover, these gums also contain calcium and magnesium, which means that these ions can also be bound to hydratable phosphatides.

In general, and for some as yet unknown reason, water degumming removes relatively more magnesium than calcium (see also Guillaumin and Drouhin, 1966). Finally, it should be mentioned that PE has been reported to need the presence of another phosphatide, such as PC, to hydrate (Kanamoto et al., 1981), whereas Segers and Van de Sande (1990) state that hydrated PC can encapsulate PA, PE, and PI (phosphatidylinositol) up to 80% of its own weight. In summary:

- PC is always fully hydratable even when bound to an alkali metal ion, such as potassium, or an alkaline earth metal ion, such as calcium or magnesium. It may also entrain other phosphatides.
- PI is also always fully hydratable.

[12] American literature can refer to NHP as β-phosphatides (Cherry et al., 1981) or 'lipoid B' (Schmitt, 1963) and to hydratable phosphatides as α-phosphatides or lipoid A. But a distinction between β-lipoids and metal phospholipid complexes is also made (Wiedermann, 1981). This notation is not to be confused with earlier literature (Halden, 1940a and 1940b), which assumed that the phosphate group could be attached to either the α-position or the β-position of the glycerol moiety, or with literature (Carr, 1978), which added to the confusion by stating that phosphatides with the phosphoric acid attached to the centre position (Beta-Lipoids) are nonhydratable (Mattikow, 1959).

- PE is only hydratable in the presence of other hydratable phosphatides. After water degumming, some PE may be left in the oil and further water degumming does not remove this PE.
- PA is hydratable when ionised, but not when neutral or as a calcium or magnesium salt.

The presence of phosphatidic acid (PA) in the NHP may indicate that phosphatides, such as phosphatidylcholine (PC), PE, and phosphatidylinositol (PI), have been hydrolysed and the most likely catalysts for this hydrolysis are the various phospholipase enzymes that have been illustrated in Figure 4.23 in Section 4.5. As shown in this figure, phospholipase D would lead to the formation of PA and it is quite logical that enzyme inactivation was felt to be the cause of the decrease in NHP when soya beans were treated with moist heat in the Alcon® process (Kock, 1978; Kock, 1981) or if soya bean flakes were extracted at a lower temperature (Kock, 1983).

However, as pointed out by Penk (1981), water degumming Alcon oil leads to an increased lecithin yield, which, according to Kock (1983), has a slightly higher PA-content. This implies that before water degumming, Alcon oil contains more PA than normal crude soya bean oil,[13] which by implication does not support the enzyme inactivation mechanism. Besides, since some PA is present in lecithin, it can apparently be removed by water degumming. Consequently, the cause of the formation of NHP should not be sought in PA formation, but rather in the formation of calcium and magnesium salts of this phosphatide.

In this context, the work by List et al. (1990; 1992; 1993) and by Rade et al. (1995) deserves to be mentioned. Although it addresses the effects of process parameters on phospholipase D activity rather than on the formation of calcium and magnesium salts of PA, it nevertheless reports (List et al., 1990) that "the differences in the degumming of oils … cannot be explained entirely by the phospholipase D activity." It also notes that "heat treatment … results in more complete extraction of phosphatides." Subsequently, List et al. (1992) noted that moisture content promoted NHP formation and in their final paper (List et al., 1993), they conclude that the adverse effects of storage conditions on NHP formation cannot be overcome by inactivation of phospholipase D prior to solvent extraction of the flakes.

Apparently, phospholipase D activity is not the overriding factor, so NHP formation and phosphatide extractability could well be more important. Soya beans already contain enough PA to form substantial amounts of NHP and changes in phospholipase D concentration have little effect on PA content. So if storage, moisture content, and temperature affect the amount of NHP observed in the oil, it may well be because these parameters affect NHP formation, the reaction between PA and calcium and magnesium ions. Copper present in crude soya bean oil is concentrated in the lecithin on water degumming and occurs mainly in the phosphatide fraction that is insoluble in chloroform/ethanol (Krog and Tolboe, 1963).

On the other hand, various (heat) treatments may affect the extractability of phosphatides (Desnuelle et al., 1951) and it could well be that different phosphatides are affected differently. Alcon® oil, for instance, contains more phosphatides but less NHP than normal oil, passing soya bean flakes through an expander has a similar effect upon phosphatide extractability (Zhang et al., 1994), and the SuperExpro® treatment of rapeseed also decreases the amounts of NHP in the resulting rapeseed oil while increasing the total phosphatide content (Dahlén, 1996; 1998). These treatments could increase the extractability of hydratable phosphatides and decrease the extractability of NHP, but there are as yet no reports on this approach in the literature.

The hydratability of phosphatides also depends upon their electric charge. Table 3.5 lists the ionisation constants of the phosphatides concerned (see also Abramson et al., 1964) and also those of two commonly used degumming acids. In Table 3.6 the charges of the various phosphatides are given in function of the pH. Table 3.6 shows that PC and PE carry a positive charge at low pH, then loose a proton from the phosphate group when the pH is increased to become a zwitterion. When the pH is raised even further, the PE can loose a further proton from its amino group, but choline, having a quaternary amino group, remains a zwitterion.

Although PC and PE have similar charges at pH values below 9, their behaviour is not the same in that PC is always fully hydratable and PE may need some "assistance." This difference in behaviour may be linked to the fact that the opposite charges in PC are quite distant from each other because of the bulky methyl groups

TABLE 3.5 Logarithmic dissociation constants of acid groups

Phosphatide	Dissociating Group	pK_a
Phosphatidylcholine (PC)	Phosphate	≤3.5
Phosphatidylethanolamine	Phosphate	≤3.5
(PE)	Amine	9.8–11.5
Phoshatidylinositol (PI)	Phosphate	≤3.5
Phosphatidic acid (PA)	Undissociated acid	2.7–3.8
	Once dissociated acid	7.9–8.7
Phosphoric acid	Neutral acid	2.16
	Once dissociated acid	7.21
	Twice dissociated acid	12.32
Citric acid	Neutral acid	3.14
	Once dissociated acid	4.77
	Twice dissociated acid	6.39

Source: Tatulian, S. (1993), in *Phospholipids Handbook*, Cevc, G., Ed., Marcel Dekker, Inc., New York, 511–552.

[13] Of course the PA content of the NHP has to be taken into account as well, but even then, there is still more PA in non-degummed Alcon® oil than in nondegummed normal oil.

TABLE 3.6 Charges of phosphatides in function of pH

pH	PC	PE	PI	PA	CaPA
2	+	+	0	0	0
3	(+)	(+)	(0)	(0)	0
4	(±)	(±)	(–)	(–)	0
5–7	±	±	–	–	0
8–9	±	±	–	2–	0
>10	±	–	–	2–	0

+: Nearly all moieties have a positive charge.
(+): Most moieties have a positive charge.
±: Nearly all moieties are zwitterions.
(±): Most moieties are zwitterions.
0: Hardly any moieties carry a charge.
(–): Most moieties have a negative charge.
– : Nearly all moieties carry a single negative charge.
2–: Nearly all moieties carry a double negative charge.

attached to the choline amino group, whereas in PE, these charges can get quite close together to form an internal salt.

Differences in hydratability of the various phosphatides have also been linked to differences in their rate of hydration with a high rate for PC and a low rate for the calcium salts of PE and PA being reported (Sen Gupta, 1986). How these rates were determined is not clear, nor whether entrainment was taken into account. Differences in the rate of hydration have also been observed by Pan et al. (2000).

3.4.2 Degumming processes

The various industrial degumming processes have different aims. An oil miller selling crude oil to refiners for a set price will aim at the maximum residual phosphorus content allowed by his crude oil sales contract and at minimising oil entrainment in the gums; he may even recuperate oil from his gums (Kellens and De Greyt, 2006). A miller with a market for lecithin should aim at ensuring a good and constant lecithin quality, whereas a refiner may aim at the total removal of all phosphatides enabling him to refine his degummed oil physically (cf. Figure 3.8).

Furthermore, the various oils and fats to be degummed vary widely in gum content and gum properties and finally, the means of gum disposal available, what equipment is needed and/or available, and the cost of auxiliaries also influence the choice of the most appropriate degumming process. Accordingly, the discussion of the various degumming processes will deal with these aspects.

3.4.2.1 Water degumming

According to Pardun (1983), the first water degumming patent was granted to Bollmann (1923). His process was quite simple and involves the addition of live steam to the miscella evaporation residue for a period of 15 minutes. This causes lecithin to precipitate so that it can be removed. As illustrated by Figure 3.9, the process has not changed much since.

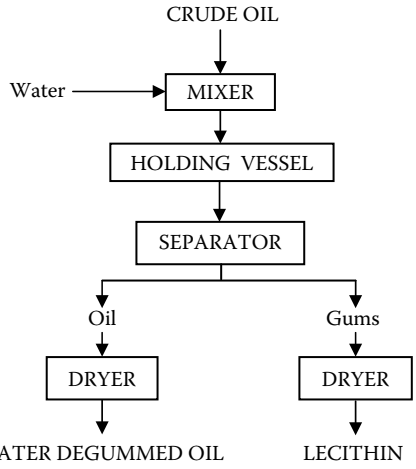


FIGURE 3.9 Water degumming process.

The water degumming process has two different aims: to produce lecithin from crude soya bean oil and to control the phosphorus content of crude oils, such as sunflower seed oil and rapeseed oil at just below 200 ppm. In principle, it can also be used to ensure that crude vegetable oils do not throw a deposit during transport and storage and, thus, avoid the formation of tank foots and their costly removal.

The equipment used in the water degumming process comprises a mixer, a holding vessel, and a separator. The mixer is apparently not very critical since the literature mentions not only high-speed mixers or thorough mixing (Whittecar, 1955; Bernardini, 1973; Van Nieuwenhuyzen, 1976; Pardun, 1983; Erickson, 1995), but also mixing by circulation (Raffaetá, 1965) or even in the tank (Carr, 1976). On a laboratory scale, the phosphatides can also be brought into contact with the degumming water by the use of ultrasonics (Moulton and Mounts, 1990). Holding times mentioned in the literature vary from a short 3 to 5 minutes (Anon., 1967) to periods of 30 to 60 minutes (Carr, 1976; Erickson, 1995). For the separation of the gums, centrifugal separators are invariably used since Ayres Jr. and Clark claimed their suitability in 1929.

The other process variables concerning the water degumming process are the amount of water to be used and the temperature. Again, there is some variation in what the literature recommends and the amount of water can vary between 1 to 2% by weight of crude oil (Andersen, 1962; Myers, 1965; Schumacher, 1972; Carr, 1976; List et al., 1978; Segers, 1990), but logically enough, the amount has also been related to the amount of gums to be removed; an equal amount is recommended (Braae, 1976). The literature also lists all kind of additives such as various salts but also products like sugars, starch, milk, amino acids, formaldehyde, urea, and many others (Diosady et al., 1982), but since these products will end up in the gums and, after drying in the lecithin, they affect lecithin properties; they also cost money and, therefore, are not used on an industrial scale.

Dissolving some phosphates in the degumming water (Thomke, 1932) could make sense, though. If allowed to react with the calcium salts of PA, these phosphates might exchange under formation of calcium phosphate and hydratable PA salts and, thus, increase the extent of degumming. However, this would require a fine dispersion of the water into the oil, which Thomke does not prescribe.

With respect to the water degumming temperature, all authors agree that it should be below 100°C to prevent the water from boiling off, but values as low as 43°C (Baylis, 1929) or 50°C (Pardun, 1983) or a range of 50 to 70°C (Van Nieuwenhuyzen, 1976) have also been advocated. Intensive mixing, more water and a high temperature promote the hydration process. Introduction of these factors allows the holding time to be reduced to the period required for the gums to agglomerate to such an extent that they can be separated. As will be discussed in Section 4.5.1, the gums resulting from the water degumming process can be further dried, bleached, modified, etc.

3.4.2.2 Dry degumming

Figure 3.8 shows that the dry degumming process allows crude oil to be fully refined in only two steps: dry degumming and physical refining. This makes the dry degumming route the cheapest provided the auxiliary costs, such as the cost of bleaching earth and its subsequent disposal, do not escalate.

The dry degumming process makes use of the fact that strong acids displace weaker acids from their salts. So, treating the calcium and magnesium salts of PA and LPA (the NHP) with an acid that is stronger than PA or LPA, as for instance nitric acid (Guillaumin and Boulot, 1960), displaces the weaker acid, and this will lead to the formation of nondissociated PA and LPA and calcium and magnesium nitrate, or rather calcium ions, magnesium ions, and nitrate ions. In the presence of bleaching earth, these metal ions will be chemisorbed by the acidic sites in the earth. A very early process (Taylor et al., 1930) using a strong acid (sulfuric acid) and bleaching earth is used for tallow and palm oil and can be regarded as the first dry degumming process. Sulfuric acid has its disadvantages, however.

Accordingly, Bock and Hommers (1967) mix phosphoric acid into crude coconut oil, add bleaching earth, and allow this to react for several hours while raising the temperature to 94°C, remove the earth by filtration and subject the clear filtrate to a vacuum steam stripping process. They obtain a good yield of fully refined oil. To obtain a good dispersion of the degumming acid into the oil, they also added some monoglycerides, but subsequent inventors omit the emulsifier during the dry degumming stage (Velan, 1968; 1970; 1971) or esterify dry-degummed oil having a high acidity with glycerol before subjecting it to the neutralisation stage (Balazs, 1969).

As one might expect, more phosphatides demand more bleaching earth and, thus, increase the cost of the dry degumming process. Accordingly, the route involving dry degumming and physical refining is especially attractive for low-phosphatide oils with appreciable acidity, since then the low neutral oil loss of the physical refining process also contributes to its attractiveness. Consequently, this route has been advocated for palm oil (Anon., 1974) for which oil degumming acid strengths have also been investigated (Amelotti et al., 1986), and also for lauric oils (Watanabe and Negishi, 1978).

Instead of bleaching earth, amorphous silica can also be used in the dry degumming process (Welsh and Parker, 1987; Van Dalen et al., 1990) and even synthetic amorphous alumina silica has been used for phosphatide removal (Lammers and Groeneweg, 1990). A combination of bleaching earth and silica can also be used to bleach palm oil, but phosphoric acid was not found to aid phosphatide removal (Siew et al., 1994). Up until then, processes were rather empirical, but then Van Dalen and Van Putte (1992) investigated what factors affect the phosphorus loading of various adsorbents, including silicas. These increase when the oil is given an acid pretreatment before the adsorptive treatment; this is fully in line with the processes listed above.

The dry degumming process constitutes the main treatment for palm oil, lauric oils, and low phosphatide animal fats, such as tallow and lard. It allows the degummed oils to be physically refined and yield bland and stable products at minimal processing cost. In Canada, it is also used to process SuperDegummed canola oil (T.K. Mag, personal communication).

3.4.2.3 Acetic acid anhydride degumming

In 1950, the American company, A.E. Staley Manufacturing Company, started developing a degumming process that aimed at producing an oil that could be physically refined. This led to "Staley's 50 oil process" (Myers, 1957). In this process (Hayes and Wolff, 1957b), soya bean oil is treated with 0.1% by weight of acetic acid anhydride at a temperature of some 60°C. After 15 to 30 minutes, an amount of 1.5 wt % water is added and the gums that are formed are removed by centrifuge; the oil is also washed with water before being bleached and physically refined. The washed oil can also be bleached and hardened.

A further patent (Hayes and Wolff, 1957a) advocates washing the oil with a tannin solution. This seems very logical since any iron present in the crude oil or resulting from equipment corrosion will end up in the oil as oil-soluble iron acetate and, thus, can act as a pro-oxidant. Given the affinity of tannin for iron, it might lead to a reduction of the iron content of the "50 oil." But it probably didn't, or at least insufficiently, because when Grothues reviewed problems with the physical refining of soya bean oil in 1982, he listed iron as a problem and

mentioned that the process was only used when followed by alkali refining.

Moreover Smiles reported (1988) that acetic acid anhydride does not degum canola oil and Paul (1968) noted only a minimal decrease in residual phosphorus content — from 178 to 160 ppm — when treating water degummed rapeseed oil with 0.31% by weight of acetic acid anhydride.

3.4.2.4 Acid degumming

Treating oil with an acid and then separating the aqueous phase from the oil phase will not remove the PA and LPA from the oil phase since this acid will cause these phosphatides to be nondissociated and nondissociated phosphatidic acids are oil-soluble and nonhydratable (Hvolby, 1971). But when the oil phase is subsequently washed with water and the environment becomes less acidic, the phosphatidic acids in the oil may dissociate and be at least partially removed with the washing water.

Accordingly, just degumming a crude oil with acidified water will only lead to a slightly lower residual phosphorus content than the water degumming process, and treating oil with acid, removing the acid, and then washing the oil with water will lead to a lower residual phosphorus content. However, the acid removal will be incomplete and not all calcium and magnesium will be removed either. If washing water is then added, these alkaline earth ions will react with the PA and LPA ions and reconstitute the original NHP, unless this NHP formation is prevented by allowing the alkaline earth ions to react with compounds that bind them so strongly that these compounds are not displaced by the phosphatidic acid anions. Examples of such compounds used industrially are the phosphate anion, the citrate anion, EDTA, etc.

Accordingly, the use of acids, such as hydrochloric acid (Guillaumin and Drouhin, 1963c; Guillaumin, 1964) or nitric acid (Defromont et al., 1962; Guillaumin and Drouhin, 1963a; Guillaumin and Drouhin, 1963b; Guillaumin, 1965; Guillaumin et al., 1965), is not to be recommended especially since the iron salts resulting from the treatment of crude oil with these acids leads to oil-soluble iron salts. Besides, oils and phosphatides can be attacked by hydrochloric acid (Ringers and Segers, 1977) and, moreover, these acids are quite corrosive.

Several authors (Freiburg, 1933; Zschau, 1982; Grothues, 1982c; MacLellan, 1983; Amelotti et al., 1986) mention the use of calcium carbonate (chalk) for the neutralization of the excess degumming acid and some of them (Amelotti et al., 1986) observe and report a higher residual phosphorus content when using chalk than when not using it. The explanation is simple: The chalk will indeed react with the acid and, in doing so, form calcium ions, which will then react with PA and LPA ions and form NHP. A similar observation was made when phosphoric acid was neutralised with an excess of magnesia. This also reacted with phosphatidic acids and caused high residual phosphorus (Dijkstra and Van Opstal, 1989).

The best known acid degumming process, and the one that has also been used industrially, is the Unilever SuperDegumming process (Ringers and Segers, 1977). In this process (Segers, 1982), crude or water degummed oil is first of all allowed to react with a concentrated aqueous solution of a degumming acid (citric acid) at a temperature of 70°C. Water is added to the acid/oil mixture and the temperature is reduced to below 40°C. According to the inventors, this causes the phosphatides to be converted to a semicrystalline phase, which also contains the acid, the water added, most of the sugar-like compounds, glycerol and waxes if present, and also the magnesium and calcium ions previously bound to the NHP; accordingly, the process also ensures a partial dewaxing of the oil. After a holding time of 1 hour, the mixture is heated to separation temperature to reduce oil viscosity and thereby decrease oil loss via the gums on centrifugal separation.

The many mixers, holding vessels and heat exchangers needed in the SuperDegumming have been represented in Figure 3.10, which also illustrates the Unidegumming extension of the process. This extension with what is essentially an acid refining process (*vide infra*) was deemed to be necessary since the acid degumming process as such leads to an unacceptably high residual phosphorus content (>30 ppm), the reason being that diluting the degumming acid does not raise the pH of the aqueous phase to such an extent that the PA and LPA dissociate to such a degree that they are substantially hydrated. Adding lecithin (Segers, 1979) or hydrolysed phosphatides (Paulitz et al., 1986) to the oil to be SuperDegummed may have the advantage of speeding up the process, but it does not affect this hydration, which therefore remains incomplete. Dispersing the degumming acid at a low temperature has no effect on hydration either (Kaji, 1988).

In addition to citric acid, there are other chelating acids that are also sufficiently strong and that have been found to be quite effective degumming acids, like maleic anhydride (Hayes and Wolff, 1956; Diosady et al., 1982; Diosady et al., 1984; Smiles et al., 1988), oxalic acid (Ohlson and Svensson, 1976; Smiles et al., 1988), tartaric acid (Dijkstra and Van Opstal, 1989), malic acid, and others. The sodium acetate and acetic acid buffer (Shenoy and Ganapathy, 1979), on the other hand, is likely to be totally ineffective, especially with respect to iron removal. Klein reports (1981) that with citric acid it was not possible to obtain oils containing less than 5 ppm phosphorus. Oxalic acid gave good results, but the toxicity of its salts precludes its use on an industrial scale.

As indicated in Figure 3.8, acid degummed oil should not be bleached and refined physically. It can either be alkali refined and then bleached and deodorised or it has to undergo a second degumming treatment before it can be safely bleached and refined physically. This makes the acid degumming process rather obsolete (Dijkstra, 1998). Nevertheless, a patent has been granted (Copeland and

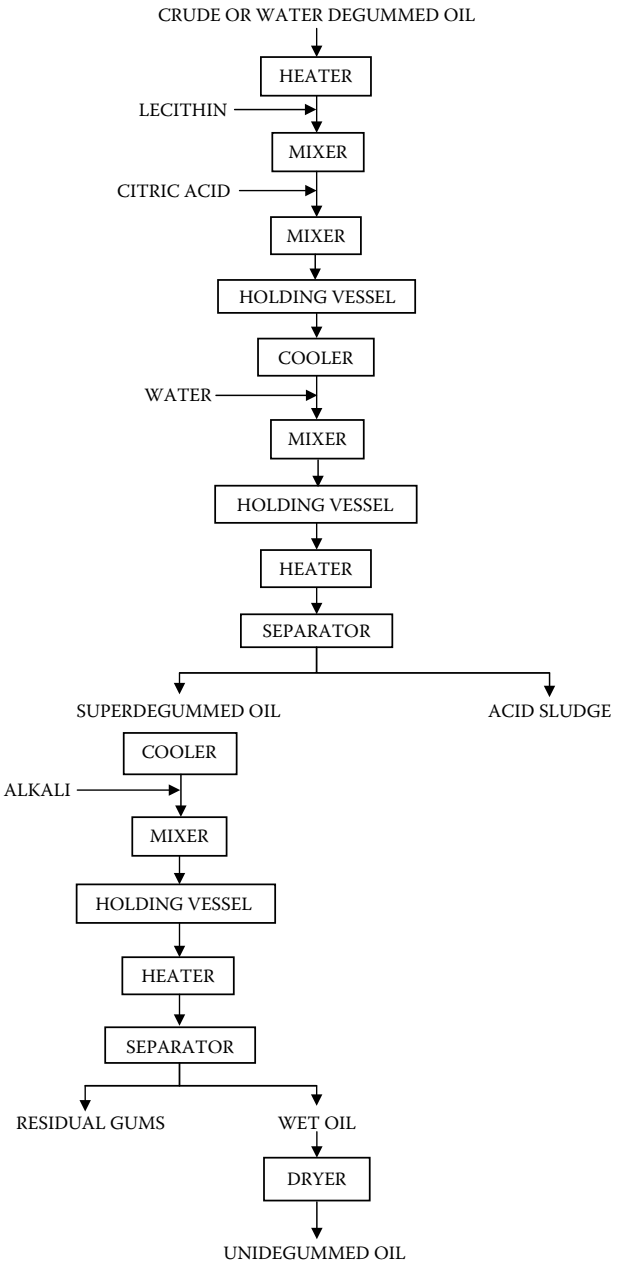


FIGURE 3.10 SuperDegumming and Unidegumming.

Belcher, 2001) describing an acid degumming process, which, quite confusingly, is also referred to as an "organic refining process." This process uses low strength (5 wt %) citric acid to decompose the NHP, although iron removal has been observed to be insufficiently complete at phosphoric acid concentrations below 20%, indicating that the NHP have been only partially decomposed (see also Dijkstra and Van Opstal, 1989, Table 4).

3.4.2.5 Acid refining

As mentioned above, diluting the strong degumming acid with water does not lead to full hydration of PA and LPA, as formed by the action of the degumming acid upon the NHP. This requires the pH to be increased further, which can be achieved by partially neutralising the degumming acid by the addition of a base; since the chemical refining process also uses a base, the degumming process in which the degumming acid is partially neutralised by a base is commonly referred to as the "acid refining process" (Dijkstra, 1993).

- H.L.S. carries out this partial neutralisation by adding an alkaline reagent, e.g., sodium hydroxide or sodium silicate (Leibovitz, 1980).
- Simon-Rosedowns neutralises the acid with an aqueous "base in such quantity that no substantial soap is formed" (Alexander, 1980; Forster and Harper, 1983, provide practical experience with the process).
- Alfa Laval adds enough caustic to neutralise the acid (Piazza, 1995) and developed an earlier acid refining process for linseed oil (Nilsson-Johansson et al., 1988).
- Vandemoortele "mixes a base into the acid-in-oil dispersion in such a quantity that the pH of the aqueous phase is increased to above 2.5, but no substantial amount of soap is formed" (Dijkstra and Van Opstal, 1986; 1987).
- Unilever adds an alkali in what became known as the Unidegumming process (Claim 9 in Van de Sande and Segers, 1989) and in an attempt to distantiate itself from Vandemoortele, mixed alkali into the acidified oil at a temperature below 70°C (Van den Broek et al., 1991).
- Finally, Krupp adds a base and provides the gums with a long period of time to develop before separating them from the oil (Rohdenburg et al., 1992; Rohdenburg and Perédi, 1993).

The acid refining concept, i.e., the neutralisation of the liberated PA and LPA to make them hydratable by the addition of a base, can also be effectively combined with the use of silica hydrogel as a means of removing the phosphatides (Nock, 1993; Schmutzler, 1993). The adsorption capacity of the silica hydrogel increased when the phosphatidic acids were neutralised so that high phosphorus oils could also be processed this way (Nock, 1994). Full-scale plant trials demonstrated that it was technically possible to remove all phosphatides without the use of centrifugal separators, but for no obvious reason, the dry acid refining process has yet to be adopted by industry.

The Vandemoortele acid refining process, which became known as TOP degumming[14] (Van Opstal et al., 1990; Cleenewerck and Dijkstra, 1992; Cleenewerck et al., 1992), differs from the other acid refining processes

[14] TOP is a Dutch acronym derived from "Totaal Ontslijmings-Proces" meaning total degumming process.

mentioned above in that it aims at a very fine dispersion of the degumming acid (Mag and Reid, 1980) and at minimising the neutral oil content of the gum phase and thereby the neutral oil loss of the degumming process. As illustrated in Figure 3.11, TOP degumming achieves the fine acid dispersion by using an in-line mixer with capacity to spare, and using this spare capacity by recycling part of the dispersion to the inlet.

The process minimises oil loss by using two centrifugal separators in series. The first separator, which governs the composition of the gums leaving the system, is tuned to minimise oil losses. This unavoidably leaves some gums in the oil exiting this first separator. Accordingly, the oil with residual gums is treated in a second separator, which can be a clarifier that fully cleans the oil and, thus, leaves oil in the gum stream. This gum stream is then recycled into the oil stream fed to the first separator. Accordingly, the streams leaving the system are a gum stream with minimal oil content and an oil stream without gums. Washing water can be added in between the two separators instead of after the two separators (Segers, 1990). This not only saves a washing centrifuge, but also retards the deposit of inorganic phosphates on the discs of the first separator and, thus, diminishes the need for manual disc cleaning.

The acid refining process is used in Europe as a preparation for physical refining of a wide variety of vegetable oils, including soya bean oil and sunflower seed oil (Kővári et al., 2000). At one stage, it was foreseen (Dijkstra, 1999) that acid refined oil could well become an article of trade, but this has as yet hardly materialised (by lack of specification?); most acid refined oil is physically refined in-house.

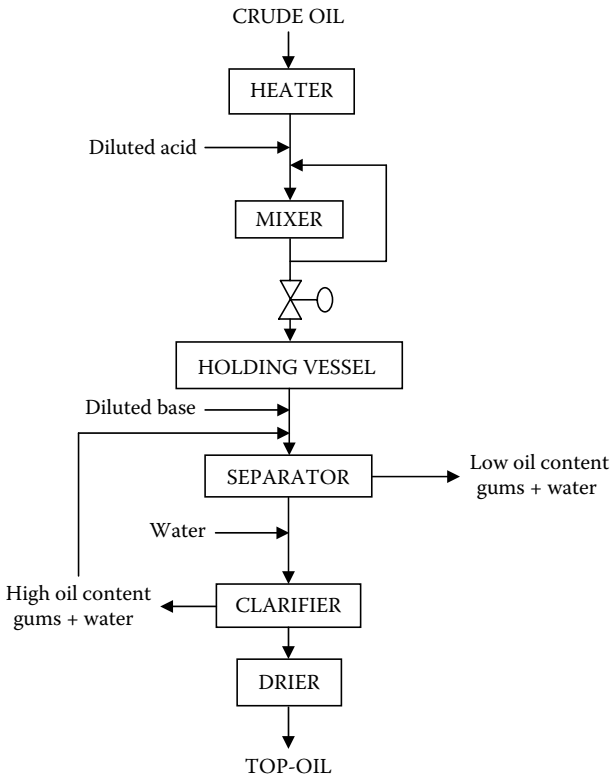


FIGURE 3.11 TOP degumming.

3.4.2.6 Enzymatic degumming

The enzymatic degumming process EnzyMax® (Aalrust et al., 1992) was launched in 1992 (Dijkstra, 1993). At that time, and as illustrated in Figure 1 (Dijkstra, 1993), oil to be degummed enzymatically had to be water degummed first. Little experience with the process was available and raised more questions than it answered:

- The process uses porcine phospholipase A_2 and this enzyme requires calcium as a co-factor. Since the process also uses a citrate buffer, the latter is likely to bind the calcium and, thus, reduce enzyme activity.
- The calcium salts of both PA and LPA are nonhydratable, so it is difficult to envisage why partial hydrolysis of NHP leads to their removal by hydration.
- The observation (Buchold, 1993) that during a citric acid treatment the residual phosphorus content, after having been halved over the course of 1 hour, increases again to close to its original value is highly unlikely.
- During the first hour of a batch experiment (see Buchold, 1993, Figure 6) citric acid on its own was about as effective as the enzyme in combination with the citrate buffer.
- The claim that the enzyme hydrolysed NHP was not substantiated by analytical results.

However, subsequent experiences with the enzymatic degumming process followed by bleaching and physical refining were published by Dahlke in 1995, and he also provided a mass balance and comparative figures for the consumption of auxiliaries. Somewhat later, he could report that enzyme usage had been reduced to only 10 g/t of oil (Dahlke, 1997), making the process highly competitive. But, since the enzyme was really a by-product of insulin production, supply was limited.

At this stage, a microbial enzyme from *Fusarium oxysporum* was developed that had phospholipase A_1 activity as opposed the phospholipase A_2 activity of the porcine material (Clausen et al., 1998; Clausen, 1999). Whereas the latter was hydrophilic and could be recycled at least partially into the degumming process, the microbial enzyme turned out to be Ca^{2+}-independent and lipophilic (Clausen and Langhoff, 1999). Therefore, it cannot be recycled (Clausen, 2001). Practical experience with this enzyme is provided by Münch (2001).

A third generation enzyme has subsequently been developed (Clausen et al., 2002) from *Thermomyces lanuginosa* that also has phospholipase A_1 activity. The degumming process using this enzyme (Lecitase Ultra) can use crude or water degummed oil and the recommended process

conditions are: water, 1.5% w/w; citric acid, 0.04% w/w; caustic soda, 0.02% w/w; enzyme, 30 ppm; temperature, 55°C; duration, 0.5 hour pretreatment with just citric acid solution followed by 2 to 5 hours enzymatic hydrolysis. The enzyme is not recycled (Münch, 2004).

Just as in the acid refining process, there is also a dry variant of the enzymatic degumming process using silica hydrogel (Nielsen and Clausen, 2002). The total amount of water including the water used to dissolve the citric acid, the sodium hydroxide and the enzyme is <0.5% w/w on oil, so that the process produces no aqueous effluent. Energy and maintenance savings and reduced oil losses are also claimed. If an aqueous acid solution is added at the end of the enzymatic reaction, this prevents fouling of the downstream centrifugal separator (Dayton et al., 2005).

More recently, an enzymatic degumming process avoiding the use of citric acid has been described (Chakrabarti et al., 2004); it uses a phospholipase A_1 from a microbial source, such as *Aspergillus oryzae.* The use of phospholipase C has also been suggested by Graille (1988a; 1988b; 1991). This enzyme would hydrolyse phospholipids to water-soluble products, like choline, ethanolamine, etc., that are easy to separate from oil, and partial glycerides that would increase the oil yield. When phospholipids of animal origin are used, enzymatic hydrolysis catalysed by phospholipase C yields diglycerides with unsaturated fatty acids at the 2 position (Wolff, 1987). *Bacillus cereus* has been mentioned as possible source of this enzyme. An early patent application (Tirtiaux et al., 1983) mentions a whole string of enzymes including phosphatases and, thus, constitutes prior art, but it never led to a granted patent.

3.4.2.7 Miscella degumming

In the 1950s, several processes have been published in the patent literature that advocate a liquid/liquid extraction of miscella, but they never reached the industrial stage. More recently, interest is being expressed in treating miscella by membrane filtration to attain a separation between phosphatides and triglycerides.

The liquid/liquid extraction processes are quite clever in that some of them also allow simultaneous or subsequent elimination of FFA. Phosphatides are reasonably soluble in lower alcohols containing a certain amount of water (Scholfield and Dutton, 1955) and soaps readily dissolve in aqueous isopropanol (Keurentjes, 1991). Accordingly, "undesirable components of the oil are efficiently removed by liquid-liquid extraction from an oil-hydrocarbon solvent miscella with aqueous methanol followed by a diluted aqueous solution of an alkali metal hydroxide, such as sodium or potassium hydroxide" (Ayers, 1951).

For the decomposition of the NHP, an acidified aqueous alcohol can be used (Weber, 1956). By reducing the amount of alcohol, the phosphatides no longer dissolve but form a separate phase ("break") that can be removed as such (Cavanagh and Bean, 1961). The extraction processes offer oil yield advantages over commonly used degumming — and also neutralisation — processes because triglyceride oil is hardly soluble in aqueous alcohols. Nevertheless, they never reached industrial operation. As mentioned in the final paragraph of (Kaufmann and Mukherjee, 1965): "Edible oil processing is today still a long way away from the beneficial application of the modern insight of the chemical process industry."

The reason why membrane filtration processes (Sen Gupta, 1977; Sen Gupta, 1983; Iwama and Kazuse, 1983; 1985; Tanahashi et al., 1988) have not become commercial is different: lack of suitable ultrafiltration membranes and their expense. The membranes must not only be hexane resistant, they must also have a certain minimum strength to withstand the filtration pressure, display a selectivity in that they should retain for instance phosphatide micelles (Sen Gupta, 1976), and allow triglycerides and the solvent to pass through and they should not foul. Of course, they can be cleaned (Bieman et al., 1999), but they will also age and then have to be replaced at substantial cost.

Suitable ultrafiltration membranes have only been reported recently in that polyvinylidene difluoride (PVdF) was found to be hexane-resistant and selective toward phosphatides (Pagliero et al., 2001; Ochoa et al., 2001). As explained by Köseoğlu et al. (2004) and illustrated by the embodiment in Figure 3.12, a prefiltration of the miscella using, for instance, candle filters is recommended to protect the ultrafiltration membranes from fouling too rapidly. The meal fines eliminated can be recycled to the extractor as illustrated, or to the marc desolventiser. The first ultrafiltration module has been provided with a circulation pump for the retentate to encourage cross-flow filtration.

The permeate leaving the first ultrafiltration module can be passed to an evaporator to yield degummed oil, which can then be bleached and physically refined to yield fully refined (RBD) oil. Filtration enthusiasts can also desolventise the miscella via another filtration process using a membrane that distinguishes between hexane and triglyceride oil and recycle the hexane obtained to the extractor, and bleaching can also take place on the miscella (Köseoğlu and Weise, 1991).

Figure 3.12 shows only a single module yielding fully degummed miscella, but in industrial practice, a number of modules will be used that can be in series or parallel. The same holds for the second ultrafiltration module shown in Figure 3.12 in which part of the first retentate is further concentrated, but to maintain its fluidity, it is diluted with hexane as resulting from the subsequent retentate evaporation. Water can also be added to the retentate to isolate the phosphatides (Gupta and Muralidhara, 2002). The permeate can be processed like

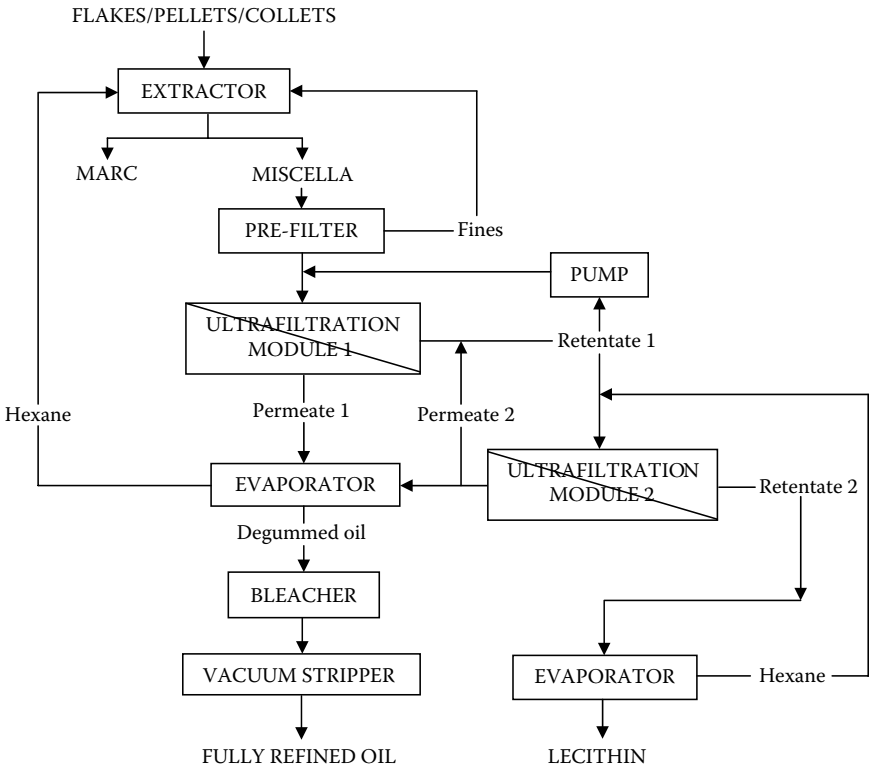


FIGURE 3.12 Membrane degumming.

the first permeate if its phosphatide content is sufficiently low; if not, it can be recycled into the main feed stream; both possibilities have been illustrated in Figure 3.12. Industrial experience will have to demonstrate what configuration is optimal.

3.4.2.8 S.O.F.T.® degumming

The use of ethylene diamine tetraacetic acid (EDTA) salts in oil degumming has already been described in a patent application (Bersworth, 1953) filed in 1948. It advocates the use of generous amounts, for instance, up to a volume of a 5% solution that equals the volume of oil to be treated, but then, it also suggests the evaporation of the solution and treatment with a strong mineral acid to recuperate the EDTA because it is a relatively expensive auxiliary product.

The S.O.F.T. degumming process (Jamil et al., 1995) launched by Deffense (1996), is also based on the use of EDTA but to disperse its solution through the oil, an emulsifier (sodium lauryl sulphate) is also used. Even before bleaching, the residual metals content is very low indeed and the S.O.F.T. degumming process also achieves these low levels with oils that are regarded as "difficult" (Gibon and Tirtiaux, 1998). The equipment required to operate the process is straightforward and processing oils at low temperatures provides an opportunity for simultaneous dewaxing (Gibon and Tirtiaux, 1999b). Moreover, when using the S.O.F.T. degumming process instead of the dry degumming process for palm oil, less bleaching earth is subsequently needed and a Golden Palm Oil, rich in carotenes tocopherols and tocotrienols can be produced (Gibon and Tirtiaux, 1999a).

Subsequently, Deffense (1999) developed a process that also makes use of the unique complexing power of EDTA, but that operates without the emulsifier deemed necessary by Jamil et al. (1995). He also mentions the possibility of incorporating his process in the detergent fractionation of palm oil, and of omitting the bleaching step in the refining of palm oil, which has been degummed by his process. Despite all these attractive advantages, EDTA is an expensive product, not only on a weight basis, but especially on a molar basis. Accordingly, its use could well be limited to polishing operations when, for instance, small amounts of iron have to be removed after storage and/or transport, and as an effective preparation for physical refining (Dijkstra, 1998).

3.4.3 Gum disposal

The degumming processes described above lead unavoidably to by-products or waste products that have to be disposed of. This disposal is least problematic in the case of water degumming since this yields lecithin for which a market exists. On the other hand, this market is in practice limited to soya bean lecithin and is also limited in size; potential availability exceeds demand, which means that soya bean phosphatides also have to be disposed of elsewhere. If they are isolated, as following a water degumming process, they can often be disposed of via the meal

since the isolation is likely to take place in the crushing plant, and in the case of soya beans, the meal drier will, in all probability, have sufficient spare capacity to accommodate the gums. Disposal via the meal is attractive in that the waste product is sold at meal value.

Spent bleaching earth resulting from the dry degumming process can also be disposed of via the meal, be it that the production of palm oil, the major oil profiting from the dry degumming process, does not lead to simultaneous production of "palm meal." In theory, the spent earth could be mixed with palm kernel meal, but then this is often produced at another location and in too small amounts. Therefore, energy recuperation by burning the spent earth in a specially designed fluidised sand bed reactor (Goemans, 2004; 2005) looks like the most attractive means of disposal. Locally, other means may also be available, such as landfill including soil improvement, biogas production by anaerobic fermentation, raw material in cement kilns, or building brick manufacture etc.

Gums resulting from acid degumming, acid refining, and other degumming processes should preferably be disposed of via their respective meals, but this is only possible if they arise in the crushing plant. If they arise in a refinery, their variable composition and availability precludes them from being processed into added value products (De Kock, 1991) for which no markets exist as yet. In this case, recovery of fatty acids (Copeland and Belcher, 2002a) or triglyceride oil (Copeland and Belcher, 2002b; Kellens and De Greyt, 2006) may be the next best solution, but incineration (Goemans, 2004; 2005) should also be contemplated.

References

Aalrust, E. et al. (1992), Enzymatisches Verfahren zur Verminderung des Gehaltes an phosphorhaltigen Bestandteilen in pflanzl. u. tierischen Ölen, European Patent 513 709, assigned to Röhm GmbH and Metallgesellschaft AG.

Abramson, M.B. et al. (1964), Ionic properties of aqueous dispersions of phosphatidic acid, *J. Biol. Chem*., **239**, 4066–4072.

Alexander, D.G. (1980), Improvements in or relating to the degumming of triglyceride oils, British Patent 1 565 569, assigned to Simon-Rosedowns Ltd.

Amelotti, G. et al. (1986), La raffinazione fisica dell'olio di palma. Effetti del pretrattamento, *Riv. Ital. Sostanze Grasse,* **58**, 25–31.

Andersen, A.J.C. (1962), *Refining of Oils and Fats for Edible Purposes,* Pergamon Press Ltd., Oxford, London, New York.

Anon. (1967), Procédé continu de démucilagination des huiles végétales et de production de lécithine à partir des boues de phosphatides obtenus, et dispositif de mise en oeuvre de ce procédé, French Patent 1.469.392, assigned to Instytut Przemystu Ttuszczowego.

Anon. (1974), Procédé de raffinage d'huiles de type de palme, French Patent 2.191.560, assigned to Showa Sangyo Co., Ltd.

Ayers, A.L. (1951), Process for refining soybean oil, US Patent 2,561,330, assigned to Phillips Petroleum Company.

Ayres Jr., E.E. and Clark, L.H. (1929a), Process of refining fatty oils, US Patent 1,737,402, assigned to The Sharples Specialty Corporation.

Balazs, E. (1969), Verfahren zum Reinigen von vegetabilen und tierischen Ölen und Fetten, Swiss Patent 469 804, assigned to Buss AG.

Baylis, W.S. (1929), Process of decolorizing and refining crude cottonseed oil, US Patent 1,725,895, assigned to Filtrol Company.

Bernardini, E. (1973), *The New Oil and Fat Technology,* Publishing House Technologia S.R.L., Rome.

Bersworth, F.C. (1953), Methods of treating and processing animal and vegetable oils, British Patent 687,843.

Bieman, H.A.C. de, et al. (1999), Cleaning method for membranes, WO Patent 99/07459, assigned to Unilever.

Bock, H. and Hommers, H. (1967), Method of refining liquid fats and oils, US Patent 3,354,188.

Bollmann, H. (1923), Verfahren zur Gewinnung des durch Auslaugen von Ölsaaten oder deren Preßkuchen mit einem Gemisch aus Alkohol und Benzol oder Benzin erhaltenen Lecithins, German Patent 382912.

Braae, B. (1976), Degumming and refining practices in Europe, *J. Am. Oil Chem. Soc.,* **43**, 353–357.

Broek, W.J. van den, et al. (1991), Method of refining glyceride oil, European Patent 456 300, assigned to Unilever.

Buchold, H. (1993), Enzymatische Phosphatidenentfernung aus Pflanzenölen, *Fat Sci. Technol.,* **95**, 300–304.

Carr, R.A. (1976), Degumming and refining practices in the U.S., *J. Am. Oil Chem. Soc.,* **53**, 347–352.

Carr, R.A. (1978), Refining and degumming systems for edible fats and oils, *J. Am. Oil Chem. Soc.,* **55**, 765–771.

Cavanagh, G.C. and Bean, R.S. (1961), Method of degumming soybean oil, US Patent 2,980,718, assigned to Ranchers Cotton Oil.

Chakrabarti, P.P. et al. (2004), Process for the pre-treatment of vegetable oils for physical refining, US Patent Application 2004/0005399 A1, assigned to Council of Scientific and Industrial Research.

Cherry, J.P. et al. (1981), A review of lecithin chemistry and glandless cottonseed as a potential commercial source, *J. Am. Oil Chem. Soc.,* **58**, 903–913.

Clausen, K. (1999), An enzymatic oil-degumming process, WO Patent Application 00/53001, assigned to Novo Nordisk A/S.

Clausen, K. (2001), Enzymatic oil-degumming by a novel microbial phospholipase, *Eur. J. Lipid Sci. Technol.,* **103**, 333–340.

Clausen, K. et al. (1998), Enzymatic oil degumming and lysolecithin preparation by novel microbial phospholipases, *Paper presented at the 89th AOCS Annual Meeting & Expo, Chicago*.

Clausen, K. and Langhoff, E.M. (1999), The chemistry of enzymatic oil degumming, *Paper presented at the 90th AOCS Annual Meeting & Expo, Orlando, FL*.

Clausen, K. et al. (2002), A new microbial phospholipase for degumming of vegetable oil, *Paper presented at the 93rd AOCS Annual Meeting and Expo, Montreal*.

Cleenewerck, B. et al. (1992), Process for the continuous removal of a gum phase from triglyceride oil, European Patent 0 507 363, assigned to N.V. Vandemoortele International.

Cleenewerck, B. and Dijkstra, A.J. (1992), The total degumming process — theory and industrial application in refining and hydrogenation, *Fat Sci. Technol.,* **94**, 317–322.

Copeland, R. and Belcher, W.M. (2001), Methods for refining vegetable oils and byproducts thereof, US Patent 6,172,248 B1, assigned to IP Holdings, L.L.C.

Copeland, R. and Belcher, W.M. (2002a), Methods for recovering fatty acids, US Patent 6,423,857 B1, assigned to IP Holdings, L.L.C.

Copeland, R. and Belcher, W.M. (2002b), Methods for treating phosphatide-containing mixtures, US Patent 6,426,423 B1, assigned to IP Holdings, L L.C.

Dahlén, J.Å.H. (1996), A way to produce crude rapeseed oil with very low content of non-hydratable phosphatides, *Poster presented at the World Conference and Exhibition on Oilseeds and Edible Oils Processing, October 6-10, 1996, Istanbul.*

Dahlén, J.Å.H. (1998), The Super Expro process, *Oléagineux Corps Gras Lipides/OCL,* **5**, 360–363.

Dahlke, K. (1997), The enzymatic degumming — EnzyMax, *Oléagineux Corps Gras Lipides/OCL,* **4**, 55–57.

Dahlke, K. et al. (1995), First experiences with enzymatic oil refining, *inform*, **6**, 1284–1291.

Dalen, J.P. van et al. (1990), Process for refining glyceride oil, European Patent 0 361 622, assigned to Unilever.

Dalen, J.P. van and Putte, K.P.A.M. van (1992), Adsorptive refining of liquid vegetable oils, *Fat Sci. Technol.,* **94**, 567–570.

Dayton, C.L.G. et al. (2005), Process for improving enzymatic degumming of vegetable oils and reducing fouling of downstream processing equipment, PCT Patent WO2005/063950 A1, assigned to Bunge Oils, Inc.

Deffense, E.M.J. (1996), Soft degumming, in *Oils-Fats-Lipids 1995,* Castenmiller, W.A.M., Ed., P.J. Barnes & Associates, Bridgewater, England, 125–128.

Deffense, E.M.J. (1999), Procédé d'élimination des métaux des corps gras et des gommes associés à ces métaux, WO 99/02630, assigned to Crystallisation & Degumming sprl.

Defromont, C. et al. (1962), Étude semi-industrielle de la démucilagination de l'huile de colza par des solutions d'acide nitrique, *Rev. Franç. Corps Gras,* **9**, 486–500.

De Kock, J. (1991), Process for the supercritical solvent extraction of a mixture of phosphatides, European Patent 431 709, assigned to N.V. Vandemoortele International.

Desnuelle, P. et al. (1951), Traitement thermique des graines oléagineuses et teneur en lécithine des huiles, *Oléagineux,* **6**, 264–267.

Dijkstra, A.J. (1993), Degumming, refining, washing and drying fats and oils, in *Proceedings of the World Conference on Oilseed Technology and Utilization,* Applewhite, T.H., Ed., AOCS, Champaign IL, 138–151.

Dijkstra, A.J. (1998), Degumming revisited, *Oléagineux Corps Gras Lipides/OCL,* **5**, 367–370.

Dijkstra, A.J. (1999), Trends in edible oil processing, *Lipid Techn.,* **11**, 53–56.

Dijkstra, A.J. and Van Opstal, M. (1986), Process for producing degummed vegetable oils and gums of high phosphatidic acid content, European Patent 0 195 991, assigned to N.V. Vandemoortele International.

Dijkstra, A.J. and Van Opstal, M. (1987), Process for producing degummed vegetable oils and gums of high phosphatidic acid content, US Patent 4,698,185, assigned to Safinco Coordination Center N.V.

Dijkstra, A.J. and Van Opstal, M. (1989), The total degumming process, *J. Am. Oil Chem. Soc.,* **66**, 1002–1009.

Diosady, L.L. et al. (1982), Chemical degumming of canola oils, *J. Am. Oil Chem. Soc.,* **59**, 313–316.

Diosady, L.L. et al. (1984), Scale-up of canola oil degumming, *J. Am. Oil Chem. Soc.,* **61**, 1366–1369.

Erickson, D.R. (1995), Degumming and lecithin processing and utilization, in *Practical Handbook of Soybean Processing and Utilization,* Erickson, D.R., Ed., AOCS Press and United Soybean Board, Champaign IL and St. Louis MO, 174–183.

Forster, A. and Harper, A.J. (1983), Physical refining, *J. Am. Oil Chem. Soc.,* **60**, 265–271.

Freiburg, A. (1933), Method of removing impurities from oils and fats, British Patent 393,108, assigned to Harburger Oelwerke Brinckman & Mergell.

Gibon, V. and Tirtiaux, A. (1998), Un raffinage S.O.F.T.®, *Oléagineux Corps Gras Lipides/OCL,* **5**, 371–377.

Gibon, V. and Tirtiaux, A. (1999a), S.O.F.T.® Degumming of crude palm oil, a process for the mill? *Paper presented at the PIPOC Conference, Emerging Technologies and Opportunities in the Next Millennium — Chemistry and Technology, Kuala-Lumpur.*

Gibon, V. and Tirtiaux, A. (1999b), The combined S.O.F.T.® degumming/dewaxing process from Tirtiaux, *Paper presented at the 90th AOCS Annual Meeting & Expo, Orlando, FL.*

Goemans, M. (2004), Thermal treatment of spent bleaching earth, filter aids, destillates, gums and soapstock: The HelioSolids® fluidised bed reactor and the Cinclus® thermal oxidizer, *Paper presented at the DGF Symposium on Refining: Handling by-products and minor compounds, Leipzig.*

Goemans, M. (2005), On-site thermal treatment of by-products from the edible oils and fats industry: a renewable energy source, *Paper presented at the 26th ISF World Congress and Exhibition, Prague,* page 8 in the abstracts.

Graille, J. (1991), Biotechnology in the oils and fats field: a few possible applications in processing and oleochemistry, *Agro-Industry Hi-Tech,* **2**, 29–35.

Graille, J. et al. (1988b), Biotechnologie des lipides: Quelques applications possibles, *Oléagineux,* **43**, 181–190.

Graille, J. et al. (1988a), Biotechnologie des lipides: quelques applications possibles, *Riv. Ital. Sostanze Grasse,* **65**, 423–428.

Grothues, B.G.M. (1982), Probleme der physikalischen Raffination von Sojaöl, *Fette Seifen Anstrichm.,* **84**, 21–28.

Guillaumin, R. (1964), Raffinage des huiles, French Patent 1.385.670, assigned to Institut des Corps Gras – I.T.E.R.G.

Guillaumin, R. (1965), Raffinage des huiles de soja, French Patent 1.388.671, assigned to Institut des Corps Gras – I.T.E.R.G.

Guillaumin, R. and Boulot, M. (1960), Démucilagination de l'huile de colza, *Rev. Franç. Corps Gras,* **7**, 506–519.

Guillaumin, R. and Drouhin, N. (1963a), Application à l'huile d'arachide du procédé de démucilagination par des solutions nitriques diluées, *Rev. Franç. Corps Gras,* **10**, 207–213.

Guillaumin, R. and Drouhin, N. (1963b), Nouveau procédé de raffinage des huiles de soja. Méthode utilisant un "dégommage" total, *Rev. Franç. Corps Gras,* **10**, 463–478.

Guillaumin, R. and Drouhin, N. (1963c), Nouvelle méthode d'élimination des mucilages des huiles de lin. Raffinage simplifié, *Rev. Franç. Corps Gras,* **10**, 671–684.

Guillaumin, R. and Drouhin, N. (1966), Calcium et magnésium dans les huiles végétales et les graisses animales. Incidence du raffinage, *Rev. Franç. Corps Gras,* **13**, 185–193.

Guillaumin, R. et al. (1965), Raffinage et stabilité des huiles de soja traités aux solutions d'acide nitrique, *Rev. Franç. Corps Gras,* **12**, 185–197.

Gupta, R. and Muralidhara, H.S. (2002), A novel approach to process crude oil membrane concentrate using a centrifuge, *J. Am. Oil Chem. Soc.,* **79**, 419–423.

Halden, W. (1940a), Über den heutigen Stand der Phosphatidforschung I., *Fette u. Seifen,* **47**, 6–9.

Halden, W. (1940b), Über den heutigen Stand der Phosphatidforschung II., *Fette u. Seifen,* **47**, 52–54.

Hayes, L.P. and Wolff, H. (1956), Novel method for refining soybean oil, *J. Am. Oil Chem. Soc.,* **33**, 440–442.

Hayes, L.P. and Wolff, H. (1957a), Processing vegetable oils, US Patent 2,792,411, assigned to A.E. Staley Manufacturing Company.

Hayes, L.P. and Wolff, H. (1957b), Refining vegetable oils, US Patent 2,782,216, assigned to A.E. Staley Manufacturing Company.

Hvolby, A. (1971), Removal of nonhydratable phospholipids from soybean oil, *J. Am. Oil Chem. Soc.,* **48**, 503–509.

Iwama, A. and Kazuse, Y. (1983), Process for the purification of crude glyceride oil compositions, US Patent 4,414,157, assigned to Nitto Electrical Industrial Co., Ltd.

Jamil, S. et al. (1995), Procédé de dégommage d'un corps gras et corps gras ainsi obtenu, WO Patent 95/00609, assigned to Fractionnement Tirtiaux S.A.

Kaji, T. (1988), Process for degumming triglyceride oils, European Patent 0 269 277, assigned to The Cambrian Engineering Group.

Kanamoto, R. et al. (1981), Phospholipid-phospholipid interaction in soybean oil, *J. Am. Oil Chem. Soc.,* **58**, 1050–1053.

Kaufmann, H.P. and Mukherjee, K.D. (1965), Neuzeitliche Technologie der Fette und Fettprodukte CX: Die Raffination der Fette, *Fette Seifen Anstrichm.,* **67**, 1027–1031.

Kellens, M.J. and De Greyt, W.F.J. (2006), Oil recuperation process, European Patent Application 1 624 047 A1, assigned to De Smet Engineering N.V.

Keurentjes, J.T.F. (1991), Physical chemistry and engineering of membranes for fat/fatty acid separations, Ph.D. thesis, Landbouwuniversiteit Wageningen, the Netherlands.

Klein, J.-M. (1981), Physical and chemical refining of soybean oil, in *Proceedings of the second A.S.A. symposium on soybean processing. Refining of soy oil, caustic or physical?* Leysen, R.J.F., Ed., American Soybean Association, Brussels, Belgium.

Kock, M. (1978), Procédé de préparation d'huile alimentaire à partir d'huile de soja brute, French Patent 2 391 272, assigned to Akzo N.V.

Kock, M. (1981), Production of an edible oil from crude soy oil, US Patent 4,255,346, assigned to Akzo N.V.

Kock, M. (1983), Oilseed pretreatment in connection with physical refining, *J. Am. Oil Chem. Soc.,* **60**, 150A–154A.

Köseoğlu, S.S. et al. (2004), Degumming of edible oils by membrane filtration, US Patent 6,797,172, B2, assigned to The Texas A&M University System.

Köseoğlu, S.S. and Weise, M.K. (1991), Soybean oil hydrogenation via a unique reactor, inform, **2**, 631–636.

Kővári, K. et al. (2000), Physical refining of sunflower oil, *Oléagineux Corps Gras Lipides/OCL,* **7**, 305–308.

Krog, N. and Tolboe, O. (1963), Über die Bindung von Kupfer in Sojabohnen-Phosphatiden, *Fette Seifen Anstrichm.,* **65**, 732–736.

Lammers, J.G. and Groeneweg, J.W. (1990), Synthetic, macroporous, amorphous alumina silica and a process for refining glyceride oils, European Patent 376 406, assigned to Unilever.

Leibovitz, Z. (1980), Completion of degumming process for fatty oils, Israeli Patent 60835, assigned to H.L.S. Ltd.

List, G.R. (1993), Supercritical CO_2 degumming and physical refining of soybean oil, *J. Am. Oil Chem. Soc.,* **70**, 473–476.

List, G.R. et al. (1992), Factors promoting the formation of nonhydratable soybean phosphatides, *J. Am. Oil Chem. Soc.,* **69**, 443–446.

List, G.R. et al. (1990), Effect of moisture, microwave heating and live steam treatment on phospholipase D activity in soybeans and soy flakes, *J. Am. Oil Chem. Soc.,* **67**, 867–871.

List, G.R. et al. (1978), Steam-refined soybean oil: I. Effect of refining and degumming methods on oil quality, *J. Am. Oil Chem. Soc.,* **55**, 277–279.

MacLellan, M. (1983), Palm oil, *J. Am. Oil Chem. Soc.,* **60**, 368–373.

Mag, T.K. and Reid, M.P. (1980), Continuous process for contacting of triglyceride oils with an acid, US Patent 4,240,972, assigned to Canada Packers Limited.

Mattikow, M. (1959), Refining methods for drying oils, *J. Am. Oil Chem. Soc.,* **36**, 491–495.

McIntosh, T.J. and Magid, A.D. (1993), Phospholipid hydration, in *Phospholipids Handbook,* Cevc, G., Ed., Marcel Dekker, Inc., New York, 553–577.

Moulton Sr., K.J. and Mounts, T.L. (1990), Continuous ultrasonic degumming of crude soybean oil, *J. Am. Oil Chem. Soc.,* **67**, 33–38.

Münch, E.-W. (2001), Practical experience of enzymatic degumming, in *Proceedings of the World Conference on Oilseed Processing and Utilization,* Wilson, R.F., Ed., AOCS Press, Champaign IL, 17–20.

Münch, E.-W. (2004), Enzymatic degumming processes for oils from soya, rape and sunflower, *Paper presented at the 3rd Euro-FedLipid Congress, Edinburgh,* page 89 of the Abstracts.

Myers, N.W. (1957), Design and operation of a commercial soybean-oil refining plant, using acetic acid anhydride as a degumming agent, *J. Am. Oil Chem. Soc.,* **34**, 93–96.

Myers, N.W. (1965), Method and apparatus for continuous degumming of vegetable oil and lecithin manufacture, US Patent 3,221,801, assigned to A.E. Staley Manufacturing Company.

Nielsen, K. (1960), The composition of difficultly extractable soybean phosphatides, *J. Am. Oil Chem. Soc.,* **37**, 217–219.

Nielsen, P.M. and Clausen, K. (2002), Degumming of vegetable oil at water content below 0.5%, *Paper presented at the 93rd AOCS Annual Meeting and Expo, Montreal*.

Nieuwenhuyzen, W. van (1976), Lecithin production and properties, *J. Am. Oil Chem. Soc.,* **53**, 425–427.

Nilsson-Johansson, L. et al. (1988), Experience of prerefining of vegetable oils with acids, *Fat Sci. Technol.,* **90**, 447–451.

Nock, A. (1993), A novel acid refining process using silica adsorbent, *Paper presented at the 84th AOCS Annual Meeting & Expo, Anaheim, CA*.

Nock, A. (1994), Acid refining using silica — A process for refining high phosphorus containing oils, *Paper presented at the 85th AOCS Annual Meeting & Expo, Atlanta*.

Ochoa, N. et al. (2001), Ultrafiltration of vegetable oils, degumming by polymeric membranes, *Sep. Pur. Technol.,* 417–422.

Ohlson, R. and Svensson, C. (1976), Comparison of oxalic acid and phosphoric acid as degumming agents for vegetable oils, *J. Am. Oil Chem. Soc.,* **53**, 8–11.

Pagliero, C. et al. (2001), Degumming of crude soybean oil by ultrafiltration using polymeric membranes, *J. Am. Oil Chem. Soc.,* **78**, 793–796.

Pan, L.G. et al. (2000), A kinetic study of phospholipid extraction by degumming process in sunflower seed oil, *J. Am. Oil Chem. Soc.,* **77**, 1273–1276.

Pardun, H. (1983), 50 Jahre Technologie pflanzlicher Öle und Fette — ein Erfahrungsbericht, *Fette Seifen Anstrichm.,* **85**, 1–18.

Paul, W. (1968), Vergleichende Untersuchungen über die Entschleimung und Bleichung von Pflanzenölen im Hinblick auf die destillative Entsäuerung, *Die Nahrung,* **12**, 429–440.

Paulitz, B.G. et al. (1986), Process relating to triglyceride oils, US Patent 4,584,141, assigned to Internationale Octrooi Maatschappij "Octropa" B.V. (Unilever).

Penk, G. (1981), Sojaflocken-Konditionierung. Verfahrenstechnik und wirtschaftliche Aspekte, *Fette Seifen Anstrichm.,* **83**, 558–563.

Piazza, J.W. (1995), Degumming of soybean oil, *Paper presented at a Texas A&M short course on edible oil refining, College Station, TX*.

Rade, D. et al. (1995), Effect of soybean pretreatment on the phospholipid content in crude and degummed oils, *Fat Sci. Technol.,* **97**, 501–507.

Raffaetá, R. (1965), Process for the continuous removal of break (mucilaginous products) from, and purification of, vegetable oils and fats, US Patent 3,206,487.

Ringers, H.J. and Segers, J.C. (1977), Degumming process for triglyceride oils, US Patent 4,049,686, assigned to Unilever.

Rohdenburg, H. et al. (1992), Entschleimungsverfahren, European Patent 0 473 985, assigned to Krupp Maschinentechnik GmbH.

Rohdenburg, H. and Perédi, J. (1993), Entschleimung und physikalische Raffination (von Raps- und Sonnenblumenöl), *Fat Sci. Technol.,* **95**, 483–487.

Sande, R.L.K. van de and Segers, J.C. (1989), Method of refining glyceride oils, European Patent 0 348 004, assigned to Unilever.

Schmitt, R.O. (1963), Process for the purification of glyceride oils, US Patent 3,102,898, assigned to The Procter & Gamble Company.

Schmutzler, L.O.F. (1993), Process for refining glyceride oil, US Patent 5,248,799, assigned to Unilever.

Scholfield, C.R. and Dutton, H.J. (1955), Purification of phosphatides, US Patent 2,727,046, assigned to USA, Secretary of Agriculture.

Schumacher, H.O. (1972), Procédé et dispositif pour éliminer les mucilages d'huiles végétales, French Patent 2.105.507.

Segers, J.C. (1979), Oil purification by adding hydratable phosphatides, US Patent 4,162,260, assigned to Unilever.

Segers, J.C. (1982), Superdegumming, A new degumming process and its effect on the effluent problems of edible oil refining, *Fette Seifen Anstrichm.,* **84**, 543–546.

Segers, J.C. (1990), Degumming — theory and practice, in *Edible Fats and Oils Processing: Basic Principles and Modern Practices*, Erickson, D.R., Ed., American Oil Chemists' Society, Champaign, IL, 88–93.

Sen Gupta, A.K. (1976), Mizelbildung von Phosphatiden als Grundlage für chromatographische Trennungen, *Fette Seifen Anstrichm.,* **78**, 111–118.

Sen Gupta, A.K. (1977), Process for refining crude glyceride oils by membrane filtration, US Patent 4,062,882, assigned to Unilever.

Sen Gupta, A.K. (1983), Refining lipids, British Patent 2 118 568, assigned to Unilever.

Sen Gupta, A.K. (1985), Separation process and products obtained thereby, US Patent 4,496,489, assigned to Unilever.

Sen Gupta, A.K. (1986), Neuere Entwicklungen auf dem Gebiet der Raffination der Speiseöle, *Fette Seifen Anstrichm.,* **88**, 79–86.

Shenoy, R.D. and Ganapathy, A. (1979), Process of degumming fatty glycerides using sodium acetate and acetic acid buffer, US Patent 4,160,774, assigned to Cadbury India Limited.

Siew, W.-L. et al. (1994), Silica refining of palm oil, *J. Am. Oil Chem. Soc.,* **71** , 1013–1016.

Smiles, A. et al. (1988), Effect of degumming reagents on the recovery and nature of lecithins from crude canola, soybean and sunflower oils, *J. Am. Oil Chem. Soc.,* **65**, 1151–1155.

Tanahashi, S. et al. (1988), Process for purification of crude glyceride oil compositions, US Patent 4,787,981, assigned to Pinoro Oil Mills Co. and Nitto Electrical Industrial Co., Ltd.

Tatulian, S. (1993), Ionization and ion binding, in *Phospholipids Handbook,* Cevc, G., Ed., Marcel Dekker, Inc., New York, 511–552.

Taylor, H.F. et al. (1930), Bleaching fats and oils, US Patent 1,744,843, assigned to Swift & Company.

Thomke, W. (1932), Verfahren zur Reinigung von Fetten, Ölen und Wachsen, German Patent 565 079, assigned to Dr. Alexander Wacker Gesellschaft für elektrochemische Industrie G.m.b.H.

Tirtiaux, A. et al. (1983), Procédé de traitement des huiles et graisses et produits ainsi obtenus, European Patent 0 070 269, assigned to Fractionnement Tirtiaux S.A.

Van Opstal, M. et al. (1990), Process for the continuous removal of a gum phase from triglyceride oil, European Patent 0 349 718, assigned to N.V. Vandemoortele International.

Velan, M.E. (1968), Procédé perfectionné pour la purification des huiles comestibles, French Patent 1.510.842, assigned to Salador Huileries Antonin Roux and Savonneries J.B. Paul Savonneries de Bourgogne.

Velan, M.E. (1970), Improved process for the purification of edible oils, British Patent 1,195,059, assigned to Salador Huileries Antonin Roux.

Velan, M.E. (1971), Process for the purification of edible oils, US Patent 3,590,059, assigned to Salador Huileries Antonin Roux and Savonneries J.B. Paul Savonneries de Bourgogne.

Watanabe, H. and Negishi, M. (1978), Method for refining palm oils, US Patent 4,113,752, assigned to Showa Sangyo Kabushiki Kaisha.

Weber, H.M. (1956), Refining of glyceride oils, US Patent 2,739,164, assigned to The Sherwin-Williams Company.

Welsh, W.A. and Parker, P.M. (1987), Method for refining glyceride oils using acid treated amorphous silica, European Patent 0 234 221, assigned to W.R. Grace & Co.

Whittecar, W.C. (1955), The recovery of phosphatides from direct extracted cottonseed and reincorporation into the meal, *J. Am. Oil Chem. Soc.,* **32**, 564–565.

Wiedermann, L.H. (1981), Degumming, refining and bleaching of soybean oil, *J. Am. Oil Chem. Soc.,* **58**, 159–166.

Wolff, R. (1987), Procédé de préparation de diglycérides contenant en position 2 un reste d'acide gras poly-unsaturé, et produits obtenus, French Patent 2 599 382, assigned to Institut des Corps Gras — I.T.E.R.G.

Zhang, F. et al. (1994), Effects of expander process on the phospholipids in soybean oil, *J. Am. Oil Chem. Soc.,* **71**, 1145–1148.

Zschau, W. (1982), Versuche zur Optimierung der Raffination von Palmöl, Fette Seifen Anstrichm., **84**, 493–498.

3.5 Alkali refining of oils and fats

According to Blank (1942), a patent was granted to Schmersahl in 1842 describing a method for the refining of cottonseed oil with caustic alkali. Other authors (O'Brien and Wan, 2001) place the invention of caustic refining in Europe around 1840 and mention the use of calcium and potassium hydroxides. Later, during the 1880s, alkali refining using caustic soda was introduced. It was used in the U.S. for the refining of cottonseed oil in open kettles. Other oils produced at that time hardly needed alkali refining and some, like olive oil, are still not alkali refined. The early alkali refining process was a batch process, but "from 1932, centrifugal refining began to replace the batch refining process" (Bailey et al., 1942).

In the alkali refining process, the oil or fat is treated with an alkali such as caustic soda (sodium hydroxide), and as illustrated in Figure 3.8, the feed can be crude oil, but the oil may also have been water degummed, acid degummed or even acid refined or S.O.F.T.® degummed before being alkali refined. The purpose of the alkali refining process is manifold. If there are still phosphatides present in the oil, the alkali refining process should remove them. The process should remove free fatty acids present by converting them into soaps that are oil-insoluble and can be separated from the oil by settling or centrifugal separation. In addition, the process should remove colouring compounds and/or their precursors so that bleaching the alkali-refined oil requires less bleaching earth and colour fixation during subsequent high temperature treatments is avoided.

The fatty acid composition of the FFA reflects the fatty composition of the oil or fat to be alkali refined and can vary considerably from oil to oil. Accordingly, soap properties will also vary from oil to oil. But a more important cause of variation in soapstock properties is the mass ratio between the sodium soaps and the phosphatides present in the soapstock. Soya bean oil that has not yet been water degummed and having only some 0.5% FFA can easily contain 1000 ppm phosphorus, which corresponds to some 3% by weight of phosphatides; this corresponds to a mass ratio of only 1/6. Sunflower seed oil with 1.5% FFA and only 200 ppm P (0.6% by weight phosphatides) after water degumming shows a mass ratio of 2.5, or 15 times the ratio observed in soya bean oil. So, whereas the sodium soaps resulting from the neutralisation of these two oils have quite similar properties, their soapstocks will behave totally differently and require different process conditions and settings.

Moreover, the phosphatide and FFA contents of crude oil vary with time since crude oil storage tanks are not agitated and their contents are far from homogenous; phosphatides, waxes, and solid contaminants may even settle on the tank bottom and form foots.[15] This variability of crude oil composition means that either process conditions must be adapted continuously to these variations, or the process must be so robust that it can cope with widely different feed streams. However, such a robust process is unlikely to be optimal in all cases with respect to usage of chemicals and/or yield. It may be in some cases, but not in others.

Accordingly, allowing the feed stream properties to control the alkali refining process would appear to be preferable, but the detailed and precise insight that should form the basis of such a control system is not available, and the literature only provides imprecise advice, such as: "Both the % excess and caustic strength are selected by the type and quality of the oil and the past experience of the refiner," (Blake Hendrix, 1990) or "It is the skill of the refiner to ensure, by the adoption of appropriate methods, that the two sources of loss, namely through emulsions and oversaponification, are minimized as far as possible, without interfering with the achievement of good results as far as quality is concerned" (Andersen, 1962).

Perhaps the development of a control system that copes with both variations in FFA content and phosphatide content, would be too difficult to achieve in practice. In that case, the alkali refining process can still be optimised by prior removal of phosphatides from the crude oil by the acid refining or even the acid degumming process. Then the FFA content would be the only variable for a given type of oil and should control the amount of caustic to be used. Caustic strength and oil temperature need not vary since they have already been optimised for the type of oil and separation equipment used. A move in this direction was made for acetic acid anhydride degummed oil (Fiala, 1963). But then, why develop such a control

[15] In the U.S., the term "foots" is also used for the soap sediment in the batch neutralisation process.

system for acid refined oil when it can also be physically refined as shown in Figure 3.8?

3.5.1 Yield loss on alkali refining

Since the purpose of the alkali refining process is to remove a range of impurities from the oil being processed, it involves a yield loss. However, this loss can be limited to the sum of the impurities (the inevitable loss) or it can also comprise loss of triglyceride oil (NOL or "neutral oil loss"). The refiner should aim at minimising his NOL because the oil selling price is always higher than what he can realise for soapstock or acid oils. Accordingly, the refiner should be in a position to calculate the NOL by determining the sum of the impurities in the crude oil and deducting this from the actual yield loss observed in the refinery, but this is where the problems start.

When reviewing the methods to determine the refining loss, James (1955) describes three methods. The oldest one is the Wesson method (Mattikow, 1942) (Desnuelle et al., 1946; Desnuelle and Micaelli, 1950). It starts with dissolving an oil sample in petroleum ether and mixing the resulting miscella with an aqueous solution of potassium hydroxide to convert the FFA into soaps. Then the mixture is extracted with aqueous alcohol to remove soaps, phosphatides, and other impurities. Subsequently, the alcoholic extract is extracted with petroleum ether to recover any oil that might have been lost and the combined petroleum ether solutions are extracted with aqueous alcohol. Evaporation of the petroleum ether leads to a residue, the weight of which is assumed to correspond to the maximum oil yield on alkali refining.

This assumption is not correct; the method overestimates the inevitable loss on refining. This has been demonstrated by adding 2 wt % FFA to an oil sample that had undergone the Wesson treatment (the evaporation residue) and to a sample that had been alkali refined industrially and determining the Wesson loss of both samples. The Wesson-treated sample showed a Wesson loss of 2% demonstrating that the treatment does not lead to saponification (Hartman and White, 1952), but the sample that had been alkali refined showed a Wesson loss that was 0.2% larger (King and Wharton, 1948). Similarly, several alkali refined groundnut oil samples showed Wesson losses (Desnuelle and Micaelli, 1950). The reason that the Wesson loss as determined overestimates the inevitable loss on alkali refining could be that partial glycerides are extracted by the aqueous alcohol used in the Wesson method, whereas they remain in the oil during alkali refining.

The second method mentioned by James (1955) is based upon the determination of the acetone insolubles (AI) of the oil and adding this to the moisture and volatile content and the FFA as determined separately. Care must be taken to determine the FFA on a phosphatide-free sample since some phosphatides also react acid during the FFA titration (see also Table 3.4). Since the result of the FFA titration is expressed as weight percent oleic acid, the figure must be converted to true percentage of FFA by correcting for the average molecular weight of the fatty acids in the oil sample. However, the acetone precipitation of phosphatides is far from complete (Desnuelle and Massoni, 1953), so the method needs improvement.

In principle, this improvement could be achieved by using another method of determining the phosphatide content of the oil sample, for instance, via its phosphorus content. With the advent of plasma emission spectroscopy (Dijkstra and Meert, 1982) in the early 1980s, the determination of phosphorus became fast and accurate for those who could afford the investment, and equipment has improved considerably since then, by being no longer susceptible to drift; the sensitivity has also been improved. In fact, the use of this equipment is strongly advocated and "the return will be evident in a matter of months with improved yields and improved quality" (Farr, 2000). But this raises the question of what conversion factor to use for converting phosphorus content to phosphatides or rather to the nontriglyceride content of the gums.

In this context, many conversion factors have been used and the literature mentions a range from about 25 to 32. The use of different conversion factors for different oils has also been suggested (Pardun and Werber, 1959). As explained by Pardun (1981), the factor 25.44 is based on the average fatty acid composition of different lecithins and their phosphatide composition. The higher factors take into account that lecithin contains other acetone insoluble compounds apart from phosphatides. Since these compounds are apparently also removed on degumming, it is fully justified to use the conversion factor of 31.5 as finally suggested (Pardun, 1981).

Although this conversion factor may be correct for oils being degummed for the first time, what about oils that have already been water-degummed? What conversion factor should be used for the residual, nonhydratable phosphatides? These consist of calcium and magnesium salts of PA and LPA and variable amounts of PE. Their molecular weights are 738 or less, so should a factor of 23.8 or less be used?

The chromatographic method also discussed by James (1955) was developed in accordance with the observations on miscella refining by adsorption (Kaufmann, 1940; Kaufmann and Schmidt, 1940) by using alumina as an adsorbent (Linteris and Handschumaker, 1950). However, this chromatographic method was also found to be inexact since this method indicated refining losses where the Wesson method showed none (Hartman and White, 1952). Finally, Naudet et al. (1953b) found that the eluate still contained free fatty acids and/or phosphatides and, therefore, demonstrated an incomplete separation; furthermore, the column also retained some triglycerides. Nevertheless, this method forms the basis

of the AOCS Official Method Ca 9f-57 (Neutral Oil and Loss, 1993).

So a new and improved chromatographic method was developed by Pardun (1959) that was indeed found to be superior. However, this author also established that the chromatographic method arrives at slightly higher values than the Wesson method. It probably also incorporates partial glycerides into the loss and it should be possible to correct for these partial glycerides by determining their content by gas-liquid-chromatography (GLC) after silylation.

Yield loss during alkali refining need not only result from NOL but can also result from the saponification of triglyceride oil "because of the excess of refining agent used. This action is autocatalytic, since any soap produced dissolves oil, which can react with the unused saponifying agent already present in the soap to produce more soap" (Sadler, 1954). Andersen (1962) reports a laboratory experiment involving neutralised groundnut oil being treated with caustic soda and various amounts of soap. He concludes that an increase in caustic strength causes more saponification and that "this was very much greater if soap were present." He also reports that loss by entrainment was less than by saponification.

A method that distinguishes between entrained and saponified oil has been developed (Naudet et al., 1953a) and followed up by a systematic study of factors affecting oil saponification during alkali refining (Naudet et al., 1957). This study concludes that the way to avoid oil loss by saponification is avoiding long contact of the oil at elevated temperature with an excess of strong lye.

Other authors have also quantified oil loss by saponification (Crauer and Sullivan, 1961; Braae, 1976), but quite a few authors (Bergman, 1961; Laat et al., 1984) just mention saponification without demonstrating its occurrence. In fact (M. Naudet, personal communication), batch alkali refining processes may well have suffered from some oil loss due to saponification of triglyceride oil, but contact times in continuous alkali refining processes are so short that oil saponification in these processes is highly unlikely.

If so desired, establishing their occurrence and determining their extent in industrial refining processes could be easily realised by alkali refining a mixture of, for example, a fully refined lauric oil and a nonlauric oil still containing free fatty acids. Saponification should then cause free lauric acid to be formed. So, taking a sample of the soapstock and immediately cooling or acidulating the sample to prevent saponification of entrained lauric oil and then analysing for lauric acid, should provide the answer.[16]

[16] The refined oil mixture can be disposed of by full hydrogenation and subsequent interesterification leading to a margarine hardstock (Delfosse, 1971; Graffelman, 1971).

The approach taken by the manufacturers of centrifugal separators when guaranteeing the performance of their equipment is usually based upon the inevitable loss or "Theoretical Loss" (TL) defined as (in percent on crude oil):

$$TL = FFA + Phosphatides + Moisture + Impurities + 0.3$$

The guarantee then limits the actual loss to $1.25 \times TL + 0.3$ (%), which may indicate that these manufacturers want to err on the safe side, like most people.

In this context, an adjustment should be made in the case of oils with a fatty acid composition having an average relative molecular mass that differs significantly from oleic acid. The FFA is commonly expressed as % oleic acid, but the analysis involves a titration. Although all fatty acids behave identically with respect to their acidity, their average relative molecular masses differ. Accordingly, FFA present in a lauric oil, like palm kernel oil, weigh less than the FFA present in, for example, soya bean oil with the same acidity. The TL has to be adjusted in accordance with the average relative fatty acid molecular mass of the oil or fat concerned (see also Section 6.3 and Table 6.2).

3.5.2 Refining processes using caustic soda

Alkali refining may originate from Europe, but it was first used in the U.S. The process developed there, and that is still in use, is referred to as the "Long-Mix" process. It started as a batch process, but with the development of centrifugal separators, their use was recommended in continuous processes (Resines, 1927). In Europe, where a wide range of oils is being processed and crude oil quality can be quite variable, the "Short-Mix" process was developed to accommodate all these different raw materials. It has almost entirely replaced the Long-Mix process.

The third industrial process to be discussed is the Pellerin-Zenith process. This process is quite different in that its starting material must be phosphatide-free, does not employ a centrifuge, and does not enjoy an economy of scale. It easily accommodates type changes so it is useful for speciality oils. Finally, the industrial miscella refining process will be discussed as well as a number of processes that have been suggested in the literature.

3.5.2.1 Long-Mix process

The Long-Mix process has been developed for the refining of cottonseed oil and colour reduction is an important aspect of the process. Since the colouring matter can be extracted with alkali, some free alkali must be present after soap formation. The alkali can be added all at once, but the "European manner of refining certain dark cotton oils, particularly Egyptian crudes, entails adding a relatively small amount of caustic solution to remove substantially all the free fatty acids and, after settling and

removal of the soapstock, giving the partially refined oil a number of washes with very dilute caustic solutions, separating the dark-coloured aqueous layer after each wash and, thus, successively reducing the colour of the oil until it meets the standard required" (James, 1958).

The Long-Mix process on the other hand, uses an excess of alkali, and to suppress saponification of triglyceride oil (?), it operates at low temperature. Just as in the batch process from which the Long-Mix process has been derived, the temperature is raised when a "break" is observed to ease separation (Sullivan, 1954). Figure 3.13 shows a flow diagram of the process. This diagram does not include the addition of a degumming acid, such as phosphoric acid, which, according to Erickson (1995), is optional but also recommended. However, adding the acid to the day tank (Erickson, 1995) is probably less effective than the SuperMix treatment that is also mentioned (Wiedermann, 1981, Table III). For cottonseed oil, a treatment with food-grade phosphoric acid lasting 4 to 8 hours is mentioned (O'Brien and Wan, 2001), but nothing is said about how it is mixed into the oil.

This raises the question of what reactions are involved in the removal of phosphatides and NHP in particular during the Long-Mix process. According to Boekenoogen (1941), phosphatides are saponified during alkali refining. Hvolby (1971) measured residual phosphorus, calcium and magnesium levels after treating soya bean oil at various pH-values with a range of complexing and precipitating reagents. He noted residual phosphatides over almost the entire pH-range, but only after a pH of 14 was reached, they suddenly dropped to zero.

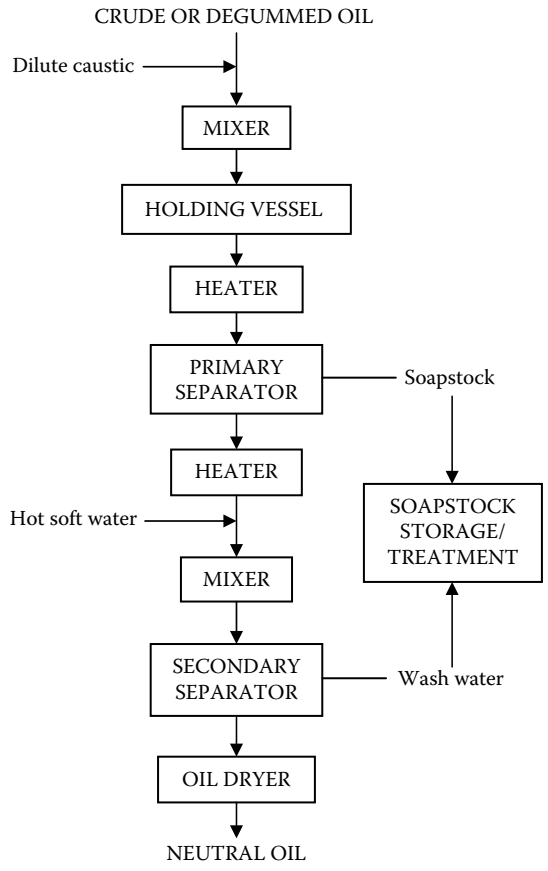


FIGURE 3.13 Long-Mix process.

However, he does not provide an explanation of this observation and a statement such as: "The sodium hydroxide in the form of a water solution will react with the FFA and, if in excess, with the phosphatidic material as well as with a portion of the triglycerides" (Sullivan, 1968) is not very specific about the reactions involved. Other statements like: "The phosphatides and gums absorb alkali and are coagulated through hydration and degradation," or "Phosphatide reduction during refining is determined largely by the amount of water present in the caustic solution" (O'Brien and Wan, 2001) are not very explicit either. Similarly, statements as: "Soybean oil, unlike hard oils or high-acid oils, requires longer effective mixing times for contact with phosphoric acid, particularly in degumming, and for contact with alkali in the refining sequence" (Wiedermann, 1981) are not explained any further.

Hvolby (1971) on the other hand, also shows that complexing reagents, such as EDTA, and reagents that precipitate calcium and magnesium cations, such as the fluoride, citrate, oxalate, tartrate, carbonate, and phosphate anions, are effective in lowering residual phosphorus levels and that their effectiveness is pH-dependent.

Accordingly, patents have been granted to include an inorganic phosphate (Rini, 1950), a tartaric compound (Rini, 1952; Carlson, 1954), EDTA and its salts (James, 1953), or EDTA and at least one "emulsion breaking agent" from the group of alkali metal borates, pyrophosphates, etc. (Julian and Iveson, 1956), citric acid (Ziels, 1954), phosphoric acid (Sullivan, 1955a), casein or albumin (Bloem, 1962), a hydrotrope, such as salts of aryl sulphonic acids (e.g*.* sodium xylene sulphonate) (Dawkins and Brownlee, 1967), or mononuclear sulphonates of alkyl aryl sulphonates (Koebner and Thornton, 1969; Marsden *et al.,* 1972) or just a soluble silicate (Hernandez and Rathbone, 2002).

The use of carbonates has also been claimed and will be discussed in Section 3.5.3. In fact, the use of these various reagents could well be necessary since "traces of phosphorus remain in soya bean and other vegetable oils even after caustic refining" (Bailey, 1946) and "it has for a long time been known that certain oils, especially soyabean and rapeseed oils, do contain phosphatides that cannot be removed completely even by repeated treatment with caustic" (Braae, 1973).

Figure 3.13 does not mention process conditions, but according to Erickson (1995), the caustic strength to be used for soya bean oil in the Long-Mix process is 14 to 18° Baumé or 2.6 to 3.6 N (see Andersen, 1962, p. 46; or

Farr, 2000, pp. 146 to 148). The excess to be added is 0.12 to 0.15% absolute and calculated on the oil for nondegummed oil and 0.10 to 0.12% absolute (or 0.01 to 0.05%, according to Farr, 2000) for degummed oil. Higher figures of 0.3% are also mentioned (Sullivan, 1968), which means that an oil with 1% FFA is then treated with a 210% excess relative to this FFA content. Other oils than soya bean oil containing less phosphatides can use stronger caustic (Blake Hendrix, 1990).

Mixing can be by an in-line mechanical mixer or by a static mixer (Bor, 1972). In the holding vessel, the oil is gently agitated to ensure uniformity, but breaking up of the emulsion or the soap agglomerates formed should be avoided; the holding time is about 15 minutes and the design of the vessel should encourage a plug flow. From the holding vessel, the oil is pumped through a heat exchanger that raises the temperature from ambient to 70 to 75°C and breaks the emulsion, to the primary centrifugal separator; oil leaving this separator should contain less than 200 ppm soap.

This soap can then be removed by a single water wash as illustrated in Figure 3.13 or two water washes, whereby water can be saved by operating counter-currently (Hund and Rowe, 1939). Washing the soapy oil with slightly acid water will convert the soap into FFA that can then be removed by steam stripping. The soaps can also be removed by treating the soapy oil with a silica hydrogel like Trisyl® or Sorbsil®. This has the advantage that residual phosphatides are removed at the same time. (Other aspects of the use of this adsorbent will be discussed in Section 3.7.4.2.) Finally, the oil is dried by spraying the oil in a vacuum dryer held at some 50 mbar, and then lowered in temperature by heating incoming oil to save on energy.

3.5.2.2 Short-Mix process

According to Braae (1973), the Short-Mix[17] process was introduced at the end of the 1940s "to process poor and unstable quality feedstocks from a wide variety of vegetable and animal oils which was typical of the 1950s European refineries" (Blake Hendrix, 1990). As shown by the flow diagram in Figure 3.14, the Short-Mix process (Klein and Crauer, 1974) starts by heating the oil to be alkali refined to 70 to 95°C. At this temperature, no real emulsion is formed by the soap, but instead, the soapstock immediately agglomerates to form large particles of low oil content.

Then a degumming acid is metered into the oil stream, whereby phosphoric acid is the most commonly used. Only refineries facing severe phosphate discharge problems or penalties will use the more expensive citric acid to decompose the NHP present in the crude oil. The amount of 85% phosphoric acid varies between 1 to 3 kg per tonne of oil.

[17] The process is also referred to as the Quick Mix process by James (1958) and as the "*De Laval Short-Mix"* or DLSM by Braae (1953).

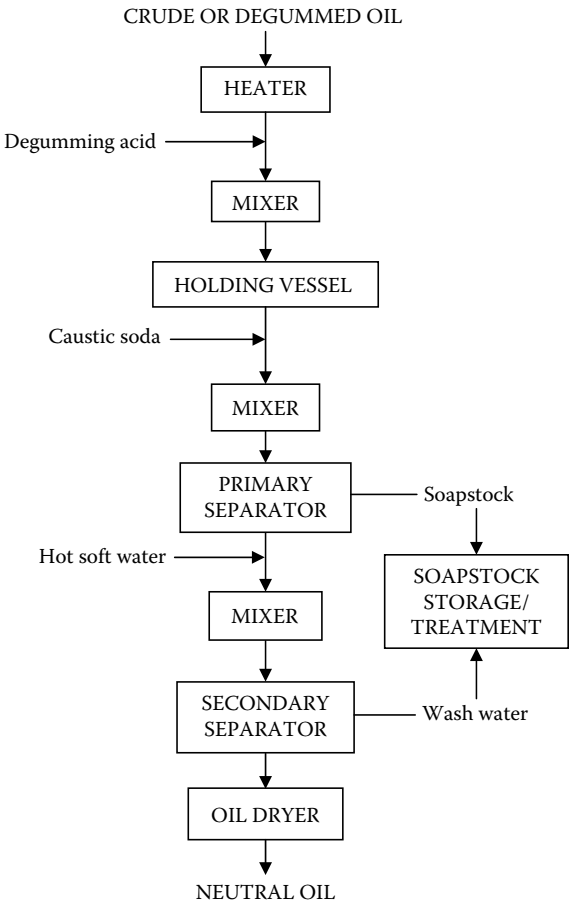


FIGURE 3.14 Short-Mix process.

The acid is mixed into the oil in a high-shear knife mixer to attain a fine dispersion that ensures a rapid reaction between the acid and the NHP as a result of the high interfacial surface and short diffusion distances. Accordingly, the holding time need not exceed 2 minutes.

Thereafter, caustic of 20 to 28° Baumé (4.1 to 6.9 normal) is mixed into the oil to neutralise both the degumming acid and the FFA. Given the pK_a of the twice-dissociated phosphoric acid of 12.32 (cf. Table 3.5), only the equivalent of two of the acid groups has to be added. A relative excess of caustic of 10% is customary. An even shorter contact time between caustic and oil can be realised in the Ultra Short Mix method where the caustic is introduced directly into the spindle of the centrifugal separator (Braae, 1973).

The oil leaving the primary separator will contain up to 500 ppm of soap, which can be removed by washing with some 5% demineralised water. With modern equipment, the soap content can be reduced by 95% per washing stage so that single water washes are now quite common. Using slightly acidified water to acidulate the soaps into FFA that are removed during the subsequent steam stripping stage is also possible. The washing water can also be regenerated by passing it through a cation exchange column (Eisenhauer et al., 1970; Beal et al.,

1973). Washing the oil with an EDTA solution (Deffense, 1996) will not only convert the soaps into FFA, but also remove other metal ions, such as iron. Removal of small amounts of residual soaps by adsorption into a silica hydrogel (Sorbsil® or Trisyl®) is also a possibility, but apparently less commonly used in Europe than in the U.S.

Instead of using an excess of caustic soda in the Short-Mix process, a stoichiometric amount or even lower amounts (Afzelius and Lindgren, 1954; Dron, 1954) can be used in the first stage of the process. Additional lye is then used in a subsequent stage of the process (Kaufmann and Mukherjee, 1965b; Kaufmann and Mukherjee, 1965c). The oil and the lye can also be processed countercurrently (Hund and Rowe, 1939). The oils can also be reheated after the first stage (Afzelius, 1956a) and instead of just caustic soda, a mixture of a strong base and a salt of a strong base and a weak acid can be used in the second stage (Afzelius, 1956b). High shear mixing was found to improve (lighten) the colour of cottonseed oil (Feuge et al., 1956).

3.5.2.3 Miscella refining processes

For logistic and cost reasons, the miscella refining process, which dates from 1952 (Cavanagh, 1958), must be carried out at the extraction plant while this is running. The strength of the miscella being refined is 65 to 80% oil, which is far higher than the 22 to 30% oil content of the full miscella leaving the extractor. Accordingly, any prepress oil present can be mixed with miscella prior to the refining stage and the miscella itself can be the concentrated product leaving the first evaporator (Blake Hendrix, 1984). A miscella strength of <70% has also been recommended (Zeitoun et al., 1962) since refining losses were found to be proportional to miscella viscosity.

As explained by Cavanagh (1990),[18] miscella refining is simple and consists of the following basic processing steps:

- The miscella strength is controlled to within 1% of target value and if necessary, its temperature is reduced to 40°C.
- A degumming acid (phosphoric acid or citric acid) is added and dispersed while using a high shear mixer. "With all other processing conditions remaining the same, an increase of acid pretreatment improves the refined and bleached oil colour" (Crauer and Pennington, 1964).
- Caustic soda at 10 to 14° Bé (Defromont and Douard, 1967) is added and dispersed with one or more static mixers (Bor, 1972).
- The miscella temperature is raised to some 65°C by pumping it through a heat exchanger.
- The mixture is allowed to react at this temperature for some 10 minutes.
- The temperature is lowered to 45°C.[19]
- The mixture is passed to a hermetically sealed centrifugal separator and split into a refined miscella stream and a soapstock stream.

The soapstock stream is preferably sent with the marc to the toaster in the extraction plant to make the meal less dusty (Cavanagh, 1956). Besides, separate acidulation and solvent recovery systems would be too expensive. The refined miscella stream is sent to the evaporator section. Its residual soap content can be lowered by passing the miscella through an enclosed filter with a precoat and body feed of 1:1 by weight of diatomaceous earth and acid-activated bleaching earth. It can also be washed with acidulated water (Cavanagh, 1958).

Although the process has, among others, the advantage that it produces a lighter coloured oil and that neutral oil loss is reduced, its being tied to a running solvent extraction plant, and the additional investment in explosion proof equipment are serious drawbacks. A further disadvantage of the process outlined above is that the soapstock can be sticky and awkward to handle (Naudet et al., 1958).

Hence, the interest in using an additional solvent, such as isopropanol, to dissolve the soaps in a separate liquid phase in a patented process (Van der Voort, 1957) and also reduce the NOL (Kaufmann and Mukherjee, 1965d). It is necessary to use a secondary alcohol like isopropanol since primary alcohols would form fatty acid esters via the base catalysed interesterification reaction, but this reaction has not prevented other inventors (Vassilevsky and Bostroem, 1968) from claiming the use of ethanol. However, as illustrated by the flow sheet of the De Smet process (Van der Voort and Debrus, 1961), there is no clear separation between the hexane phase and the isopropanol phase so that an interphase has to be regularly withdrawn and treated to recover the solvent (Defromont and Douard, 1967).

Another miscella refining process (Keating, 1973) that is also tied to the extraction process employs a single-phase mixture of acetone, hexane, and water (King and Frampton, 1960). If water or an aqueous solution of caustic soda is added to this mixture, it will form two separate phases, one of which is the soapstock phase. This can be separated by centrifuge; it is unlikely the process has ever been industrialised.

[18] Cavanagh presented this paper at an AOCS World Conference held in 1989 in Maastricht. However, the paper on "Cottonseed oil processing and utilization" presented by O'Brien and Wan at a similar conference held in 2000 at Cancun, no longer mentions miscella refining.

[19] A brochure (JMF/svh/270696) by De Smet-Ballestra mentions a temperature of 60–65°C during the separation stage. It also gives a refining factor of 1.10 for the miscella refining process as opposed to a factor of 1.33 for the conventional process. On the other hand, Blake Hendrix (1984) limits the temperature to below the hexane boiling point of 59°C.

The Vaccarino process (1960), on the other hand, has been used industrially (Vaccarino and Vaccarino, 1957; 1958; 1959; 1961). It is also tied to an extraction plant using acetone as the extraction solvent. The resulting miscella is treated with strong (45 to 50%) caustic soda and, then, hot water is added to the resulting mixture. This leads to a solution of soap in an acetone/water mixture and refined oil still containing 8 to 10% acetone. Recovering the acetone from the soap solution does not require that much energy since the acetone has the lowest boiling point. Prior acidulation of the solution is recommended to prevent foaming. The process does not suffer from a phosphatide interphase since the phosphatides, being insoluble in acetone, have not been extracted from the oilseeds.

3.5.2.4 Low caustic strength processes

Whereas the Long-Mix process utilises a lye strength >2.6 N and the Short-Mix process employs even higher lye strengths, there are also processes employing much lower strength caustic soda solutions. There is the Unilever high temperature alkali refining process (Seip, 1967; Seip, 1970) that employs temperatures above 105°C and a caustic soda strength between 0.05 N and 2.0 N. Another Unilever process (Eichler et al., 1966) employs the same caustic strengths, but operates at a lower temperature in a kind of counter-current packed extraction column. Both processes claim low loss of neutral oil but are not used industrially.

In this respect, these processes differ from the Pellerin-Zenith process that is still offered for sale by Campro International Inc. (Cavanagh, 1990). In this process (Bergman, 1964; 1965; 1968), the oil to be refined is allowed to rise as small droplets through a dilute caustic solution that in industrial installations has a height of 3 m. By the time they float on top of the lye, the FFAs have been converted to soaps that dissolve in the 0.35 N (2.0 Bé) caustic.

However, it is necessary to remove all gums before introducing the oil into the lye column. This is done by adding 0.01 to 0.03% by weight of phosphoric acid to lauric oils and animal fats or 0.1 to 0.4% by weight to seed oils and drying by evacuation, so that the gums can then be removed either by filtration while using a filter aid (diatomaceous earth) or by centrifuge.

Moreover, the oil droplets must all have the same diameter so that they rise at the same speed. If not, large droplets would rise faster, meet smaller ones, coalesce, rise still faster and still contain FFAs when reaching the top of the lye column. Consequently, droplets must be spaced so that the throughput of a Zenith installation is only 1.0 to 1.5 tonnes/m^2/h. The throughput of the equipment designed for a co-current variant (De Man, 1968) might well be even lower. Since the construction of such installations hardly profits from an economy of scale, their use is limited to low tonnage, speciality oils and/or high-acidity oils, such as palm oil (Hoffmann, 1974). Finally, it should be mentioned that the data provided in the patent examples (Bergman, 1965) do not support the claim that the NOL in the Zenith process is quite low (Hoffmann, 1973). The high fatty acid content of the acidulated soaps is due to oil saponification rather than too a low NOL.

3.5.3 Refining processes using bases other than caustic soda

Using potassium hydroxide instead of the much cheaper sodium hydroxide is not that obvious, but it has advantages in that the aqueous effluent resulting from the splitting of the potassium soaps can be profitably used as a fertiliser in agriculture (Daniels, 1990). If phosphoric acid is used as degumming acid, this effluent will also contain phosphates in addition to the sulphates resulting from the soapstock acidulation with sulfuric acid. Furthermore, the alkali refining step using potassium hydroxide is claimed to lead to a lower NOL, a lower soap content after the primary separator and a less viscous soapstock (Daniels, 2003).

3.5.3.1 Ammonia

The idea to use ammonia in the alkali refining of edible oils originates from Germany in that Wilhelm suggested in 1925 the use of mixtures of equal parts of water and alcohol into which some gaseous ammonia had been dissolved as a solvent for the ammonia soaps. This mixture is heavier than oil so that the neutral oil floats on the soap solution. If the mixture contains only 4 to 10% of water, the oil or fat lies below the solution. Subsequently, Wilhelm (1929) suggested the use of his ammoniacal solution in a miscella refining process. One of the advantages he mentioned (Wilhelm, 1925) is that the ammonia can be regenerated because the ammonium soaps of fatty acids decompose on distillation into ammonia and FFA. This means that no soapstock acidulation plant is necessary and that its high BOD effluent is thereby also avoided.

The use of ammonia in an extractor to immobilise any soaps present and produce acid-free oil has also been suggested (Leimdörfer, 1931). In the U.S., Rosenstein (1933; 1934) suggested the use of liquid ammonia as a neutralising agent and when ethanolamine was put forward as neutralising agent, it was also suggested (Ashworth, 1939) to treat the oil with a weak alkali solution after the amine treatment, whereas Van Dijck (1942) suggests a counter-current extraction of oil with ammoniacal isopropanol, whereby additional ammonia is injected at the last extraction stage. It was much later that Pardun (1979) carried out a systematic study demonstrating that the ammonia treatment itself is about as effective in removing phosphatides as water degumming.

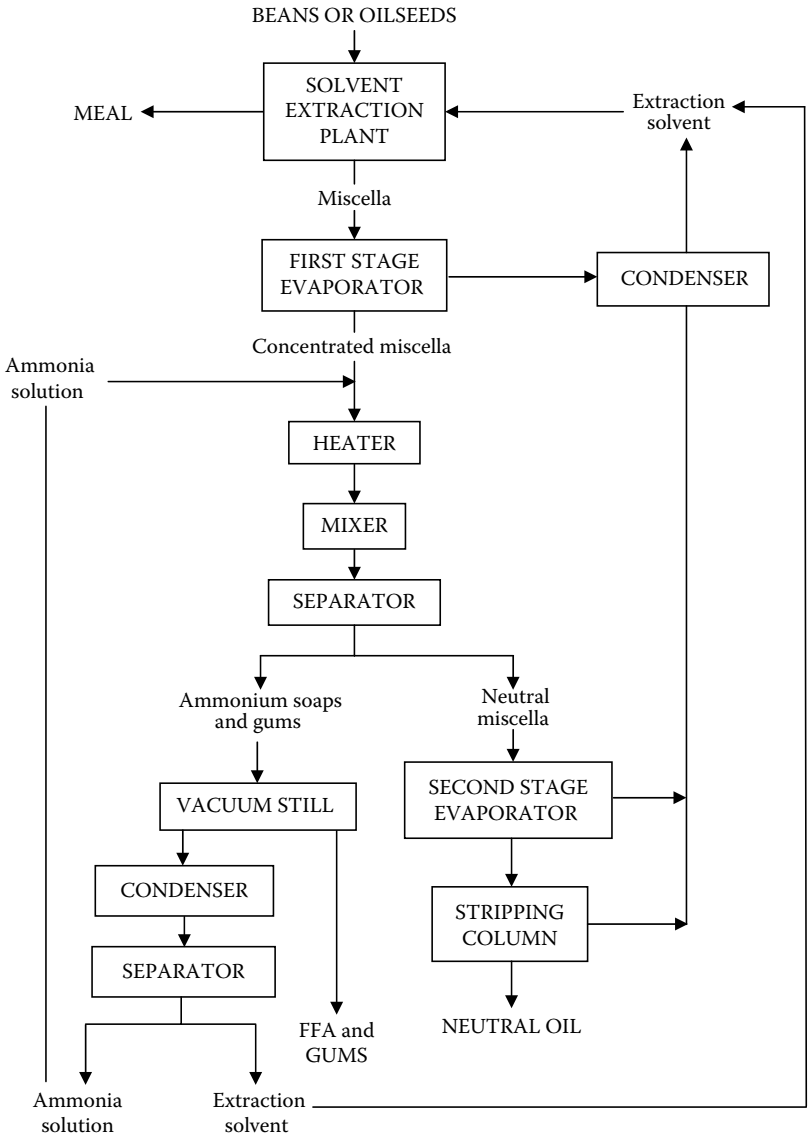


FIGURE 3.15 Flow diagram of miscella neutralisation by ammonia.

However, when Clayton studied the use of ammonia as the neutralising agent (1954; 1956), little was known about NHP as yet. The process according to the flow sheet as shown in Figure 3.15, leads to neutral oil that still contains some NHP. In this process, the refinery is an integral part of the extraction plant although the patent itself (Clayton, 1954) is much wider.

Miscella is first of all concentrated in the first stage evaporator before being treated with aqueous ammonia, which removes the FFA by converting them to ammonium soaps and also removes the hydratable phosphatides. After their removal, the neutral oil is recovered from the miscella by further evaporation and steam stripping as in the production of crude oil. The extraction solvent is also recuperated from the ammonium soaps and gums fraction. Heating this fraction in the still will also decompose the ammonium soaps so that the ammonia can also be recuperated and recycled.

The presence of NHP in the neutral oil can be avoided by neutralising an oil that has already been fully degummed by acid refining, or by treating the oil with a caustic soda solution after it has been neutralised with ammonia (see Pardun, 1979, Table 4). Such a caustic treatment is advocated for oil colour reasons anyway (Zeitoun et al., 1963).

3.5.3.2 Soda ash

Such a caustic treatment is also necessary when a weak base, like sodium carbonate, is used to neutralise the FFA (Clayton, 1940). This weak base has the advantage that it does not saponify neutral oil and, in addition, the soapstock entrains little neutral oil so that a high yield is claimed (Mattikow, 1942). The original full soda

ash process can be divided into the following stages (James, 1958):

1. A continuous stream of crude oil, heated to some 60°C, is mixed with an amount of soda ash solution that is proportional to the FFA, but is also in two- to threefold excess to prevent the formation of carbon dioxide.
2. The mixture is then heated to 90 to 95°C and dried in a dehydrating tank kept under vacuum. Free carbon dioxide also evolves there.
3. From the dehydrating tank, the mixture of oil and partially dehydrated soapstock is rehydrated by means of 3 to 4% of strong (20° Bé) sodium carbonate solution.
4. This mixture is then passed to a centrifugal separator and split into an oil stream and a soapstock stream.
5. The oil stream is cooled to below 40°C before being mixed with strong caustic (20 to 30° Bé) to reduce the oil colour.
6. After removal of the dark caustic solution, the oil is washed with water.

The process outlined above was subsequently simplified by omitting the dehydration and rehydration stages (Clayton, 1952). Less soda ash could be used when a de-aeration tank was included (Smith and Ayres, 1956; Ayres and Smith, 1958), by removing carbon dioxide in the absence of substantial dehydration (Thurman, 1959a; 1959b), or by pressurising the system (Sullivan, 1955b).

As pointed out by Schmitt (1963), soda ash hardly removes any NHP (which he refers to as "lipoid B"). Washing a miscella with a saturated solution of aqueous sodium carbonates only removes 10 to 15% of the phospholipids (Stefanov and Gardev Marinov, 1979). The use of a degumming acid is hardly feasible when using soda ash since the acid will cause a violent evolution of carbon dioxide. Accordingly, Schmitt uses a mixture of soda ash (about 3% of a 24° Bé solution) and caustic soda (0.65 to 1.45% of 24° Bé) to neutralise the non-degummed soya bean oil and remove the NHP. The reactions involved in their removal are anybody's guess. Perhaps this is the reason why the process fell into disuse in the 1960s, but the current availability of fully degummed oils could in principle re-awaken interest in the process. It has a high yield and avoids soapstock splitting since the soapstock can be included in animal feed products as such (Clayton, 1961).

3.5.3.3 Calcium oxide

Apart from caustic soda, ammonia and soda ash, the literature mentions quite a few other reagents that have been used to remove free fatty acids from edible oils. Among the bases used, Andersen (1962) and Kaufmann et al. (1965a) mention lime (calcium hydroxide). "Although refining losses generally are small, the alkaline earth neutralization processes have not been used widely in practice; filtration difficulties arise through slimy soaps unless the lime soaps are quite dry and in suitable granular form." In addition, residual FFA levels attainable with just calcium oxide can be quite high (Myers, 1997).

However, by first adding a small amount of lye to the oil to be neutralised and then adding quicklime (calcium oxide), Myers (1997; 2000) observes very low residual FFA levels. Heating the reaction mixture will cause the calcium soaps to "curdle" so that they can then easily be removed by filtration, whereby the soaps retain very little neutral oil. The process is referred to as the "Dry Refining Process" (DRP). Since calcium oxide is cheap and the calcium soaps can be added to animal feed, the DRP certainly offers potential advantages.

Another instance whereby soaps are reported to retain very little oil is the Bataille vacuum neutralisation process (see Fritsch, 1931, p. 687; or Andersen, 1962, p. 50). Oil is neutralised with only a small excess of lye and then dried under vacuum. This generates dry granular soap in such a form that very little oil is occluded. The reason why this seemingly simple process has not reached industrialisation is not immediately obvious.

3.5.4 Miscellaneous refining processes

All refining processes discussed above aim at the neutralisation (also referred to as "saponification") of the FFA present in the oil. As will be discussed in Section 3.9.4, free fatty acids can also be removed from the oil by evaporation during a vacuum stripping process. Other means of removal comprise solvent extraction and esterification, which will be discussed below.

3.5.4.1 Refining by solvent extraction

In the early 1940s, several solvent fractionation processes were developed for the mineral oil industry. In the edible oils industry, the term "solvent fractionation" has acquired a specific meaning and nowadays stands for "fractional crystallisation from a solvent" (Section 4.4.2.1). Formerly, the term also included processes during which oils and molten fats were subjected to a liquid-liquid extraction process employing one or more extraction columns. The purpose of such a "solvent fractionation" using furfural could be the production of two fractions of different IV from soya bean oil (Gloyer, 1949). The high IV fraction would be ideal for paints and varnishes and the low IV fraction would hopefully be more stable than straight soya bean oil (Hixson and Miller, 1953).

Treating oils (Mattikow, 1950) and tallow (Moore, 1950) with liquid propane removed gums and colouring compounds and thereby caused the acidity to decrease; hardly any fatty acids were removed. Passino (1957)

performed this in a two-step process that removed the high molecular FFA by solvent extraction under paracritical conditions with a low boiling solvent, such as propane, and the residual FFA by vacuum stripping.

Witte and Sipos (1962) used anhydrous methanol to extract soya bean oil at temperatures well below the methanol boiling point to minimise oil dissolution, and produce a xanthophyll concentrate to be used in poultry feed. This concentrate also contained 58% triglycerides and 25% FFA, some acetone insoluble and, of course, the xanthophyll pigments. The oil phase may contain from 5 to 8% methanol, which can easily be removed by vacuum stripping. If the raw material is degummed, the extracted oil no longer needs refining or, in other words, it has already been neutralised by the methanol extraction. However, this extraction will also remove partial glycerides and have the disadvantage of giving a lower yield than alkali refining.

Zosel (1974) is more specific about FFA removal in his extraction process for degummed oils employing supercritical carbon dioxide. This process also removes malodorous compounds and a lot of oil. An amount of 1.5 kg was lost from a starting amount of 5 kg. Given also the innate expense of supercritical extraction (see Section 3.3.2.5), it is not surprising that it has not been exploited. Nevertheless, Brunetti et al. (1989) tried to extract FFA from various olive oils with supercritical carbon dioxide and noted a low selectivity and, thus, a poor yield; these findings were confirmed by Gonçalves et al. (1991). An improvement was attained by using up to 20% ethanol as an entrainer when extracting comminuted sunflower seeds (Cocero and Calvo, 1996). A good selectivity was observed for the removal of polychlorinated biphenyls from fish oil by supercritical carbon dioxide extraction (Krukonis, 1989), but the author does not mention what happens to the FFA. Seed oils extracted with supercritical carbon dioxide turn out to be less stable than normally processed oils (List and Friedrich, 1989).

Aqueous isopropanol is a much better solvent for extracting FFA from oil (Shah and Venkatesan, 1989). Aqueous isopropanol (30% IPA) can also be used to extract soaps (Defromont and Douard, 1967) by dissolving an alkali into the aqueous IPA; phosphatides are also quite soluble in aqueous IPA. Since catalysis of ester interchange by alkali is limited to primary and secondary alcohols, there is no risk of triglyceride alcoholysis. Triglyceride oils are totally insoluble in aqueous IPA and the IPA itself will hardly dissolve in the oily phase. It has been suggested (Dijkstra, 2004) to use ammonia as a base in this refining process by extraction, since this would allow soapstock splitting by heating and reusing the ammonia. As pointed out, such a novel process would have to compete against the economy of scale of the established processes and involve a risk as well, which does not make it an attractive proposition.

Extracting a hexane miscella with aqueous ethanol that has been saturated with hexane (Türkay and Civelekoğlu, 1991a; 1991b) has also been studied and a mathematical model has been built that allows the extract and raffinate compositions to be calculated as a function of the water content of the ethanol, the solvent/miscella ratio and the number of stages. On the other hand, Andersen (1962) points out that a complete separation between fatty acids and triglycerides has been found to be impossible since the solubility of triglycerides in alcohol increases as and when their fatty acid content increases. Moreover, given the perceived desirability of avoiding the use of solvents, adoption of FFA removal by solvent extraction seems unlikely.

3.5.4.2 Refining by esterification

Andersen (1962) mentions deacidification by esterification as a way to reduce the neutral oil loss during the alkali refining of high acidity oils, such as olive husk oil, and as a means of combating fat shortages in, for instance, wartime Germany. This even led to ethylene glycol being used instead of glycerol (Schlinck, 1919) because the latter compound was urgently needed for explosive manufacture. Nowadays, fatty acid esters of ethylene glycol are no longer food grade.

Heating a fat comprising both free hydroxyl groups and FFA will lead to esterification when it is heated to 220 to 240°C, but the use of a catalyst, such as tin chloride or zinc chloride, allows a lower temperature to be used. However, these catalysts also catalyse the interesterification of the triglycerides (Van Loon, 1926) so that their use may lead to a quite different oil or fat. The use of acid activated bleaching earth as the esterification catalyst has also been studied (P.J. Maes, personal communication) when an attempt was made to arrive at an oil with a low partial glyceride content. Since the rate of esterification will slow down when the partial glyceride content has decreased by esterification, a large excess of FFA was added to the reaction mixture. And indeed, heating the "acidified" oil with 2% bleaching earth to 150 to 170°C under vacuum causes the oil to boil because the water being formed evaporates.

A low partial glyceride content can be achieved this way, but, unfortunately, the acid activated bleaching earth also catalyses the *cis-trans* isomerisation of unsaturated fatty acid moieties. This does not matter when re-esterifying high acidity rice bran oil when this re-esterified oil, subsequently, will be partially hydrogenated to provide a hardstock for shortening and the like, but in other applications, the formation of *trans* isomers may be unacceptable. Recently, Meng et al. (2006) report the use of a heteropolyacid, such as tungstosilicic acid, as a catalyst in the esterification of plant sterols with fatty acids. Like bleaching earth, this immobilised heteropolyacid has the advantage that it can be removed from the reaction mixture by filtration and reused. The paper (Meng et al., 2006) does not mention

whether any *trans* isomers are formed from oleic or linoleic acid. A direct question put to the author provided the answer that this aspect would be investigated in a later stage of the research project.

References

Afzelius, I.A. (1956a), Method for continuous refining of fatty oils, US Patent 2,729,662, assigned to Aktiebolaget Separator.

Afzelius, I.A. (1956b), Method of decolorizing neutralized fatty oils, US Patent 2,754,310, assigned to Aktiebolaget Separator.

Afzelius, I.A. and Lindgren, H.O. (1954), Method of refining vegetable and animal oils, US Patent 2,678,936, assigned to Aktiebolaget Separator.

Andersen, A.J.C. (1962), *Refining of Oils and Fats for Edible Purposes*, Pergamon Press Ltd, Oxford, London, New York, Paris.

Ashworth, D.I. (1939), Process for purifying vegetable and animal oils, US Patent 2,157,882, assigned to The De Laval Separator Company.

Ayres, A.U. and Smith, F.H. (1958), Refining fatty oils, US Patent 2,838,553, assigned to The Sharples Corporation.

Bailey, A.E. (1946), Flavor reversion in edible fats, *Oil & Soap*, **23**, 55–58.

Bailey, A.E., et al. (1942), The grading of crude vegetable oils by means of refining tests: a review and evaluation of the method, *Oil & Soap*, **19**, 97102.

Beal, R.E. et al. (1973), Water-recycle washing of refined soybean oil: plant scale evaluation, *J. Am. Oil Chem. Soc.*, **50**, 260–263.

Bergman, L.O. (1961), *Procédé et installation pour le raffinage des graisses*, French Patent 1.274.675, assigned to Aktiebolaget Pellerins Margarinfabrik.

Bergman, L.O. (1964), Method for purifying fat, US Patent 3,147,291, assigned to Aktiebolaget Pellerins Margarinfabrik.

Bergman, L.O. (1965), Process for acid and then alkaline refining fatty oils, US Patent 3,226,407.

Bergman, L.O. (1968), Apparatus for neutralizing fatty oils, US Patent 3,362,794, assigned to Aktiebolaget Aritmos.

Blake Hendrix, W.M. (1984), Current practices in continuous cottonseed miscella refining, *J. Am. Oil Chem. Soc.*, **61**, 1369–1372.

Blake Hendrix, W.M. (1990), Neutralization I. Theory and practice of conventional caustic (NaOH) refining, in *Edible Fats and Oils Processing: Basic Principles and Modern Practices*, Erickson, D.R., Ed., American Oil Chemists' Society, Champaign IL, 94–100.

Blank, E.W. (1942), Chronological list of important dates in the history of the fats and waxes, *Oil & Soap*, **19**, 110–113.

Bloem, E. (1962), *Procédé de désacidification des huiles et des graisses*, French Patent 1.302.929, assigned to Unilever.

Boekenoogen, H.A. (1941), Die Adsorptionsentsäuerung, *Fette u. Seifen*, **48**, 59–60.

Bor, T.P. (1972), Der Kenics Static Mixer® und seine Anwendungsmöglichkeiten in der Verfahrenstechnik, *Fette Seifen Anstrichm.*, **74**, 449–453.

Braae, B. (1953), Kontinuierliche Raffination von Ölen und Fetten, *Fette Seifen Anstrichm.*, **55**, 859–862.

Braae, B. (1973), The vegetable oil refining process. I. Alkali refining. II. Bleaching and deodorisation, *Oléagineux*, **28**, 193–195.

Braae, B. (1976), Degumming and refining practices in Europe, *J. Am. Oil Chem. Soc.*, **43**, 353–357.

Brunetti, L. et al. (1989), Deacidifaction of olive oils by supercritical carbon dioxide, *J. Am. Oil Chem. Soc.*, **66**, 209–217.

Carlson, J.A. (1954), Alkali-refining of fatty glycerides in the presence of a tartaric compound, US Patent 2,678,325, assigned to Unilever.

Cavanagh, G.C. (1956), New integrated refining process for edible oils, *J. Am. Oil Chem. Soc.*, **33**, 528–531.

Cavanagh, G.C. (1958), Refining triglyceride oils, US Patent 2,824,885, assigned to Ranchers Cotton Oil.

Cavanagh, G.C. (1990), Neutralization II: Theory and practice of non-conventional caustic refining by miscella refining and the Zenith process, in *Edible Fats and Oils Processing: Basic Principles and Modern Practices*, Erickson, D.R., Ed., American Oil Chemists' Society, Champaign, IL, 101–106.

Clayton, B. (1940), Refining of animal and vegetable oils, US Patent 2,190,594, assigned to Refining Inc.

Clayton, B. (1952), Refining of glyceride oils, US Patent 2,607,788.

Clayton, B. (1954), Ammonia treatment of glyceride oils, and products resulting therefrom, US Patent 2,686,794.

Clayton, B. (1956), Purification of glyceride oils, US Patent 2,769,827.

Clayton, B. (1961), Process of producing feed products, US Patent 2,991,178.

Cocero, M.J. and Calvo, L. (1996), Supercritical fluid extraction of sunflower seed oil with CO_2-ethanol mixtures, *J. Am. Oil Chem. Soc.*, **73**, 1573–1578.

Crauer, L.S. and Pennington, H. (1964), Continuous refining of crude cottonseed miscella, *J. Am. Oil Chem. Soc.*, **41**, 656–659.

Crauer, L.S. and Sullivan, F.E. (1961), Determining refining loss by the sodium balance method, *J. Am. Oil Chem. Soc.*, **38**, 172–174.

Daniels, R.S. (1990), Acid water recovery; an environmental treatment alternative, in *Edible Fats and Oils Processing: Basic Principles and Modern Practices*, Erickson, D.R., Ed., AOCS Press, Champaign, IL, 430–431.

Daniels, R.S. (2003), Agricultural oils processing using potassium hydroxide, US Patent 6,632,952,B1, assigned to Carrie Lee Mahoney.

Dawkins, A.W. and Brownlee, W.H. (1967), Improvements in the refining of oils, British Patent 1,077,557, assigned to Marchon Products Limited.

Deffense, E.M.J. (1996), Soft degumming, in *Oils-Fats-Lipids 1995*, Castenmiller, W.A.M., Ed., P.J. Barnes & Associates, Bridgewater, England, 125–128.

Defromont, C. and Douard, F. (1967), Quelques progrès récents du raffinage des huiles et graisses sur miscella, *Rev. Franç. Corps Gras*, **10**, 587.

Delfosse, J.K.F. (1971), Margarine fat composition, British Patent 1 244 868, assigned to Unilever.

Desnuelle, P. and Massoni, R. (1953), Pouvoir antisiccatif de certains phosphatides vis-à-vis de l'huile de lin, *Bull. mens. ITERG*, 263–264.

Desnuelle, P. and Micaelli, O. (1950), Quelques remarques au sujet des pertes subies pendant la neutralisation des huiles par les alcalis, *Oléagineux*, **5**, 161–163.

Desnuelle, P. et al. (1946), Sur quelques aspects de la neutralisation des huiles d'arachide par les alcalis, *Industries des Corps Gras*, **2**, 304–309.

Dijck, W.J.D. van (1942), Process for refining fatty compounds, US Patent 2,268,786.

Dijkstra, A.J. (2004), Edible oil processing *quo vadis*, *Eur. J. Lipid Sci. Technol.*, **106**, 77–78.

Dijkstra, A.J. and Meert, D. (1982), Determination of trace elements in oils by plasma emission spectroscopy, *J. Am. Oil Chem. Soc.*, **59**, 199–204.

Dron, D.W. (1954), Refining of vegetable, marine and animal oils containing phosphatides, US Patent 2,683,155, assigned to The De Laval Separator Company.

Eichler, F. et al. (1966), Procédé et appareil pour le raffinage d'huiles glycéridiques, French Patent 1.432.909, assigned to Unilever.

Eisenhauer, R.A. et al. (1970), Water recycle method for washing alkali-refined soybean oil, *J. Am. Oil Chem. Soc.*, **47**, 137–140.

Erickson, D.R. (1995), Neutralization, in *Practical Handbook of Soybean Processing and Utilization*, Erickson, D.R., Ed., AOCS Press and United Soybean Board, Champaign, IL and St. Louis, MO, 184–202.

Farr, W.E. (2000), Refining of fats and oils, in *Introduction to Oils and Fats Technology*, O'Brien, R.D., Farr, W.E., and Wan, P.J., Eds., AOCS Press, Champaign, IL, 136–157.

Feuge, R.O. et al. (1956), Refining cottonseed oil at high rates of shear, *J. Am. Oil Chem. Soc.*, **33**, 344–347.

Fiala, R.J. (1963), Refining soybean oil, US Patent 3,093,667, assigned to A.E. Staley Manufacturing Company.

Fritsch, J. (1931), Raffinage des huiles, in *Fabrication et raffinage des huiles végétales*, Amédée Legrans, Paris, 686.

Gloyer, S.W. (1949), Refining and fractionating soybean oil with furfural, *J. Am. Oil Chem. Soc.*, **26**, 162–166.

Gonçalves, M., et al. (1991), On the application of supercritical fluid extraction to the deacidification of olive oils, *J. Am. Oil Chem. Soc.*, **68**, 474–480.

Graffelman, H.A. (1971), Margarine fat and process for preparing same, US Patent 3,617,308, assigned to Unilever.

Hartman, L. and White, M.D.L. (1952), Wesson loss as a measure of the degree of refining, *J. Am. Oil Chem. Soc.*, **29**, 177–180.

Hernandez, E. and Rathbone, S.J. (2002), Refining of glyceride oils by treatment with silicate solutions and filtration, US Patent 6,448,423 B1, assigned to The Texas A&M University System, College Station, TX.

Hixson, A.W. and Miller, R. (1953), Fractionation of triglycerides, US Patent 2,631,157, assigned to The Chemical Foundation, Incorporated.

Hoffmann, Y.B.L. (1973), Experiences in refining rapeseed oil by the Zenith Process, *J. Am. Oil Chem. Soc.*, **50**, 160A–167A.

Hoffmann, Y.B.L. (1974), Le procédé Zenith et le raffinage de l'huile de palme, *Oléagineux*, **29**, 259–262.

Hund, W.J. and Rowe, D.H. (1939), Fatty matter refining process, US Patent 2,164,189, assigned to Shell Development Company.

Hvolby, A. (1971), Removal of nonhydratable phospholipids from soybean oil, *J. Am. Oil Chem. Soc.*, **48**, 503–509.

James, E.M. (1953), Method of refining oils with "Versene", US Patent 2,638,476, assigned to Unilever.

James, E.M. (1955), The determination of refining loss, *J. Am. Oil Chem. Soc.*, **32**, 581–587.

James, E.M. (1958), The refining and bleaching of vegetable oils, *J. Am. Oil Chem. Soc.*, **35**, 76–83.

Julian, P.L. and Iveson, H.T. (1956), Hydration method of refining glyceride oils, US Patent 2,752,378, assigned to The Glidden Company.

Kaufmann, H.P. (1940), Adsorptionstrennungen auf dem Fettgebiet IV: Die Trennung von Glyceriden durch Adsorption, *Fette u. Seifen*, **47**, 460–462.

Kaufmann, H.P. and Mukherjee, K.D. (1965a), Neuzeitliche Technologie der Fette und Fettprodukte CIX: Die Raffination der Fette, *Fette Seifen Anstrichm.*, **67**, 954–958.

Kaufmann, H.P. and Mukherjee, K.D. (1965b), Neuzeitliche Technologie der Fette und Fettprodukte CVI: Die Raffination der Fette, *Fette Seifen Anstrichm.*, **67**, 614–620.

Kaufmann, H.P. and Mukherjee, K.D. (1965c), Neuzeitliche Technologie der Fette und Fettprodukte CVII: Die Raffination der Fette, *Fette Seifen Anstrichm.*, **67**, 697–701.

Kaufmann, H.P. and Mukherjee, K.D. (1965d), Neuzeitliche Technologie der Fette und Fettprodukte CX: Die Raffination der Fette, *Fette Seifen Anstrichm.*, **67**, 1027–1031.

Kaufmann, H.P. and Schmidt, O. (1940), Adsorptionstrennungen auf dem Fettgebiet III. Die Trennung von Glyceriden und Fettsäuren und die "Adsorptionsentsäurung" der Fette, *Fette u. Seifen*, **47**, 294–296.

Keating, E.J. (1973), Process for refining glyceride oils and fats, US Patent 3,746,731, assigned to USA, Secretary of Agriculture.

King, R.R. and Wharton, F.W. (1948), A comparison of Wesson loss and cup refining loss. Analyses of crude cottonseed and soybean oils, *J. Am. Oil Chem. Soc.*, **25**, 66–68.

King, W.H. and Frampton, V.L. (1960), Production of cottonseed meal of improved quality, US Patent 2,950,198, assigned to USA, Secretary of Agriculture.

Klein, K. and Crauer, L.S. (1974), Further developments in crude oil processing, *J. Am. Oil Chem. Soc.*, **51**, 382A–385A.

Koebner, A. and Thornton, T. (1969), Method of refining vegetable and animal oils, US Patent 3,440,253, assigned to Marchon Products Limited.

Krukonis, V.J. (1989), Supercritical fluid processing of fish oils: extraction of polychlorinated biphenyls, *J. Am. Oil Chem. Soc.*, **66**, 818–821.

Laat, C.I. de et al. (1984), Process for refining glyceride oils, European Patent 0 099 201, assigned to Unilever.

Leimdörfer, J. (1931), Verfahren zur Extraktion säurefreier Öle und Fette, German Patent 525 946.

Linteris, L. and Handschumaker, E. (1950), Investigation of methods for determining the refining efficiency of crude oils, *J. Am. Oil Chem. Soc.*, **27**, 260–264.

List, G.R. and Friedrich, J.P. (1989), Oxidative stability of seed oils extracted with supercritical carbon dioxide, *J. Am. Oil Chem. Soc.*, **66**, 98–101.

Loon, C. van (1926), An improved process for the conversion of neutral or nearly neutral triglycerides, mutually or with other esters, British Patent 249,916.

Man, W. de (1968), Process for continuously deacidifying oils, US Patent 3,419,588, assigned to Unilever.

Marsden, F.C. et al. (1972), Procédé pour le raffinage d'huiles de glycérides, French Patent 2.091.308, assigned to Albright & Wilson Ltd.

Mattikow, M. (1942), A new method of refining oils with non-saponifying alkalies: the Clayton process, *Oil & Soap*, **19**, 83–87.

Mattikow, M. (1950), Refining fatty oils with liquid propane, *J. Am. Oil Chem. Soc.*, **27**, 11–14.

Meng, X. et al. (2006), Synthesis of plant sterol esters catalyzed by heteropolyacid in a solvent-free system, *Eur. J. Lipid Sci. Technol.*, **108**, 13–18.

Moore, E.B. (1950), Decolorization of tallow by liquid-liquid extraction with propane, *J. Am. Oil Chem. Soc.*, **27**, 75–80.

Myers, H. (1997), Dry refining process, *Poster presented at the 88th AOCS Annual Meeting & Expo, Seattle.*

Myers, H. (2000), Method of refining oils and fats, US Patent 6,111,120, assigned to Hyam Myers Consulting PTY. Ltd.

Naudet, M. et al. (1958), Sur quelques essais de neutralisation de miscella, *Rev. Franç. Corps Gras*, **5**, 74–83.

Naudet, M. et al. (1953a), Étude analytique de la neutralisation alcaline des huiles III' Établissement des bilans de neutralisation, *Bull. mens. ITERG*, 378–383.

Naudet, M. et al. (1957), Étude analytique de la neutralisation alcaline des huiles VI. Influence de divers facteurs sur les pertes par saponification au cours de la neutralisation alcaline continue, *Rev. Franç. Corps Gras*, **4**, 142–148.

Naudet, M. et al. (1953b), Étude analytique de la neutralisation alcaline des huiles II. Dosage des glycérides (huile neutre) dans les huiles brutes et neutralisées, Bull. *mens. ITERG*, 274–280.

O'Brien, R.D. and Wan, P.J. (2001), Cottonseed oil: processing and utilization, in *Proceedings of the World Conference on Oilseed Processing and Utilization*, Wilson, R.F., Ed., AOCS Press, Champaign, IL, 90–144.

Pardun, H. (1979), Die Entsäuerung von Pflanzenölen mit Ammoniak. Eine umweltfreundliche Raffinationsmethode, *Fette Seifen Anstrichm.*, **81**, 297–302.

Pardun, H. (1981), Neuberechnung der zur Bestimmung des Phosphatidgehalts benötigten Umrechnungfaktoren, *Fette Seifen Anstrichm.*, **83**, 240–242.

Pardun, H. and Werber, O. (1959), Laboratoriums-Methoden zur Bestimmung des Raffinations-Verlustes von Ölen und Fetten, *Fette Seifen Anstrichm.*, **61**, 1010–1017.

Passino, H.J. (1957), Refining soybean oil, US Patent 2,802,849, assigned to The M.W. Kellogg Company.

Resines, F.J. (1927), Process of refining oils, US Patent 1,619,486, assigned to Byproducts Recovery Company.

Rini, S.J. (1950), Alkali-refining of fatty glycerides in the presence of an inorganic phosphate compound, US Patent 2,507,184, assigned to Unilever.

Rini, S.J. (1952), Improvements in the refining of fatty glycerides, British Patent 676,574, assigned to Unilever.

Rosenstein, L. (1933), Process of refining, US Patent 1,900,132.

Rosenstein, L. and Hund, W.J. (1934), Process for the refining of vegetable and animal oils, US Patent 1,963,505.

Sadler, F.S. (1954), Refining fatty oils, US Patent 2,666,074, assigned to The Sharples Corporation.

Schlinck, J. (1919), Verfahren zur Neutralisation von Fetten und Ölen durch Veresterung der freien Fettsäuren, German Patent 315222, assigned to H. Schlinck & Cie AG.

Schmitt, R.O. (1963), Process for the purification of glyceride oils, US Patent 3,102,898, assigned to The Procter & Gamble Company.

Seip, P.J. (1967), Procédé de raffinage de glycérides huileux, French Patent 1.471.772, assigned to Unilever.

Seip, P.J. (1970), Glyceride oils, US Patent 3,510,510, assigned to Unilever.

Shah, K.J. and Venkatesan, T.K. (1989), Aqueous isopropanol alcohol for the extraction of free fatty acids from oils, *J. Am. Oil Chem. Soc.*, **66**, 783–787.

Smith, F.H. and Ayres, A.U. (1956), Vegetable oil refining by the modified soda ash process, *J. Am. Oil Chem. Soc.*, **33**, 93–95.

Stefanov, K. and Gardev Marinov, M. (1979), Removal of free fatty acids and sugars from petroleum miscella of vegetable oils by washing with a solution of sodium carbonate, *Rev. Franç. Corps Gras*, **26**, 269–272.

Sullivan, F.E. (1954), The hermetic separator as a new approach to vegetable oil refining, *J. Am. Oil Chem. Soc.*, **31**, 361–363.

Sullivan, F.E. (1955a), Refining of fatty oils and fats, US Patent 2,702,813, assigned to The De Laval Separator Company.

Sullivan, F.E. (1955b), Some further data on pressure system centrifugal refining, *J. Am. Oil Chem. Soc.*, **32**, 121–123.

Sullivan, F.E. (1968), Refining oils and fats, *J. Am. Oil Chem. Soc.*, **45**, 564A–615A.

Thurman, B.H. (1959a), Process for refining fatty oils, US Patent 2,876,242, assigned to Benjamin Clayton.

Thurman, B.H. (1959b), Process for refining glyceride oils, US Patent 2,917,525, assigned to Benjamin Clayton (Refining Unincorporated).

Türkay, S. and Civelekoğlu, H. (1991a), Deacidification of sulfur olive oil I. Single-stage liquid-liquid extraction miscella with ethyl alcohol, *J. Am. Oil Chem. Soc.*, **68**, 83–86.

Türkay, S. and Civelekoğlu, H (1991b), Deacidification of sulfur olive oil. II. Multi-stage liquid-liquid extraction of miscella with ethyl alcohol, *J. Am. Oil Chem. Soc.*, **68**, 818–821.

Vaccarino, C. (1958), Nouveau procédé pour l'extraction des huiles par l'acétone et pour la neutralisation au sein du miscella, *Oléagineux*, **13**, 133–136.

Vaccarino, C. (1959), Emploi de l'acétone en huilerie, *Oléagineux*, **14**, 367–369.

Vaccarino, C. (1961), A new industrial process for cottonseed, *J. Am. Oil Chem. Soc.*, **38**, 143–147.

Vaccarino, C. and Vaccarino, G. (1960), Process for neutralizing vegetable or animal oils or fats in a water-soluble solvent, US Patent 2,944,072.

Vaccarino, G. and Vaccarino, S. (1957), Un procédé nouveau pour le raffinage des huiles végétales ou animales, *Oléagineux*, **12**, 623–626.

Vassilevsky, A.N. and Bostroem, T. (1968), Procédé pour retirer les acides gras libres des huiles et graisses animales et végétales, French Patent 1.522.617.

Voort, F.R. van der (1957), Method for the refining of oils and fatty substances, US Patent 2,786,858, assigned to Extraction Continue De Smet.

Voort, F.R. van der and Debrus, O.J. (1961), Procédé de raffinage des huiles végétales, *Rev. Franç. Corps Gras*, **8**, 457–459.

Wiedermann, L.H. (1981), Degumming, refining and bleaching of soybean oil, *J. Am. Oil Chem. Soc.*, **58**, 159–166.

Wilhelm, K.F. (1925), Improvements in or relating to the elimination of fatty acids, resins, bitter and mucuous substances from oils and fats, British Patent 226,767.
Wilhelm, K.F. (1929), Improvements in or relating to the removal or elimination of fatty acids, resins, and bitter and mucuous substances from oils and fats, British Patent 289,801.
Witte, N.H. and Sipos, E. (1962), Method of treating soybean oil, US Patent 3,069,443, assigned to Central Soya Company, Inc.
Zeitoun, M.A. et al. (1962), Refining crude cottonseed oil dissolved in hexane, *J. Am. Oil Chem. Soc.*, **39**, 286–289.
Zeitoun, M.A. et al. (1963), Gaseous ammonia as a refining agent for cottonseed oils, *J. Am. Oil Chem. Soc.*, **40**, 279–283.
Ziels, N.W. (1954), Method of refining oils with polycarboxylic acids, US Patent 2,678,326, assigned to Unilever.
Zosel, K. (1974), Procédé de désodorisation de graisses et d'huiles, French Patent 2.190.909, assigned to Studiengesellschaft Kohle m.b.H.

3.6 Soapstock and by-product treatments

Soapstock[20] originates from the alkali refining of edible oils and fats. It is a complicated and variable mixture (Sonntag, 1990) of water, soaps, phosphatides, and their hydrolysis products, triglycerides and partial glycerides; inorganic and/or organic salts stemming from the use of degumming acids and alkali; particulate matter, such as meal fines, colouring matter, odoriferous matter (e.g., originating from fish oil); and minor constituents, such as sterols and tocopherols, some sugars and glycolipids, etc. (Keith Jr. et al., 1954; Stansbury et al., 1957; Pabst, 1964; Dowd, 1996). It has to be handled with care and attention because when total fatty acids (TFA) are above 30%, the soapstock may solidify and cause pipes to block (Woerfel, 1983).

In a refinery environment, the soapstock may be combined with other waste streams, such as those originating from deodoriser hotwells, grease traps, and incidental streams resulting from tank cleaning operations, deodoriser boil-out treatments, etc., so that a single processing unit can cope with all waste streams containing fat. Several processes to recover fat from aqueous effluents have been described (Nettli, 1977; Jollez et al., 2001; Garro et al., 2003).

According to Boyer (2000) several methods of soapstock disposal are employed routinely, whereby use as an animal feed ingredient is by far the biggest outlet (Woerfel, 1995). It uses acid oils obtained by soapstock acidulation, dried soapstock (Witte and Sipos, 1960; Kruse and Cravens, 1960; Beal, 1981), or soapstock that has been kept fluid and pumpable by the addition of some propionic acid (Evans, 1990). Other outlets comprise sale as raw soap on the open market, disposal by spraying on meal as a fatty additive, and, finally, a neutralisation and partial dewatering treatment that allows the concentrated material to be transported to a central treatment plant for processing. Soapstock from miscella refining is commonly sent to the toaster (Kuk and Tetlow, 2005).

A more recent means of disposal recovers the energy content of the soapstock by burning it (Goemans, 2004; 2005). This avoids the investment involved in soapstock splitting, drying or concentrating, eliminates the need for effluent treatment and saves on fossil fuel. If other refinery by-products or residues can be co-fired with the soapstock, waste management costs are reduced to an absolute minimum.

[20] In 1992, the NOPA (National Oilseed Processors Association) *Trading Rules* were changed to substitute the name *refining byproduct lipid* for *soapstock* in line with the Association of American Feed Control Officials' (AAFCO) definition of a feed ingredient (Woerfel, 1995).

3.6.1 Fatty acid recuperation processes

Fatty acid moieties are present in soapstock as soaps but also in triglycerides because of the neutral oil loss (NOL) during alkali refining, and in phosphatides. Their concentrations depend very much upon the raw material that has been alkali refined and also on the refining process. A low-acidity, nondegummed soya bean oil for example, will yield a soapstock with a relatively high phosphatide content and a low soap content, whereas the alkali refining of high acidity oils, such as palm oil, leads to a soapstock with a relatively high soap content. Because the soapstock is stored at a temperature above 60°C to prevent solidification (Woerfel, 1995) and because excess alkali is used during alkali refining, this excess is available for saponification and causes the soap content of soapstock to increase during storage.

Formerly, soapstock was often sold to a soap factory that was preferably located next door and which could belong to the company running the refinery. With the increase in refinery capacities, this symbiosis dwindled and soapstock had to be treated on site. Whatever fatty acid recuperation treatment is chosen, it leads to an aqueous effluent with a very high biological (BOD) and chemical oxygen demand (COD), which often requires on-site treatment before it can be discharged. Consequently, there is a shift toward acid refining at the oil mill and trade in acid refined oil since the latter can be physically refined and, thus, obviates soapstock treatments and effluent treatment (Dijkstra, 1999a; 1999b).

3.6.1.1 Acidulation

Although batch acidulation units (Levin and Swaeringen, 1953) can still be found in small refineries, most refineries operate continuous acidulation units often comprising centrifugal separators. Understandably, their use was promoted by their manufacturers (Keith Jr. et al., 1954; 1955; Sadler, 1957; Crauer, 1965; Stahle, 1965; Todd and Morren, 1965; Morren, 1969; Crauer, 1970; Crauer, 1972; Steinwallner, 1979), but they are not essential (Mag et al., 1983). The continuous processes are quite simple and several of them have been illustrated in Figure 3.16. Warm

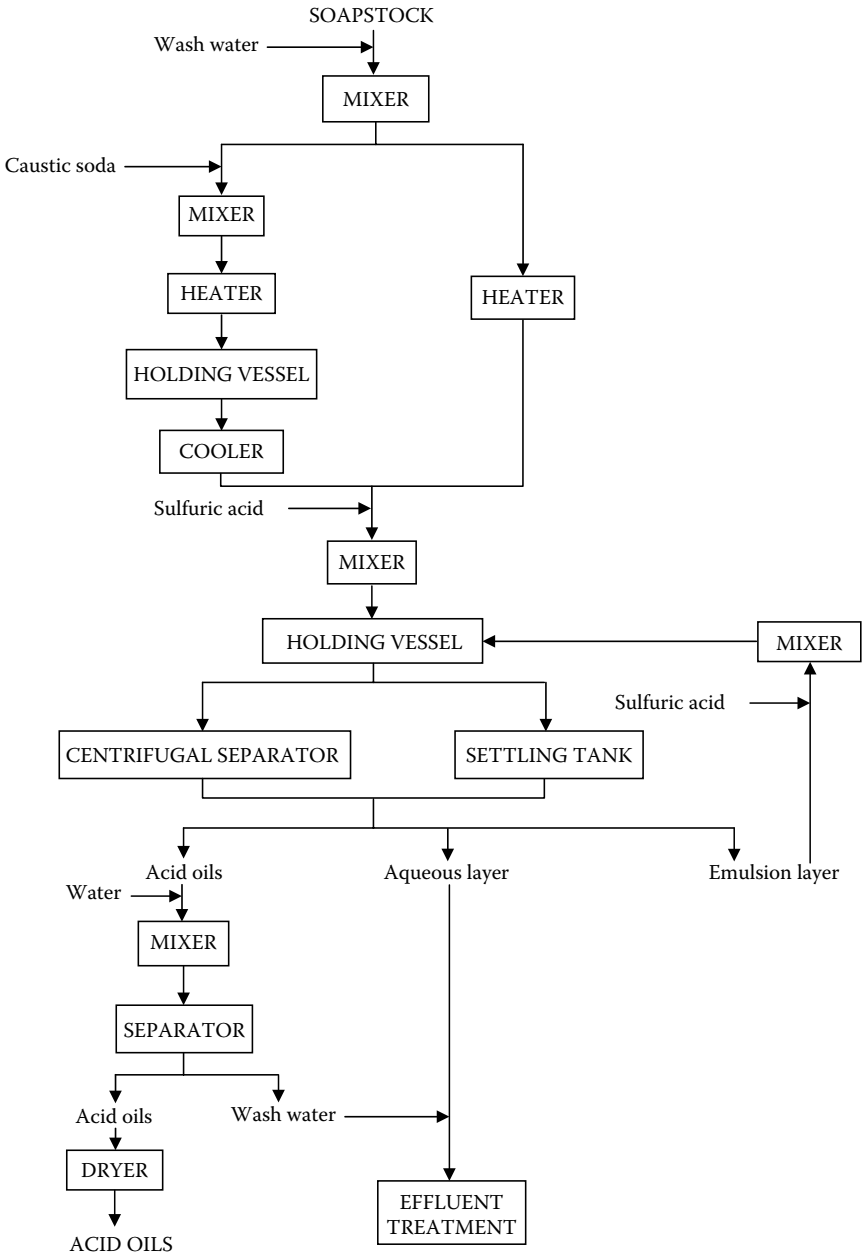


FIGURE 3.16 soapstock splitting.

soapstock is mixed with wash water from the alkali refining process if available and can also be mixed with other waste streams (not illustrated) to be processed in the soapstock splitting unit.

The soapstock requires heating to the acidulation temperature of 90 to 95°C, as illustrated on the right-hand side of Figure 3.16. Heating can be in a heat exchanger, such as a steam jet heater (Crauer, 1965), but can also be with sparge steam after the addition of sulfuric acid, so that the holding vessel then also acts as a heater and the steam as an agitator (Woerfel, 1983).

The sulfuric acid must be well mixed into the soapstock since the acidulation acid forms an oily film around the soap granule. This film protects the soap inside from further reaction (Naudet et al., 1955). An in-line static mixer made of porcelain can be used for the purpose, but a special aspiration process based on the Venturi principle has also been described (Gadefaix and Klère, 1974).

The acid supply can be controlled by downstream pH-measurement (Duff and Segers, 1982). Because of the corrosive nature of aqueous sulfuric acid, tanks are preferably made of glass fibre reinforced polyester. The amount of acid to be used should bring the pH of the aqueous layer after completion of the acidulation reaction to a value of about 2 (Mag et al., 1983). This implies that the phosphoric acid, if present, is also converted to its non-dissociated form, the first pK_a being 2.16. So higher values of 3.5 are also used (Crauer, 1965). It can be added as

concentrated or diluted acid (Keith Jr. et al., 1955). When the soapstock is mixed with hexane or originates from miscella refining, a higher acidulation pH (3 to 8.5) suffices (Kelley, 1959).

As illustrated in Figure 3.16, the acidulation reaction product can be separated in a centrifugal separator or in a simple settling tank. The latter is preferably supplemented with a coalescer to treat the aqueous phase and ensure a fat content of < 100 ppm (Mag et al., 1983). As pointed out by several authors, the problem with the acidulation process is the formation of an emulsion layer in between the aqueous layer and the layer of acid oils. This layer can be combined with the oily layer and dried to yield a fatty material that can be separated centrifugally (Brister, 1987). This emulsion layer can also be given a separate treatment, either continuous as illustrated by Figure 3.16, or batchwise when a larger amount has been collected (Gosewinkel and Knuth, 1988). This figure also shows that the acid oils can be water washed and dried before being sent to storage. The aqueous layer is too acid to be discharged as effluent. It is commonly neutralised with lime, which has the additional advantage that phosphates and, to a lesser extent, sulfates are also removed as precipitated calcium salts.

3.6.1.2 Saponification

If the acid oils resulting from the acidulation step are to be distilled to yield a fatty acid distillate, the yield of this distillate can be increased by saponifying the neutral oil present in the soapstock before this acidulation step. A patent describing this prior saponification (McClain, 1948) also prescribes the addition of some potassium persulphate during the saponification to improve the colour of the distilled fatty acids. Extracting the saponification product with an organic solvent, such as hexane, also leads to less colour in the distilled fatty acids and also allows the unsaponifiables to be recovered (Garrett et al., 1974).

If the soapstock contains a large proportion of phosphatides (as is the case when nondegummed soya bean oil has been alkali refined), a saponification step must also be included (Balazs, 1987) because alkali is more effective than acid in breaking down the phosphatide emulsion layer (Keith Jr. et al., 1954); this step may comprise adding additional caustic as illustrated on the left-hand side of Figure 3.16. For the saponification to proceed sufficiently quickly, a temperature of 150 to 220°C is recommended (Clayton, 1959; Spannuth, 1974). This implies operating at super-atmospheric pressure.

In the process outlined by Keith et al. (1955), the temperature is not raised above the boiling point of water. Moreover, a separation stage and a washing stage are included before the acidulation proper. This has the advantage that the aqueous caustic streams resulting from the separation and washing operations can be used to neutralise the aqueous stream resulting from the acidulation stage, which in that case also requires less acid.

Red and Ilagan (1978) have described another process operating at atmospheric pressure. In this process, a base like sodium hydroxide is added to the soapstock held at 80 to 95°C. Then the reaction product is acidulated by the addition of an inorganic acid and the acid oils are isolated. Part of the acid water is neutralised and mixed with the soapstock to build up a high salt concentration. The aqueous effluent can then be further concentrated and sold to the paper industry for kraft paper pulp processing. If the salts cannot be sold, saponification is expensive on reagents. It uses additional alkali and this alkali must also be neutralised, so that the process also uses more acid than acidulation without prior saponification.

3.6.1.3 Hydrolysis

When Pabst reviewed the treatment, purification, properties, and utilisation of soapstock in 1964, he mentioned an autoclave treatment of the soapstock as such. After Naudet et al. (1954) showed that gums can be completely hydrolysed by heating them to 225°C, the same authors also heated soapstock in an agitated autoclave (Naudet et al., 1955). They noted that soapstock hydrolyses somewhat faster than gums, probably because the water in soapstock is better dispersed/dissolved than in gums; soapstock also hydrolyses much faster than acid oils; a temperature of 170°C suffices (Denise, 1982, p. 205). They also noted that acidulating the reaction product does not lead to an emulsion layer at all. In fact all ester bonds in the raw material had been hydrolysed. Acidulating to a pH of 4 suffices to obtain a clear separation between the fatty acids and the aqueous layer (J. De Kock, personal communication).

Of course, this aqueous layer has a high BOD in that it not only contains the glycerol resulting from triglyceride and phosphatide hydrolysis, but also choline, ethanolamine, etc., originating from the complete hydrolysis of all ester bonds in the phosphatides. On the other hand, it is less acid than effluent originating from straight acidulation processes. The complete hydrolysis also leads to the formation of phosphoric acid, so when gums are hydrolysed, some soda ash is preferably added to the reaction mixture to mop up this acid and prevent the fatty acids formed from darkening under the influence of this acid (Naudet et al., 1954). The reason why the hydrolysis process has not been adopted is not so much this high-BOD effluent, but rather (M. Naudet, personal communication) that it was developed when there was no market for it, since French refineries could dispose of their soapstock to soap works at that time; the process having been published in French may also have been part of the reason.

3.6.1.4 Esterification

If the acid oils (also referred to as "black grease") are not destined for animal feed but to be used as industrial raw material, purity and colour can be quite important. So when preparing high-grade soaps from black grease,

Starrells (1929) first of all converted this raw material into FAME by adding an excess of alcohol and 1 to 2% sulfuric acid, and then he purified the FAME by washing and distillation, hydrogenated the distillate, and finally saponified the hydrogenation product. To increase the yield, Starrells suggests a prior Twitchell treatment of the black grease to increase its FFA content by hydrolysis of triglycerides.

For the same reason, Mattikow and Perlman (1955a) subject their black grease to a combined splitting and esterification step employing methanol and a strong mineral acid. To enhance the purification, they also wash the FAME with caustic alkali before their purification by distillation. Another process that starts with acid oils still containing some triglyceride oil operates at elevated temperatures (115 to 125°C) and superatmospheric pressure. It uses a Twitchell-type catalyst (Eaves et al., 1959; Eaves and Spadaro, 1961) and also converts the triglyceride oil to FAME. A plant of 10,000 tpa capacity using this process was built in Dupo, IL, U.S. (Anon., 1960).

Choo and Goh (1985), on the other hand, employ a dual catalyst system when converting sludges containing both FFA and TAG into FAME. For the acid catalysed esterification of the FFA, they use solid sodium bisulphate, which they remove by filtration from the esterification product consisting of FAME, TAG, and free methanol before adding sodium hydroxide to this mixture as an interesterification catalyst. Glycerol can also be used for the removal of acidic esterification catalysts, such as *p*-toluene sulphonic acid or methane sulphonic acid, and sodium methoxide for the interesterification (Lepper and Friesenhagen, 1986).

In a laboratory process using soapstock as the starting material for FAME preparation (Haas et al., 2000), the soapstock is first saponified by addition of lye. Then the water is removed by lyophilisation and acidified methanol is added. After the esterification reaction, an amount of FAME corresponding to 60% of theory separates as a distinct layer. Extracting the methanol layer and washing the solids (sodium sulphate) releases further FAME.

3.6.2 Sterol recuperation processes

A number of naturally occurring sterols have been widely used as starting materials for the production of pharmaceuticals, such as vitamins and steroid hormones, for more than 60 years. They are also incorporated into cosmetics and more recently, stanols (hydrogenated sterols) or rather their fatty acid esters (Miettinen et al., 1996, listing 46 references) are included into food products, such as spreads and yogurt drinks that are effective in lowering serum LDL and total cholesterol levels by partly inhibiting the absorption of both dietary and biliary cholesterol from the digestive tract (Wester, 2002).

However, the concentration of sterols in raw materials which could be used for their recuperation, tends to be low so that, in principle, large amounts of raw material would have to be processed to arrive at appreciable amounts of sterols. Fortunately, some common industrial processes already lead to a concentration of sterols. Treating pine wood with sodium sulfate in a kraft pulp mill leads to the by-product tall oil into which the phytosterols have been concentrated. Further concentration is achieved when this crude tall oil is distilled to yield fatty acids so that the tall oil pitch (distillation residue) contains some 32% unsaponifiables (Table 3.7) of which 45% is sitosterol (Watkins, 2000). Accordingly, tall oil pitch is widely used to provide the stanols added to nutraceuticals. Fat splitting to produce fatty acids and even methanolysis to make biodiesel also lead to a concentration of the unsaponifiables and, thus, to raw materials for sterol recuperation.

TABLE 3.7 Composition of tall oil pitch

Type of Compound	%
Unsaponifiables	32
Rosin acids (free)	22
Rosin acids (esterified)	8
Fatty acid (free)	6
Fatty acids (esterified)	23
"Lignous" acids	9

During the distillation of fatty acids from tall oil or from acid oils, such as resulting from soapstock acidulation, the free sterols present will react with the FFA present under ester formation. In wool grease, a substantial fraction of the sterols appear in the form of esters (Yoder, 1943) so that often a preliminary saponification of the sterol esters is necessary before they can be recovered.

3.6.2.1 Cholesterol

Cholesterol can be recovered from slaughterhouse offal and especially brains and spinal cords, but the recovery processes to be discussed in the present section will be limited to the use of wool wax as the raw material. Since some isolation and purification processes for cholesterol can also be used for recovering plant sterols, the former will be discussed in chronological order, except for (Lifschütz, 1903; Frick, 1929) and (Anon., 1933) because it is not clear that the processes described therein yield cholesterol.

- After having saponified his wool grease, Yoder (1943) prepares an adduct of the wool alcohols with hydrogen chloride or bromide and achieves a separation between the cholesterol adduct and the iso-cholesterol adduct by fractional crystallisation from, for example, ethylene dichloride.
- A year later, Yoder finds that oxalic acid also forms an adduct with cholesterol and can be separated from other sterols not having a single reactive double bond in the hydrophenantrene nucleus via this adduct. Upon heating with water, the adduct decomposes and practically pure cholesterol results.

- Adducts can also be formed with manganese chloride in the presence of 1 to 2% of water (Overhoff and Hackmann, 1948).
- Because oxalic acid is poisonous, the use of succinic acid that also forms an adduct with cholesterol is preferred (Katscher, 1948).
- Cholesterol can be recovered from wool wax alcohols by first allowing the iso-cholesterol to crystallise from methanol (Hewett, 1952b) or a mixture of benzene or xylene with methanol (Hewett, 1952a), and purifying the evaporation residue of the filtrate by recrystallisation from, for instance, glacial acetic acid.
- Cholesterol can also be recuperated from wool wax alcohols via an urea adduct that can easily be decomposed with water. The crude cholesterol obtained can be further purified by recrystallisation from alcohol (Hackmann, 1951).
- By first precipitating lanosterol from a solution of lanolin alcohols in a mixture of acetone and an alcohol, evaporating the solvent mixture, and dissolving the evaporation residue in ethylene dichloride, a cholesterol adduct can be formed by adding a mixture of maleic and oxalic acids. The adduct crystallises when the temperature is lowered. It can be removed by filtration, decomposed, and purified by recrystallisation (Vaterrodt and McNellis, 1952).
- A German patent (Overhoff and Hackmann, 1952) with the same priority date as the Dutch patent (Overhoff and Hackmann, 1948) lists many more salts capable of forming adducts with cholesterol: manganese chloride, calcium chloride, aluminum nitrate (used to recover cholesterol from cattle brain), aluminum chloride, zinc chloride, stannous chloride, ferric chloride, chromic chloride, cobaltous chloride, and cadmium chloride can all be used to prepare adducts that can subsequently be decomposed by the addition of water.
- Subsequently (later priority date), Knol (1949; 1952) found that zinc chloride behaved differently from the other salts listed above in that if used in four to sixfold excess, a much higher cholesterol yield is attained.
- The finding that a solution of hydrochloric acid also leads to a cholesterol adduct and that the acid need not be anhydrous forms the basis of yet another Dutch patent.

3.6.2.2 Phytosterols

When investigating how to produce value-added products from refinery by-products, the French Edible Oil Institute developed a process to isolate sterols from soapstock (Uzzan and Trizis, 1958). The process starts by fully saponifying the soapstock, washing the soaps with concentrated brine, and drying the washed soaps. These are then extracted with a solvent, and anhydrous acetone was found to be the most effective among the solvents investigated. Subsequently, the isolation of sterols from gums and from still bottoms resulting from the distillation of fatty acids from acid oils was also investigated by the same research institute (Sisley et al., 1958).

The isolation method can be by precipitation from an alcoholic solution and subsequent recrystallisation from pure alcohol (Schwieger, 1936), precipitation from aqueous butanol (Bennett, 1940), extraction with an excess of alcoholic alkali, diluting with water and extracting the solution with an organic solvent (Fernholz, 1942) or by simply collecting the distillation residue (Trent, 1947).

Sterols present in the residue resulting from the distillation of FAME will be esterified with fatty acids. They can be saponified with methanolic caustic soda, the reaction product can be diluted with water and the aqueous/alcoholic soap liquor can then be extracted with a solvent like petroleum ether. Extracting the petroleum ether again with aqueous methanol removes the last soap traces so that on evaporation of the petroleum ether, a sterol-rich residue results. If this is then extracted with hot methanol, the extract will yield crystalline sterols on cooling (Böhm, 1950). Saponification of the soapstock or "pitch" (still bottoms left after distillation of FFA from acid oils), followed by drying the soaps and extracting the dry soaps with, for instance, acetone, also leads to a solution of sterols that can be evaporated to dryness and further purified by recrystallisation (Laquer, 1956).

Sterols can also be isolated from gums by very similar processes comprising splitting the ester bonds by using an acid catalyst in an alcoholic environment and allowing the resulting solution to cool as a result of which the sterols will crystallise (Mattikow and Perlman, 1952). Another sterol source is deodoriser "sludge" that on a similar treatment also yields sterols (Mattikow and Perlman, 1955b). Deodoriser distillates can also be treated by first saponifying the distillate, separating the organics by acidulation, and isolating the sterols by extraction and crystallisation (Brown and Rawlings, 1958). If methyl esters are made from this sludge, which can be done by enzymatic esterification (Ramamurthi and McCurdy, 1993), they can also be subjected to liquid/liquid extraction using a suitable solvent mixture (Brown and Smith, 1964). The process was subsequently improved by diluting the mixture of water, alcohol and fatty acid esters of this alcohol, sterols and tocopherols with water, and cooling the diluted mixture, which causes the sterols to crystallise (Smith, 1967). Using water-saturated hexane with small amounts of ethanol leads to a high sterol recovery (Moreira and Baltanás, 2004).

A different purification approach was taken by Struve (1979), who isolated the sterols by forming an adduct with calcium chloride in a specified solvent mixture. This

adduct is easy to isolate from the solvent and can then be decomposed.

Because the tall oil pitch is also a distillation residue, the sterols present in this residue will have been esterified (Struve and Schuh, 1985). Accordingly, all isolation processes comprise a saponification stage to obtain free sterols. In an early isolation process (Steiner and Fritz, 1958), tall oil pitch was fractionated in a propane tower resulting in an overhead fraction containing more than 80% of the sterols in the feed. After propane removal, the evaporation residue was refluxed with caustic ethanol, and allowed to cool to ambient. The free sterols crystallised and were isolated, and after washing with methanol and water, were found to be quite pure. Warm water can also be added to the caustic ethanol solution, which on gradual cooling will throw a sterol deposit (Albrecht and Herrlinger, 1955).

In a process starting with saponification (or with soapstock), an aqueous solution of the soaps is extracted counter-currently with an organic ester, such as isopropyl acetate. Evaporation of the solvent from both fractions yields soaps and sterols (Chase and Bowers, 1958). In another sterol isolation process (Julian, 1972; Julian, 1974), the pitch is, first of all, extracted with a solvent to remove free acids, then the sterol esters in the extraction residue are saponified by an excess of caustic soda in methanol at quite high temperatures (about 200°C) under pressure. The saponified mixture is diluted with water, which leads to a precipitate that is isolated and dried. It is then purified by dissolving it in ethylene dichloride and discarding the insolubles (soaps) and evaporating to dryness.

3.6.3 Tocopherol recuperation processes

According to a supplier brochure (Frandsen, 1996), the value of deodoriser distillate is entirely a function of its tocopherol content. In this context, the tocopherol composition does not matter that much since "natural" vitamin E (α-tocopherol) can be produced by conversion of the other tocopherols also present in the distillate. Tocopherols can also be synthesized, but then a mixture of stereoisomers results (Walsh et al., 1981). The tocopherols are normally recovered from the distillate by saponification, extraction, and crystallisation processes (Zilch, 2000), but enriched fractions can also be obtained by extraction with supercritical carbon dioxide (Willner et al., 1997).

In another type of process, volatility differences are first of all introduced by esterifying the free sterols present in the deodoriser sludge with the fatty acids that are also present in this sludge (Fizet, 1996). This reduces the volatility of the sterols so that the tocopherols can be separated from the esterified sterols by molecular distillation. The residual free fatty acids present in the sludge will also be volatilised, but esterifying them with glycerol (Takagi and Kai, 1984) can prevent this. In that case the tocopherols become the most volatile constituent of the esterified sludge and can be isolated by distillation. The process comprising esterification and molecular distillation can also be combined with purification by crystallisation (Pramparo et al., 2005).

References

Albrecht, H. and Herrlinger, R. (1955), Production of sterols from tall oil pitch, US Patent 2,715,638, assigned to American Cyanamid Company.

Anon. (1933), Werkwijze voor het winnen van l- en d-cholesterine uit wolvet, Dutch Patent 29780, assigned to N.V. Oliefraffinaderij "Zuilen".

Anon. (1960), Esters méthyliques à partir de soapstocks, *Rev. Franç. Corps Gras*, 7, 452.

Anon. (1967), Werkwijze voor het winnen van sterolen uit sterolbevattend materiaal, Dutch Patent 67 06583, assigned to N.V. Koninklijke Veenendaalsche Sajet- en Vijfschachtfabriek v/h Wed. D.S. van Schuppen & Zoon.

Balazs, I. (1987), Refining and use of byproducts from various fats and oils, *J. Am. Oil Chem. Soc.*, 64, 1126–1128.

Beal, L. (1921), The neutralised dried soapstock process as an improvement to the traditional sulfuric acid process, *Proceedings of the Second ASA Symposium on Soybean Processing. Refining of Soy Oil, Caustic or Physical*? Leysen, R.J.F., Ed., American Soy bean Association, Brussels, Belguim.

Bennett, W.G. (1940), Extraction of sterols, US Patent 2,223,398, assigned to Standard Brands Incorporated.

Boyer, M.J. (2000), Environmental considerations in fats and oils technologies, in *Introduction to Fats and Oils Technology*, O'Brien, R.D., Farr, W.E., and Wan, P.J., Eds., AOCS Press, Champaign, IL, 285–301.

Böhm, O. (1950), Process for the production of sterols from oils, fats, and fatty acids, US Patent 2,516,834, assigned to Severočeske tukové závodu n.p., Ústí nad Labem.

Brister, B.C. (1987), Process for obtaining fatty acid product from glyceride oil soapstock, US Patent 4,671,902, assigned to The Procter & Gamble Company.

Brown, W. and Rawlings, H.W. (1958), Separation of sterols from deodorizer sludge, US Patent 2,843,610, assigned to Eastman Kodak Company.

Brown, W. and Smith, F.E. (1964), Process for separating tocopherols and sterols from deodorizer sludge and the like, US Patent 3,153,055, assigned to Eastman Kodak Company.

Chase, R.R. and Bowers, J.C. (1958), Separating non-acids from soapstocks, US Patent 2,866,781, assigned to Union Bag-Camp Paper Corporation.

Choo, Y.M. and Goh, S.H. (1985), Catalytic esterification of carboxylic acid/glyceride mixtures, British Patent 2 148 897, assigned to Institut Penyelidikan Minyak Kalapa Sawit Malaysia.

Clayton, B. (1959), Upgrading of soda ash soapstock, US Patent 2,902,501.

Crauer, L.S. (1965), Continuous recovery of acid oil, *J. Am. Oil Chem. Soc.*, 42, 661–663.

Crauer, L.S. (1970), Continuous treatment of refinery waste waters, *J. Am. Oil Chem. Soc.*, 47, 210A–235A.

Crauer, L.S. (1972), Continuous miscella soapstock acidulation process, *J. Am. Oil Chem. Soc.*, 49, 681–682.

Denise, J. (1982), Traitement des sous-produits de la neutralisation, in *Le raffinage des corps gras*, Westhoek Editions, Les éditions des Beffrois, Dunkerque, France, 204–206.

Dijkstra, A.J. (1999a), Integrated pollution control in seed oil refining, *Fett/Lipid*, **101**, 132–137.

Dijkstra, A.J. (1999b), Trends in edible oil processing, *Lipid Techn.*, **11**, 53–56.

Dowd, M.K. (1996), Compositional characterization of cottonseed soapstocks, *J. Am. Oil Chem. Soc.*, **73**, 1287–1295.

Duff, A.J. and Segers, J.C. (1982), Continuous soapstock splitting, US Patent 4,361,517, assigned to Unilever.

Eaves, P.H. and Spadaro, J.J. (1961), Process for the methylation of vegetable oil soapstocks, US Patent 3,010,977, assigned to USA, Secretary of Agriculture.

Eaves, P.H. et al. (1959), Methyl esters directly from acidulated soapstock, *J. Am. Oil Chem. Soc.*, **36**, 230–234.

Evans, J.C. (1990), Fluidization of soapstock, European Patent 367 473, assigned to Cargill Incorporated.

Fernholz, E. (1942), Method of obtaining sterols, US Patent 2,280,815, assigned to E.R. Squibb & Sons.

Fizet, C. (1996), Process for tocopherols and sterols from natural sources, US Patent 5,487,817, assigned to Hoffmann-La Roche Inc.

Frandsen, S.S. (1996), *Supplier brochure, Revision 3*, Henkel Corporation, LaGrange, IL.

Frick, F. (1929), Verfahren zur Gewinnung einheitlicher Verbindungen aus den Alkoholen des Wollfetts, German Patent 485 198, assigned to I.G. Farbenindustrie AG.

Gadefaix, R. and Klère, J. (1974), Process for splitting soapstock and apparatus therefor, US Patent 3,787,460, assigned to Unilever.

Garrett, R.L. et al. (1974), soapstock reclamation process for producing fatty acids, glycerine and salts, US Patent 3,830,789, assigned to Adams Laboratories Inc.

Garro, J.M. et al. (2003), Method for fractionating grease trap waste and uses of fractions therefrom, WO Patent 03/038020, assigned to Prolab Technologies Inc.

Goemans, M. (2004), Thermal treatment of spent bleaching earth, filter aids, destillates, gums and soapstock: the HelioSolids® fluidised bed reactor and the Cinclus® thermal oxidizer, *Paper presented at the DGF Symposium on Refining: Handling by-products and minor compounds,* Leipzig.

Goemans, M. (2005), On-site thermal treatment of by-products from the edible oils and fats industry: a renewable energy source, *Paper presented at the 26th ISF World Congress and Exhibition, Prague,* page 8 in the abstracts.

Gosewinkel, L. and Knuth, M. (1988), Seifenspaltung — kontinuierlich und umweltfreundlich, *Fat Sci. Technol.*, **90**, 155–158.

Haas, M.J. et al. (2000), Simple, high-efficiency synthesis of fatty acid methyl esters from soapstock, *J. Am. Oil Chem. Soc.*, **77**, 373–379.

Hackmann, J.T. (1951), Verfahren zur Gewinnung von Sterinen oder ihren Additionsverbindungen aus Sterine enthaltenden Mischungen oder Lösungen, German Patent 929 424, assigned to N.V. Koninklijke Veenendaalsche Sajet- en Vijfschachtfabriek v/h Wed. D.S. van Schuppen & Zoon.

Hewett, C.L. (1952a), Verfahren zur Abtrennung des Cholesterins aus Wollwachsalkoholen, German Patent 859 620, assigned to Organon Laboratories Limited.

Hewett, C.L. (1952b), Verfahren zur Abtrennung von Cholesterin aus Wollwachsalkoholen, German Patent 833 815, assigned to Organon Laboratories Limited.

Jollez, P. et al. (2001), Production of high grade and high concentration of free fatty acids from residual oils, fats and greases, WO Patent 01/83655, assigned to Prolab Technologies Inc.

Julian, D.V. (1972), Process for preparing sterols from tall oil pitch, US Patent 3,691,211, assigned to The Procter & Gamble Company.

Julian, D.V. (1974), Process for preparing sterols from tall oil pitch, US Patent 3,840,570, assigned to The Procter & Gamble Company.

Katscher, E. (1948), Improvements in or relating to the production of cholesterol, British Patent 613,778, assigned to United Domestic Industries Limited.

Keith Jr., F.W. et al. (1955), Continuous purification and acidulation of vegetable oil soapstock, *J. Am. Oil Chem. Soc.*, **32**, 517–519.

Keith Jr., F.W. et al. (1954), Impurities in vegetable oil refining soapstock, *J. Am. Oil Chem. Soc.*, **31**, 298–302.

Kelley, T.H. (1959), Fatty acid recovery from soapstock, US Patent 2,877,249, assigned to The Buckeye Cellulose Corporation.

Knol, H.W. (1949), Werkwijze voor het winnen van sterolen, Dutch Patent 65260, assigned to N.V. Koninklijke Veenendaalsche Sajet- en Vijfschachtfabriek v/h Wed. D.S. van Schuppen & Zoon.

Knol, H.W. (1952), Verfahren zur Gewinnung von Sterine, German Patent 827 199, assigned to N.V. Koninklijke Veenendaalsche Sajet- en Vijfschachtfabriek v/h Wed. D.S. van Schuppen & Zoon.

Kruse, N.F. and Cravens, W.W. (1960), Poultry treatment for skin pigmentation, US Patent 2,924,525, assigned to Central Soya Company.

Kuk, M.S. and Tetlow, R. (2005), Gossypol removal by adsorption from cottonseed processing, *Paper presented at the 26th ISF World Congress and Exhibition, Prague.*

Laquer, F.O. (1956), Sterol recovery, US Patent 2,772,297.

Lepper, H. and Friesenhagen, L. (1986), Process for the production of fatty acid esters of short-chain aliphatic alcohols from fats and/or oils containing free fatty acids, US Patent 4,608,202, assigned to Henkel KGaA.

Levin, H. and Swaeringen, J.S. (1953), An inexpensive soapstock conversion plant, *J. Am. Oil Chem. Soc.*, **30**, 85–88.

Lifschütz, J. (1903), Verfahren zur Zerlegung des Wollfetts in einen Wasser leicht und einen dasselbe schwer absorbierenden Teil, German Patent 163 254.

Mag, T.K. et al. (1983), Continuous acidulation of soapstock and recovery of acid oil, *J. Am. Oil Chem. Soc.*, **60**, 1008–1011.

Mattikow, M. and Perlman, D. (1952), Recovery of sterols, US Patent 2,585,954.

Mattikow, M. and Perlman, D. (1955a), Refining of low-grade fats, US Patent 2,721,872, assigned to Benjamin Clayton (Refining Unincorporated).

Mattikow, M. and Perlman, D. (1955b), Treatment of fatty material, US Patent 2,704,764, assigned to Benjamin Clayton, doing business under the fictitious name and style of Refining Unincorporated, a sole proprietorship.

McClain, H.K. (1948), Fatty acids from cottonseed foots, US Patent 2,435,456, assigned to The Procter & Gamble Company.

Miettinen, T. et al. (1996), Use of a stanol fatty acid ester for reducing serum cholesterol level, US Patent 5,502,045, assigned to Raisio Tehtaat Oy AB.

Moreira, E.A. and Baltanás, M.A. (2004), Recovery of phytosterols from sunflower oil deodorizer distillates, *J. Am. Oil Chem. Soc.*, **81**, 161–167.

Morren, J.E. (1969), Process for recovering fatty acids and triglyceride oil from soapstock, US Patent 3,428,660, assigned to Baker Perkins Inc.

Naudet, M. et al. (1954), Récupération des acides gras contenus dans les mucilages, *Bull. mens. ITERG*, 112–115.

Naudet, M. et al. (1955), Les acides gras des pâtes de neutralisation, *Rev. Franç. Corps Gras*, **2**, 222–224.

Nettli, P. (1977), A method for recovering and stabilizing fat and fatty substances as well as proteins and proteinous substances from aqueous effluents, British Patent 1 468 091, assigned to A/S Apothekernes Laboratorium for Specialpraeparater.

Overhoff, J. and Hackmann, J.T. (1948), Werkwijze voor het bereiden van sterolen en additieproducten daarvan uit sterolen bevattende materialen, Dutch Patent 60735, assigned to N.V. Koninklijke Veenendaalsche Sajet- en Vijfschachtfabriek v/h Wed. D.S. van Schuppen & Zoon.

Overhoff, J. and Hackmann, J.T. (1952), Verfahren zur Gewinnung von Sterinen oder deren Additionsprodukten aus Sterine enthaltenden Ausgangsstoffen, German Patent 882 090, assigned to N.V. Koninklijke Veenendaalsche Sajet- en Vijfschachtfabriek v/h Wed. D.S. van Schuppen & Zoon.

Pabst, M. (1964), Pâtes de neutralisation, huiles acides et acides gras distillés : traitements, purification, propriétés, emplois, *Rev. Franç. Corps Gras*, **11**, 399–403.

Pramparo, M. et al. (2005), Estudio de la purificación de ácidos grasos, tocoferoles y esteroles a partir del destillado de desodorización, *Grasas Aceites*, **56**, 228–234.

Ramamurthi, S. and McCurdy, A.R. (1993), Enzymatic pretreatment of deodorizer distillate for concentration of sterols and tocopherols, *J. Am. Oil Chem. Soc.*, **70**, 287–295.

Red, J.F.P. and Ilagan Jr., J.B. (1978), Fatty acid recovery from soapstock, US Patent 4,118,407, assigned to SCM Corporation.

Sadler, F.S. (1957), Production of fatty acids from soaps, US Patent 2,802,845, assigned to The Sharples Corporation.

Schwieger, A. (1936), Method of producing concentrated phytosterin-containing products, US Patent 2,046,345, assigned to Hanseatische Mühlenwerke A.G.

Sisley, J.-P. et al. (1958), Revalorisation des sous-produits de l'huilerie II. Étude de différents autres sous-produits en vue de la récupération des stérols, *Rev. Franç. Corps Gras*, **5**, 307–315.

Smith, F.E. (1967), Separation of tocopherols and sterols from deodorizer sludge and the like, US Patent 3,335,154, assigned to Eastman Kodak Company.

Sonntag, N.O.V. (1990), Fatty acids, utilization and disposal, in *Edible Fats and Oils Processing: Basic Principles and Modern Practices*, Erickson, D.R., Ed., AOCS Press, Champaign, IL, 406–412.

Spannuth, D. (1974), Verfahren zur Aufbearbeitung von Soapstock, German Patent 2 344 683, assigned to Oelmühle Hamburg AG.

Stahle, C.W. (1965), Engineering and economic aspects of continuous soapstock acidulation, *J. Am. Oil Chem. Soc.*, **42**, 566–568.

Stansbury, M.F. et al. (1957), Composition of acidulated cottonseed soapstocks as influenced by commercial methods of processing seed and oil, *J. Am. Oil Chem. Soc.*, **34**, 539–544.

Starrells, J. (1929), Manufacture of high-grade soaps from low-grade fats, US Patent 1,701,703.

Steiner, C.S. and Fritz, E. (1958), Sterol recovery process, US Patent 2,835,682, assigned to Swift & Company.

Steinwallner, F. (1979), Eine neue kontinuierliche Anlage zur Soapstock-Spaltung, *Fette Seifen Anstrichm.*, **81**, 286–290.

Struve, A. and Schuh, R. (1985), Steringewinnung aus Rückständen der Fettspaltung, *Fette Seifen Anstrichm.*, **87**, 103–106.

Struve, A. (1979), Process for isolating sterols from fat processing residues, US Patent 4,148,810, assigned to Henkel KGaA.

Takagi, Y. and Kai, Y. (1984), Process for the preparation of tocopherol concentrates, US Patent 4,454,329, assigned to The Nisshin Oil Mills Ltd.

Todd, D.B. and Morren, J.E. (1965), Continuous soapstock acidulation, *J. Am. Oil Chem. Soc.*, **42**, 172A–178A.

Trent, W.R. (1947), Vitamin recovery, US Patent 2 432,181, assigned to Colgate-Palmolive-Peet Company.

Uzzan, A. and Trizis, C. (1958), Revalorisation des sous-produits de l'huilerie I. Fraction des stérols contenus dans certains résidus de raffinage de l'huile de colza, *Rev. Franç. Corps Gras*, **5**, 24-29.

Vaterrodt, P. and McNellis, J. (1952), Method for the production of cholesterol, US Patent 2,598,468, assigned to Botany Mills, Inc.

Walsh, L. et al. (1981), Optimizing deodorizer distillate tocopherol yields, *inform*, **9**, 78–83.

Watkins, C. (2000), Tall oil fatty acids, *inform*, **11**, 580–588.

Wester, I. (2002), Plant sterols for cholesterol lowering, *Plenary lecture presented at the 2nd EuroFedLipid Congress, Strasbourg*.

Willner, T. et al. (1997), Einfluss der Prozessparameter auf die Tocopherolbilanz bei der Gewinnung von pflanzlichen Ölen, *Fett/Lipid*, **99**, 138–147.

Witte, N.H. and Sipos, E. (1960), Treating soapstock, US Patent 2,929,714, assigned to Central Soya Company, Inc.

Woerfel, J.B. (1983), Alternatives for processing of soapstock, *J. Am. Oil Chem. Soc.*, **60**, 310–313.

Woerfel, J.B. (1995), Soybean oil processing byproducts and their utililization, in *Practical Handbook of Soybean Processing and Utilization*, Erickson, D.R., Ed., AOCS Press, Champaign, IL, 297-313.

Yoder, L. (1943), Isolation of sterols from fats and oils, US Patents 2,322,906 and 2,362,605 (1943), assigned to Iowa State College Research Foundation.

Zilch, K.T. (2000), By-product utilization, in *Introduction to Fats and Oils Technology*, O'Brien, R.D., Farr, W.E., and Wan, P.J., Eds., AOCS Press, Champaign, IL, 542–556.

3.7 Bleaching of oils and fats

In Figure 3.8, showing the various processing routes from crude oil to RBD oil, bleaching operations are indicated after alkali refining and before deodorisation, after several degumming processes (acid refining, S.O.F.T.® degumming, and enzymatic degumming), and preceding physical refining. The dry degumming process, which also employs bleaching earth, is shown as well. Accordingly, all fully refined oil has been subjected to one bleaching process or another.

The reason for this omnipresence is that the bleaching process does more than just increase the transmission of light through the oil. For that reason, Patterson (1976) prefers the term "adsorptive cleansing." As pointed out by Erickson (1995), the bleaching process is often the first filtration encountered by the oil, so it ensures the removal of soaps, residual phosphatides, trace metals, and some oxidation products, and it catalyses the decomposition of β-carotene, whereby peroxides act as oxygen source (Tollenaar and Hoekman, 1964; see also Venkatarao and Achaya, 1969).

It also catalyses the decomposition of peroxides (Henderson et al., 1991), which Wiedermann (1981) regards as the most important function of bleaching earth; he advocates using a level of bleaching earth that will assure total peroxide decomposition. However, subsequent authors (Estes et al., 1995a) questioned the need for total peroxide decomposition and demonstrated experimentally that "provided that soap, phosphorus, and trace metals are removed to sufficiently low levels, it is not necessary to convert the hydroperoxides during bleaching to achieve a stable bland oil."

To complicate the understanding of the bleaching process even further, the bleaching earth itself should not be regarded as a chemical compound with a well-defined structure, but as a semi-natural product with a far from uniform structure and variations between batches. Accordingly, the literature describes the bleaching process as "an art of witchcraft" (Rich, 1964), "a mystery" (Rich, 1967), "the least understood process in edible oil processing" (Naudet, 1965) and reports that "the physical and chemical adsorbent properties that determine adsorbent efficiency are poorly understood" (Chapman and Pfannkoch, 1992). It, therefore, is not surprising that the bleaching literature is full of half-truths, apparent contradictions and above all, unwarranted generalisations.

3.7.1 Types of adsorbent

The edible oil industry uses three different types of adsorbent: various forms of bleaching earth, activated carbon, and amorphous silica. Other materials have also been studied on a laboratory scale, such as alumina (Pons Jr. et al., 1962), which can be pretreated with sulfurous acid (Pons Jr et al., 1963) and which can also be reactivated (Kuck et al., 1962) or mixed with water before adding it to the oil (Eaves, 1967), attapulgite (Boki et al., 1994), soy hull carbon (Proctor and Harris, 1996), combusted rice hull (Gingras et al., 1998), etc.

Among these adsorbents, activated carbon is by far the oldest since it was used industrially in 1828 to decolourise sugar solutions (Aehnelt, 1957). The use of bleaching earth dates from 1880, when W.B. Albright and H. Eckstein of the N.K. Fairbank Company, Chicago, introduced fuller's earth for refining cottonseed oil (Blank, 1942) after D. Wesson had discovered its decolourising effect on vegetable and animal oils and fats (Zschau, 1987). The use of silica hydrogel in edible oil processing is much more recent given the date of its launch (Welsh and Bogdanor, 1986).

3.7.1.1 Bleaching earths

As recounted by Zschau (1987), it was a chance discovery that a mixture of clay and mineral acid left overnight in a water bath caused the material (after washing and drying) to become much more active in removing colour from oil. Since then, acid activation has become standard. The raw material used for the production of acid activated bleaching earth is a particular clay ore called bentonite having as major component the mineral montmorillonite (Taylor, 1993), a smectite-type mineral of volcanic origin. It is a lamellar aluminosilicate with each layer consisting of two sheets of silicium surrounded by oxygen tetrahedra and one central sheet of aluminum ions surrounded by oxygen octahedra (Zschau, 2000). Due to the occasional (about 1 in 5) replacement of an Al^{3+} ion by a divalent Mg^{2+} or Fe^{2+} ion, the aluminum silicate obtains a negative charge that is neutralised by a mobile, hydrated alkali cation. During acid activation, protons not only replace these mobile cations, but they also replace structural cations from the octahedral layer. This causes the clay particles to disintegrate from the edges, so that their surface area increases substantially (Taylor and Jenkins, 1988). It also provides the acid activated material with its acidity and ion exchange properties.

In the U.S., the clay raw material is mined in open pits because the overburden is usually only 1.5 to 15 m. The clay seams can range from a few centimetres to 4.5 m or more. The raw bentonite is moved by truck to the acid activation plant where the clay lumps are crushed, dried, and then slurried with acid water and pumped into large agitated reaction vessels that can be heated to near boiling temperature; the use of sulfuric acid is common in the U.S., whereas in Europe, the activation acid tends to be hydrochloric acid. When the desired degree of activation has been reached, the slurry is dewatered and washed with water before being dried, ground, and classified (Kögler, 1963). Acid activation plants face severe effluent problems.

As mentioned by Dijkstra (2002), bleaching earth has a totally different cost structure from for example edible oils. Its production entails a high initial investment leading to high fixed costs, whereas the variable costs are relatively

low. Developing a process or a product requiring substantially less bleaching earth would for a company manufacturing bleaching earth, therefore, amount to filing for bankruptcy. This is probably why the development effort by those companies is more directed towards filtration improvement and technical service.

3.7.1.2 Activated carbon

The edible oils and fats industry is far less important to the manufacturers of activated carbon than it is to the manufacturers of bleaching earth, since the 0.5 million tonnes of activated carbon produced annually worldwide have many other, widely differing applications. Besides, the usage level of activated carbon in edible oils processing is far lower than the usage level of bleaching earth.

Because of its unique properties, activated carbon is an essential adjuvant for the edible oils and fats industry: It is the only material known that effectively removes polycyclic aromatic hydrocarbons (PAH) that are not removed from oil during its deodorisation.

As discussed by Aehnelt (1957), several processes are used to produce activated carbon from a large variety of preferably cheap raw materials with a carbon content of 40 to 80%, such as blood, animal bones, sawdust, peat, etc. These raw materials are pyrolysed at a temperature of 850 to 950°C, at which temperature water gas is formed, especially when superheated steam is also injected into the incandescent material. This is the so-called steam activation process that yields a product that no longer contains any oxygen or hydrogen and that has become very porous (Ostrejko, 1902). The total surface area (BET) is then some 900 m^2/g. A coal-, lignite- or peat-based and steam-activated carbon is the product recommended for PAH removal.

Carbon can also be activated by the addition of chemical reagents. Formerly, the use of zinc chloride was widespread, but for environmental reasons, the industry decided to discontinue this practice. Phosphoric acid is now the most commonly used reagent.

3.7.1.3 Silica hydrogel

Silica hydrogel is not really a novel product since Joseph Crosfield was already producing it in 1927 (Leake, 1997b). Its manufacturing process is reasonably simple, but process control is critical in ensuring consistent quality. The raw materials are sulfuric acid and sodium silicate (water glass) as made by fusing pure quartz sand and soda ash. The sodium silicate is dissolved in water, the sulfuric acid is diluted with water and both solutions are mixed at high shear in the presence of excess acid. This destabilises the polysilicic acids present in solution, causing them to be liberated in a colloidal form as a silica hydrosol (Nock, 1996).

Within 30 minutes, this hydrosol becomes more robust so that it can be broken into small pieces that permit the sodium sulfate and excess sulfuric acid to be removed by washing with water. After washing, the hydrogel is milled, classified to a uniform particle size to ensure good filtration characteristics, and bagged. The final product as supplied to oil refineries contains some 67% volatiles (water) and has a surface area (BET) of some 800 m^2/g. A comparative chemical analysis of silica hydrogel and acid activated bleaching earth is given in Table 3.8 (Nock, 1996).

TABLE 3.8 Properties of silica hydrogel and acid-activated bleaching earth

Property	Silica Hydrogel	Activated Bleaching Earth
Total volatiles (1000°C; % wt)	67	16
Mean particle size (Malvern; µm)	18	16
BET surface [m^2/g]	800	260
Chemical Analysis [% wt on volatile-free basis]		
SiO_2	99.6	73.9
Al_2O_3	<0.1	10.9
Fe_2O_3	<0.1	5.4
MgO	<0.1	3.3
CaO	<0.1	2.2
K_2O	<0.1	2.1
Na_2O	<0.1	2.2

3.7.2 Physical aspects of bleaching

Activated carbon, the manufacture of which has been described above, is used in gas masks to purify the air inhaled by the person wearing the mask and is also used to adsorb obnoxious flue gas components. Adsorption isotherms are used to quantify the extent to which the amount being adsorbed and thus the purity of the gas that has been in contact with the adsorbent depend upon its concentration in the flue gas and the characteristics of the adsorbent.

However, as will be explained below, the adsorption isotherms concerned, the *Langmuir* and *Freundlich* isotherms, only apply in ideal circumstances where all active sites on the adsorbent are identical and the adsorbate (the substance being adsorbed) is a pure compound, such as a gas; also, the isotherms only deal with equilibria and certainly not with the rate at which this equilibrium is attained. Moreover, the isotherms do not cater for competitive adsorbates and if these happen to have different rates of adsorption, phenomena can be observed that bear no relation whatsoever to the adsorption isotherms. A decrease in colour followed by an increase is just one example of this limitation of adsorption isotherms. Nevertheless, and despite these fundamental limitations, adsorption isotherms can have their use in facilitating the understanding of what happens (or could happen) when edible oils are subjected to a bleaching treatment.

But they can only be useful in this respect if oil colour is measured in such a way that the analytical data are proportional to the amount of colour compound present. The *Lovibond* scale, having been designed for judging beer without drinking it (Stillman, 1955), is not linear and, therefore, useless for studying the bleaching process.

Fortunately, other methods of measuring oil colour have been developed and validated (Naudet and Sambuc, 1968). One such method is based on measuring the luminance Y (also called "integral transmission") of the oil sample. The luminance itself is a nonadditive quantity, but the logarithm of its reciprocal [$\delta = \log(100/Y)$], which by analogy may be named the "integral optical density," is linearly additive when determined at constant, dominant wavelength. It, therefore, can be used for studying decolourisation isotherms (Naudet and Drap, 1960a; Drap, 1961).

When studying these isotherms, two phenomena affecting the "colour balance" may have to be reckoned with (Transfeld, 1986). There is the phenomenon that heating oil without an adsorbent can already lead to a less intense colour (heat bleaching) and there is the phenomenon that bleaching oil over and over again still may not lead to a colourless oil (Drap, 1961). Both the disappearance of colour during heat bleaching and its persistence as "unbleachable" colour must be taken into account when calculating the data to be plotted in an adsorption isotherm.

3.7.2.1 Langmuir adsorption isotherm

The adsorption isotherm derived by Langmuir in 1916 is based on the assumption that the adsorbent has a limited and fixed number of identical adsorption sites; this assumption has been referred to as the "multistory car park" situation (Dijkstra, 2002). The rate at which these sites adsorb the adsorbate molecules is proportional to the number of unoccupied sites and the concentration c [mol/l] of the adsorbate in the surrounding solution. The rate at which the adsorbate molecules leave the adsorbent is proportional to the number of occupied sites. The proportionality factors equal the rate constants of the adsorption rate and the desorption rate, respectively, and the ratio of these rate constants equals the equilibrium constant b [l/mol]. By introducing the adsorbent loading x/m [mol/kg] and an adsorbent parameter a [mol/kg] that is indicative of the number of adsorption sites per unit of weight or adsorption capacity, the *Langmuir* adsorption isotherm can be expressed as:

$$\frac{x}{m} = a\frac{bc}{1+bc} \tag{3.1}$$

Writing the *Langmuir* isotherm this way shows that when the adsorbate concentration (c) increases, the fraction on the right-hand side approaches unity. Accordingly, the adsorbent loading approaches the adsorption capacity. To facilitate the graphical determination of the *Langmuir* constants a and b (or rather a and ab), Equation 3.1 can be rewritten as:

$$\frac{c}{x/m} = \frac{1}{ab} + \frac{c}{a} \tag{3.2}$$

Plotting the ratio of the adsorbate concentration (c) and the adsorbent loading (x/m) against the adsorbate concentration (c) results in a straight line that has a slope equal to $1/a$ and that cuts a piece of the ordinate that is equal to $1/ab$.

3.7.2.2 Freundlich[21] adsorption isotherm

A purely empirical correlation between the adsorbent loading x/m [mol/kg] and the adsorbate concentration c [mol/l] is the *Freundlich* adsorption isotherm:

$$\frac{x}{m} = kc^n \tag{3.3}$$

Like the *Langmuir* isotherm 3.1, the *Freundlich* isotherm 3.3 can also be written in a form that permits graphical determination of its constants:

$$\log(x/m) = \log k + n\log c \tag{3.4}$$

and like the *Langmuir*-isotherm, the *Freundlich* isotherm has also two constants: the dimensionless exponent n and the constant k, the dimensions of which depend upon the magnitude of n. For every value of $n > 0$, the adsorbent loading (x/m) goes on increasing when the concentration c increases. So whereas according to the *Langmuir* approach (multistory car park with a fixed number of spaces), there is a maximum adsorbent loading that is approached at infinite adsorbate concentration, there is no such maximum adsorbent loading according to the *Freundlich* isotherm. This is the reason why the latter has been likened to an old-car dump where cars can be stacked on top of each other (Dijkstra, 2002).

However, stacking cars is more cumbersome than just driving them into a parking space. In mathematical terms, this means that $n < 1$ since an increase in adsorbate concentration must lead to a smaller increase in adsorbent loading. Accordingly, the statement that "a good affinity is expressed by an n value between 2 and 10" (De Greyt and Kellens, 2000, p. 98.) should be disregarded. A paper dealing specifically with the *Freundlich* isotherm (Proctor and Toro-Vazquez, 1996) does not comment on n having to be <1, either.

Values for n reported in the literature differ widely. They can be <1 (King and Wharton, 1949b; Armstrong and Ireland, 1958; Jancik, 1958; Trizis and Uzzan, 1958; Drap and Naudet, 1960; Naudet and Drap, 1960a; Lachamp and Naudet, 1963; Gutfinger and Letan, 1978; Brown and Snyder, 1985; Achife and Ibemesi, 1989; Palaniappan and Proctor, 1991; Boki et al., 1992a). However, values >1 have also been reported (Hassler and Hagberg, 1939; Baldwin,

[21] Andersen (1962) still refers to this formula as the *Van Bemmelen-Freundlich* equation and, thus, includes the name of its actual originator *J.M. van Bemmelen*, a professor of inorganic chemistry in Leiden, The Netherlands.

1949; Stout et al., 1949; Feuge and Janssen, 1951; Pons Jr. et al., 1961; Okpala and Nwoke, 1989; Suzuki and Nishioka, 1993; Boki et al., 1994). Some authors (Fontana and Colagrande, 1964; Fontana and Colagrande, 1965; Sanelli, 1980) and more recently (Bayrak, 2003) even report negative values for n without apparently realising that these imply that a lower colour results from using less bleaching earth.

Why these wide differences? Why can some authors (Hinners et al., 1946) establish a correlation between the titratable acidity of a bleaching earth and its bleaching capacity for chlorophyll, and report fully acceptable values of 0.35 for n, while others are stuck with observations that do not fit into the generally accepted model of what happens when an adsorption equilibrium is established? There are as yet no straightforward answers to these and similar questions, but competition between co-adsorbing components could well play a role (Chapman and Pfannkoch, 1992).

3.7.2.3 Adsorption kinetics

Early authors reported in qualitative terms about the kinetics of bleaching that "adsorption of color is practically instantaneous" (Rich, 1964) or that 95% of the ultimate decolouration was attained within 5 minutes (Guillaumin and Pertuisot, 1967). By using this phraseology, Guillaumin and Pertuisot demonstrate that they realise that said ultimate decolouration is reached asymptotically. Accordingly, industrial bleaching processes may not reach adsorption equilibrium as exemplified by the so-called "press effect" (Henderson, 1993). This term refers to the observation that additional bleaching can take place in the filter cake, which then acts as a fixed-bed adsorption column.

Among the attempts to quantify the kinetics of the bleaching process, the efforts by Brimberg (1981; 1982) should be mentioned since subsequent authors (Brát and Zajíc, 1992; Topallar, 1998; Langmaack and Eggers, 2002) refer to them. Brimberg likens the bleaching process to the aggregation of colloid particles and, thus, proposes a rate equation according to:

$$\ln \frac{C_1}{C_0} = -k\sqrt{t} \tag{3.5}$$

where t is the time from the addition of the adsorbent, c_t is the pigment concentration at time t, c_0 is the concentration at $t = 0$, and k is the rate constant.

However, she apparently did not realise that when $t = \infty$, $c_t = 0$ or, in other words, that with patience, all pigment (colour) will disappear; it won't. Perhaps that is why her experimental data start to deviate from the straight line with slope $-k$ when $\ln (c_t/c_0)$ has decreased by about unity (to 40% residual colour). Topallar (1998) did not allow his colour to decrease that far, so this could be why he observes straight lines when plotting his absorbance values against $\sqrt{t}$. Whether the activation energy (3 kJ/mol) he derives has any significance, is to be doubted.

The rate equation used by Brát and Zajíc (1992):

$$\frac{dc}{dt} = k_1 e^{k_2} \tag{3.6}$$

cannot be a correct representation of reality either since the use of this equation leads to most unlikely numerical values: In a series of bleaching experiments with increasing bleaching earth dosages spanning one decade, the values of k_1 do not increase proportionally to the bleaching earth dosage, but cover a range of nine (!) decades. Similarly, the exponent k_2, in Equation 3.6 varies between 3.79 and 24.00 instead of being more or less constant.

Therefore, the approach taken by Proctor and Snyder (1988) is to be preferred. They just looked at the adsorption process as if it were a chemical reaction that is first order in both reagents provided their concentrations are below a certain limit. Similar kinetics were also observed for the removal of β-carotene from an acetone solution by acid activated bleaching earth (Khoo et al., 1979). Moreover, this approach also predicts that equilibrium will be more rapidly reached in a column or filter than when a small amount of bleaching earth is suspended in a large volume of oil.

A similar approach was also taken by de Oliveira and Porto (2005). They use absorbance data and assume that active adsorption sites decrease during the bleaching process. This brings them to a kinetic model that is second order in the dimensionless pigment concentration and that is in good agreement with their experimental data. They also report activation energies (heat of adsorption) of 52 to 78 kJ/mol, which is high for physical adsorption.

3.7.3 Chemical aspects of bleaching

3.7.3.1 Types of adsorption

The literature distinguishes two types of adsorption: physical adsorption and chemisorption, but there is no clear borderline between the two. During physical adsorption, van der Waals' forces cause the adsorbate to attach itself to the adsorbent. These forces are relatively weak and their heat of adsorption is low. For gases, values of 5 to 10 kcal/mol (20 to 40 kJ/mol) have been found.

Chemisorption on the other hand is characterised by much higher heats of adsorption, which should really be regarded as heats of reaction. Sarier et al. (1988) report a heat of adsorption for β-carotene on montmorillonite of 193 kJ/mol and describe this adsorption as a chemisorption stemming from the interaction of β-carotene with acid sites (Sarier and Güler, 1989). Similarly, Liew et al. (1993) describe the adsorption of β-carotene onto rice hull ash that had been given an acid wash as "caused by chemical interactions involving the adsorbed acid and the carotene."

Because a given amount and type of adsorbent has a fixed number of acid sites, this chemisorption follows a *Langmuir* isotherm. However, Sarier at al. (1988) noted that increasing the β-carotene concentration in the oil to above the maximum loading of the adsorbent still led to some carotene adsorption. For this adsorption, they calculated a heat of adsorption of only 21 kJ/mol and they, therefore, describe this secondary adsorption as physical.

Another instance of chemisorption is given by Güler et al. (1992) who report on chlorophyll adsorption. After mentioning that the acid bleaching earth will first of all remove the magnesium ion from chlorophyll under formation of phaeophytin, they note that phaeophytins are weak bases. Consequently, the adsorption of phaeophytin on acid-activated clay is mainly a chemical process stemming from the interaction of a weak base with the Lewis and Brönsted sites of the clay.

Thus, a distinction can be made between chemisorption and physical adsorption on the basis of this interaction and not on the basis of heat of adsorption. Chapman et al. (1992) use model systems (protoporphyrin IX dimethyl ester and several triglycerides and fatty acids) to determine single-component isotherms and find that the affinities of their adsorbates increase with their basicities; they, therefore, conclude that the predominant mechanism of the adsorption they studied is an acid-base reaction.

The mechanism by which phospholipids are adsorbed is not clear from the literature. Besides, several authors do not distinguish between hydratable and nonhydratable phosphatides (Brown and Snyder, 1989; Proctor et al., 1992). However, when introducing silica hydrogel (Trisyl™) as a novel refining material, Welsh and Bogdanor (1986) did not only report residual phosphorus levels, but also residual calcium and magnesium levels and compared these with the levels in the oil at the outset. This report shows that Trisyl™ could have an affinity for nonhydratable phosphatides since not only the phosphorus level but also both the calcium and magnesium levels decreased considerably; no bonding mechanism was proposed though.

In a subsequent presentation, Welsh et al. (1990) use mainly acid refined oil for their bleaching experiments. Consequently, the residual phosphorus and trace metals present in this oil could well stem from a poor separation after the acid degumming stage. This could imply that they are hydratable at sufficiently high pH and, therefore, does not prove the adsorption of NHP by silica.

For bleaching earth Zschau (2000) suggests that "in the presence of water, activated bleaching earth acts like phosphoric or citric acids to chelate trace metals. In both cases, either with bleaching earth or the chelating acids, the 'nonhydratable' calcium and magnesium salts of PA are split and become water soluble for removal either with the water phase or by absorption on the bleaching earth." His experimental evidence is represented in Table 3.9.

TABLE 3.9 Bleaching of canola oil: dry vs. wet

Bleaching Earth	Amount [%]	Chlorophyll [ppm]		Phosphatides [ppm P]	
		dry[a]	wet[b]	dry[a]	wet[b]
Untreated		5.63	5.63	34.6	34.6
Tonsil Opt.210 FF	1	0.21	0.08	24.5	3.1
Tonsil Opt 210 FF	2	0.02		18.6	1.5
Tonsil Opt 210 FF	3			9.2	1.5

[a] Dry bleaching: 1 to 3% bleaching earth at 90°C, 30 min, 60 hPa.
[b] Wet bleaching: 1% water and 1 to 3% bleaching earth at 80°C, 20 min, 600 hPa, afterwards conditions as for dry bleaching.
Source: Zschau, W. (2000), in *Introduction to Fats and Oils Technology*, O'Brien, R.D., Farr, W.E., and Wan, P.J., Eds., AOCS Press, Champaign, IL, 158–178; and Study Group, "Technologies for Industrial Processing of Fats and Oils" (2001), *Eur. J. Lipid Sci. Technol.*, **103,** 505–550.

That the presence of moisture could be desirable had already been discovered (King et al., 1947; Christenson and Harpt, 1951). If neutral bleaching earths are used, a chelating polycarboxylic acid must be added to remove the NHP (Brooks et al., 1992).

On the other hand, Nock (1996) uses water-degummed soya bean oil containing about 50 ppm magnesium and 150 ppm of calcium and notes that these levels decrease to quite low values after a treatment with 1.2 wt % (wet basis) silica hydrogel (Sorbsil®). This proves the adsorption of NHP. The same histogram (Nock, 1996, Figure 4.4) also shows the effect of using 1.2 wt % bleaching earth. This also lowers magnesium and calcium levels and, thus, removes NHP, but is less effective than the silica; possibly no water was present during the bleaching treatment. By using diffuse reflectance Fourier transform infrared spectroscopy, Proctor et al. (1996) conclude that silica hydrogel binds PC through the PC carbonyl and phosphate groups.

Soap removal by bleaching earth has been a matter of controversy. The earth could just act as a filter aid for the insoluble soaps. The possibility of this mechanism was demonstrated by Newby (1947), who observes the same low residual soap content when treating oil with bleaching earth as when using a filter aid. Sodium soaps could also be converted to FFA by the ion exchange capability of the acid activated earth (Arbeitskreis "Technologien der industriellen Gewinnung und Verarbeitung von Speisefetten", 199s3).

Sodium soaps could also be adsorbed by bleaching earth, but this possibility was rejected on the grounds that the presence of soaps does not affect discolouration (Odeen and Slosson, 1935; Newby, 1947; Naudet et al., 1961; Ottesen, 1981) meaning that the soap and the colour molecule do not compete for the same active sites on the adsorbent. However, more recently, Taylor shows in his review article (1993) two graphs showing residual red and residual chlorophyll, respectively, as a function of clay dosage for different soap levels, and these graphs indicate

lower residual colour levels for lower soap content. According to their source (Shaw et al., Paper presented at the 83rd AOCS Annual Meeting & Expo, Philadelphia, 1985[22]), soap does compete with colouring bodies for the active sites on the bleaching earth. Even more recently, Dijkstra (2002) reported on some results obtained by ITERG, Pessac: the FFA (acidity) of a sample of oil containing both soaps and FFA does not change on bleaching, so no FFA are formed by ion exchange.

For the soap removal by silica hydrogel, a mechanism has been proposed that just assumes that the soap dissolves in the water trapped by the hydrogel. This proposal is supported by the observation that the soap is liberated again when the water is removed from the mixture by heating under reduced pressure.

3.7.3.2 Bleaching earth as a catalyst

Acid-activated bleaching earth not only has a large surface area per unit of mass (around 300 m^2/g), but it also has acid sites that can bind molecules by polarising them, and it can extract metal ions from NHP (*vide supra*) and chlorophyll and convert the latter into pheophytins (Zschau, 2000). The acid sites can also cause the UV (ultraviolet) adsorption around 230 nm to decrease and the absorption around 270 to increase (Drap et al., 1961). This phenomenon, therefore, forms the basis of a method of detecting whether or not lard has been refined, i.e., treated with bleaching earth (Kaufmann et al., 1956).

The presence of an isosbestic point when more bleaching earth is used (Guillaumin and Pertuisot, 1968) points to a conversion of a limited amount of a precursor present in oil to be bleached, into a conjugated double bond system. Guillaumin and Pertuisot (1968) describe the isolation of this precursor with the absorption maximum at 232 nm, which they demonstrate to consist of dienes with an hydroxy- or hydroperoxy-group at an allylic position. These precursors then form conjugated trienes absorbing at 268 nm by a dehydration reaction that is catalysed by the acid sites on the bleaching earth. Aldehydes and ketones are also formed as demonstrated by the rise in the Anisidine Value (AV) of oil being bleached (Mag, 1990).

The acid sites in bleaching earth also catalyse the dehydration of other hydroxy compounds present in the oil, such as plant sterols (Kaufmann et al., 1970; Kaufmann and Hamza, 1970) and, thus, lead to nonpolar sterols. In addition, Kaufmann et al. (1970) also noted dehydration under formation of an ether linkage between two sterols. Disitosterol ethers were detected in processed oils, whereas none were detected in the unsaponifiable of freshly extracted oils; bleaching the unsaponifiable dissolved in hexane led to their formation.

Subsequently, Homberg (1974; 1975) continued studying the effect of bleaching earth on sterols and concludes that disterols are only formed when treating the unsaponifiable with bleaching earth. In oils, dehydration under formation of double bonds is the only reaction observed. This catalytic action is also used to detect the adulteration of virgin olive oil by admixture of refined olive oil. In refined olive oil, significant amounts of stigmasta-3,5-diene have been formed by the dehydrative action of bleaching earth, whereas this compound is completely absent in virgin oil (Lanzón et al., 1989).

Because bleaching earths are acid catalysts, they can also protonate double bonds, and this reaction, being reversible, can lead to both positional and geometrical isomerisation. Ney (1964) has studied both types of isomerisation and concludes that below 150°C bleaching earth does not cause such isomers to be formed.

Another reaction requiring an acid catalyst is the formation of an ester bond between for instance a free fatty acid and partial glyceride. In theory, it should be possible to neutralise high-acidity oils by reesterification and use acid activated bleaching earth as the esterification catalyst. On paper, this looks an attractive process since the FFA bound by reesterification are not lost. However, in practice the process suffers from the formation of *trans*-isomers because the temperature required for the reesterification is also above 150°C.

The opposite reaction, acid catalysed hydrolysis of triglycerides, has also been mentioned (Study Group "Technologies for Industrial Processing of Fats and Oils," 2001) and indeed, several authors report an FFA increase during bleaching (Kögler, 1968; Morgan et al., 1985). On the other hand, both FFA increases and FFA decreases on bleaching have also been reported (Zschau, 1979), which Baldwin (1949) explains by assuming a preferential adsorption of FFA combined with hydrolysis if water is present (Boki et al., 1992b). Mag (1990) concludes that variations in clay dosage and contact time have little or no effect, but that the bleaching temperature significantly raises the FFA concentration during bleaching.

Bleaching earth also acts as a catalyst in spent earth, the filter cake resulting from the removal of the earth from the oil by filtration. Especially when air is present and the oil is unsaturated, the oil retained in between the earth particles tends to oxidise and/or polymerise. These reactions are catalysed by the bleaching earth and can lead to self-combustion (see also Taylor and Jenkins, 1990).

3.7.3.3 Chemical bleaching

In 1929, Vosmaer uses ozone to bleach linseed oil, palm oil, and soya bean oil. Sodium chlorite (Jacini, 1955) and chlorine dioxide have been used (Anon., 1947) on "brown grease" and tallow. Blowing oxygen through the oil for a considerable period of time (2 hours) is also quite effective provided some phosphoric acid and silicate are present (Henderson and Libby, 1949a), but in a later patent

[22] It is a pity that I have only the reference to this paper, so I lack data about the type of clay used and cannot speculate to what extent this might explain the conflicting evidence.

(Henderson and Libby, 1949b) with an earlier application date, the same inventors claim the use of compounds liberating nascent oxygen, such as hydrogen peroxide, sodium chlorite, and sodium perborate. Brown grease and fish oil can also be bleached with 30% hydrogen peroxide (Jespersen, 1950; Barsky, 1955). Sodium perpyrophosphate can be used on its own or in combination with hydrogen peroxide (Noder, 1941) and the effect of hydrogen peroxide can be optimised by controlling the pH at around 8.5 (Scheller, 1940) or by adding water (Bates and Endres, 1967).

Even more drastic oxidation processes have been claimed for treating edible oils: Aschenbrenner (1953) uses chromium trioxide and sulfuric acid. A mixture of chromium trioxide and hydrogen peroxide has been claimed (Elston, 1957) as well as chlorine compounds liberating oxygen, chromates, and permanganates (Bouquet and Faur, 1960). Using potassium dichromate, potassium permanganate, or dilute aqueous solutions of nitric acid (Sims et al., 1959) substantially retards the development of flavour reversion of soya bean oil. In their review on chemical bleaching, Kaufmann et al. (1967b) also mention potassium permanganate. Andersen (1962) mentions the use of oxygen, ozone, peroxides, light, dichromate, and permanganate, and finally chlorine compounds. In addition, he mentions reduction methods employing sulfites and points out that the hydrogenation process can also lead to colour reduction and is to be regarded as a chemical bleaching process. Yet another chemical process employs ferric chloride for the removal of gossypol (Yatsu et al., 1970; Yatsu et al., 1972).

After 1960, hardly any publications on chemical bleaching seemed to have appeared. However, Jones et al. (1982), who are predominantly concerned with bleaching raw materials for soap making, mentioned that sal and rice bran oils, which are important raw materials in the Indian subcontinent, are currently being bleached with chlorine dioxide. They described the use of polar bleaching agents, such as hypochlorites, peroxides, peroxoacids, and chlorites in the presence of a phase transfer catalyst, such as a quarternary ammonium compound. Of course, hydrogen peroxide is still being used, but then strictly for non-food applications, except of course for lecithin used as a food additive. Double bleached lecithin has also been treated with benzoyl peroxide (Pardun, 1988).

Heat bleaching can also be considered a form of "chemical bleaching," but since it does not require the addition of "chemicals," the general public is unaware that quite a lot of chemistry is involved in the thermal breakdown of colouring compounds. Accordingly, it is still acceptable, which is fortunate since heat bleaching during deodorisation would be difficult to prevent.

3.7.4 Bleaching processes

By comparing vacuum bleaching equipment (see Zschau, 2000, Figure 9.9, p. 173) with an industrial bleacher, Dijkstra (2002) showed the latter to be nothing but a scaled-up version of the former. He concluded that "the bleaching process has been insufficiently exposed to proper process engineering" and could be ripe for development that should preferably be based upon insight into the fundamental aspects of the process.

3.7.4.1 Theoretical considerations

Most fatty consumer products like spreads and shortenings are based upon blends of different fatty components, such as one or more hydrogenated hardstocks, perhaps some interesterified products, or even fractionated products, and one or more liquid oils. Before being processed into said consumer products, these blends will have been deodorised and the deodorisation process is always preceded by a bleaching step. The question to be discussed now is what intermediates should be bleached: components or blends. The two possibilities are illustrated in Figure 3.17.

Both possibilities start with neutral components that may be liquid oils or modified hardstocks. The possibility shown on the left-hand side of Figure 3.17 prepares a blend from these neutral components, then bleaches the various blends, and obtains blends that are ready to be sold or processed by deodorising these bleached blends. Another possibility is depicted in the remainder of Figure 3.17. The components are bleached individually and then stored (which is not recommended (Erickson, 1995) since bleached oil is reported to be highly susceptible to oxidation), blended and deodorised as blends (centre of the figure), or bleached and deodorised individually, stored as such and then weighed into blends as and when required (right-hand side of the figure). The question to be answered now is whether these possibilities require different amounts of bleaching earth and, if so, which possibility requires the least. By defining:

r = amount of colouring matter after bleaching ("r" = residual)
s = amount of colouring matter before bleaching ("s" = start)
t = amount of oil ("t" = tonnes)

and by looking at a simple system comprising two components (subscripts 1 and 2) only, the bleaching earth requirement (m_1) needed by the first component to reach a final colouring matter concentration of $c_1 = r_1/t_1$ after bleaching, requires the adsorption of an amount of colouring matter equal to (s_1 - r_1), so that according to the *Freundlich* adsorption isotherm and its bleaching earth parameters k and n:

$$\frac{s_1 - r_1}{m_1} = k\left(\frac{r_1}{t_1}\right)^n = p_1 \qquad (3.7)$$

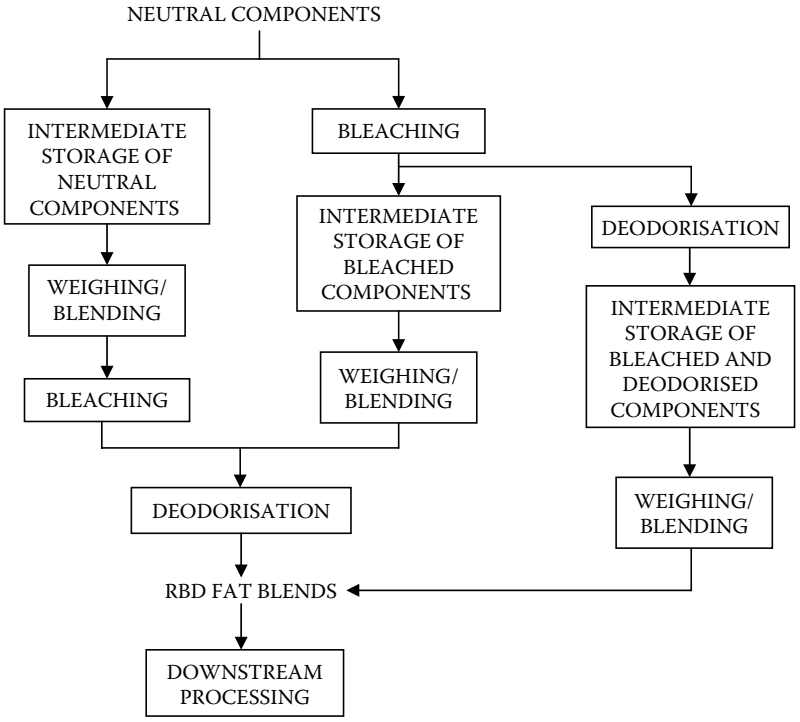


FIGURE 3.17 Bleaching components or blends?

For the second component, the same equation holds when the subscript 1 has been changed to subscript 2. Since the two components will be bleached to the same residual colour levels:

$$\frac{r_1}{t_1} = \frac{r_2}{t_2} \tag{3.8}$$

Accordingly:

$$p_1 = p_2 = p \tag{3.9}$$

so that the amounts of bleaching earth required to bleach the components are:

$$m_1 = \frac{s_1 - r_1}{p} \quad \text{and} \quad m_2 = \frac{s_2 - r_2}{p} \tag{3.10}$$

On the other hand, if the two components containing s_1 and s_2 amounts of starting colour are blended before being bleached and then bleached as a blend weighing $(t_1 + t_2)$, an amount of m_3 bleaching earth is needed according to:

$$\frac{s_1 - r_1 + s_2 - r_2}{m_3} = k\left(\frac{r_1 + r_2}{t_1 + t_2}\right)^n = p \tag{3.11}$$

Like Equation 3.9, the above Equation 3.11 has again been equaled to p since the fraction between brackets indicates the residual colour of the blend, which equals the colours of the bleached components discussed before. Accordingly,

$$m_3 = \frac{s_1 - r_1 + s_2 - r_2}{p} = \frac{s_1 - r_1}{p} + \frac{s_2 - r_2}{p} = m_1 + m_2 \tag{3.12}$$

or in other words, the amount of bleaching earth required to bleach a blend to a certain residual colour is the same as the sum of the amounts required to bleach the blend components to the same residual colour.

This finding is not really surprising since an adsorbent in equilibrium with oil having a given residual colour concentration will, according to the adsorption isotherm, have a set colour loading. Since each component and the blend were bleached to the same residual colour concentration, the spent earth resulting from all these bleaching steps has the same colour loading and, because the amount of colour removed is the same for all routes, the amount of earth required is also the same for all routes. Consequently, the same result would have been reached with the assumptions underlying the *Langmuir* adsorption isotherm as with the *Freundlich* isotherm as used above.

Similarly, bleaching one component to its set residual colour level and mixing this bleached component with an unbleached component and bleaching the mixture to the same set residual colour level does not affect the total amount of adsorbent required. However, bleaching one component that is considered "difficult to bleach" to a residual colour level that is higher than target, and

compensating this by blending this relatively dark component with another component that has been bleached to a colour level below target, will require more bleaching earth than bleaching both components to the same target level since the reduced usage for the "dark" component is outweighed by the additional usage for the "light" component. If, on the other hand, the relatively dark bleached component is mixed with the other, unbleached component and the mixture bleached to target residual colour, the total amount of bleaching earth would be slightly less, since the colour loading on the earth used for the "dark" component would be higher than when that component is bleached to target colour.

The above, of course, only holds when the same adsorbent is used for each component and to remove the same adsorbate(s). If, for instance, activated carbon is used to remove heavy PAHs from coconut oil and activated bleaching earth used to remove chlorophyll from another component that does not contain any PAH, minimal adsorbent usage results from treating the coconut oil separately with an amount of activated carbon that ensures that the ultimate blend meets specification even if the treated coconut oil on its own does not. That way, the PAH loading on the active carbon is much higher than when this adsorbent is used to treat the blend, and a high adsorbate loading implies a low adsorbate usage. However, the EU Scientific Committee on Food conclude that "in view of the nonthreshold effects of genotoxic substances, the levels of PAH in foods should be reduced as low as reasonably achievable." Consequently, the way to save on activated carbon as described above is, in fact, prohibited.

Similarly, a high bleaching earth usage can be caused by a low adsorbate loading and this corresponds to a low adsorbate concentration as observed when oil has been diluted with a solvent. On practical grounds, bleaching a miscella would have advantages since the bleaching earth filter cake will retain less oil and it can be easily disposed of by mixing it with the marc. In theory, it might have the disadvantage of using more bleaching earth. However, experiment does not substantiate this theoretical possibility. When Feuge and Janssen (1951) bleached a cottonseed oil miscella in hexane, they observed lower oil colours than when they omitted the hexane.

They thereby confirmed their findings of a patent applied for in 1947 (Marmor and Moyer, 1952), which also reports a better colour after miscella bleaching than after oil bleaching for a wide range of oils and adsorbents. In a later patent (Fabre, 1968), the adsorbent is also used to remove phosphatides so that the resulting oil can be physically refined. In addition, the spent earth is even regenerated by extraction with acetone and treatment with sulfuric acid; these processes are unlikely to work. On the other hand, it is somewhat surprising that the recent review (Study Group "Technologies for Industrial Processing of Fats and Oils," 2001) only mentions a column chromatography process (Strauss et al., 1975; 1976; 1977) when discussing miscella bleaching. Perhaps the lack of specifications for anything but crude oil mentioned in the Introduction (Section 3.1) is partially responsible for the apparent segregation of oil treatments between oil milling and refining.

A high adsorbate loading on spent adsorbent leading to reduced adsorbent usage is also the aim of countercurrent bleaching processes. The following treatise shows what this reduction can amount to. Using the same definitions of *r, s,* and *t* as above, the *Langmuir* adsorption isotherm (Equation 3.1) can be rewritten as:

$$\frac{s-r}{m_1} = a\frac{br/t}{1+br/t} \tag{3.13}$$

Accordingly, the amount (m_1) of bleaching earth with an adsorbed amount of colouring matter (s - r) that is in equilibrium with a residual amount of colouring matter (r) that is dissolved in an amount of oil (t) equals:

$$m_1 = \frac{(s-r)(t+br)}{abr} \tag{3.14}$$

This is the standard co-current situation whereby spent earth is more or less in adsorption equilibrium with bleached oil. If it were to be in equilibrium with nonbleached oil, the equilibrium concentration of the adsorbate would increase from r/t to s/t and the amount of earth required would decrease to m_2:

$$m_2 = \frac{(s-r)(t+bs)}{abs} \tag{3.15}$$

By defining the variable p as the fraction of the total amount of adsorbate that is not removed by adsorption ($p = r/t$), the variable r can be eliminated. The potential "adsorbent saving" can then be worked out as:

$$m_1 - m_2 = \frac{t}{ab}\frac{(1-p)^2}{p} = \frac{t}{ab}(1-p)\left(\frac{1}{p}-1\right) \tag{3.16}$$

As to be expected, this equation shows the adsorbent savings to be proportional to the amount of oil t being bleached. The saving is also inversely proportional to the *Langmuir* constants a and b. The constant a is indicative of the surface area or the number of active adsorption sites per unit of mass, so, if less adsorbent is required to achieve a certain degree of discolouration, the saving when using that particular adsorbent will also be less. It is also less when the adsorbate is strongly bound as indicated by the constant b, being the ratio of the rate constants of the adsorption and the desorption, respectively.

The variable p also affects the adsorbent saving. As shown by the above equation, a small value of p

(extensive colour removal) leads to increased savings. On qualitative grounds this was also to be expected since extensive bleaching increases the difference in adsorbent loading between adsorbents in equilibrium with nonbleached oil and with bleached oil.

Quantifying the savings is not yet possible since the literature lacks pertinent data on the *Langmuir* constants. According to data by Trizis and Uzzan (1958), who quantified oil colour by measuring its absorbance, *a* could be about 100 and *b* could be around 0.1 when expressed in the units used by the authors. Using different units and a different adsorbent leads to different constants (Fontana and Colagrande, 1962). Subsequent bleaching earth development may have made these data obsolete.

3.7.4.2 Industrial processes

The bleaching equipment used industrially has been the subject of several reviews (Kaufmann and Mukherjee, 1967a; Study Group "Technologies for Industrial Processing of Fats and Oils," 2001). An aspect not mentioned in these reviews is the replacement of air by nitrogen (Liebing et al., 1975) in the silos or day-hoppers storing the bleaching earth and it is not clear whether this has ever been used in practice.

With respect to the bleaching temperature, a range of 90 to 100°C is generally recommended, but this may be raised slightly to 90 to 110°C (Patterson, 1976) or 95 to 110°C (Mag, 1990) for oils that prove to be difficult to bleach. (Mag, 1990; Arbeitskreis "Technologien der industriellen Gewinnung und Verarbeitung von Speisefetten," 1993; Patterson, 1993; Taylor, 1993; Erickson, 1995; De Greyt and Kellens, 2000; Study Group "Technologies for Industrial Processing of Fats and Oils," 2001). When raising the temperature does not lead to a lower colour and "colour fixation" is suspected, a lower temperature may be beneficial. For palm oil, a temperature of up to 130°C was quoted as usual at that time and raising this to at least 175°C was claimed as an improvement since less fuller's earth was required (Brinckman, Sr. et al., 1959). Henderson (1997) reports that with a given amount of clay (2%) and bleaching time (20 min), raising the temperature in the bleacher from 230°F (110°C) to 290°F (143°C) substantially reduces the final chlorophyll level (from 320 to 120 ppm). However, it also increases FFA, so using the lowest temperature is recommended.

The duration of the industrial bleaching process should be 20 to 30 minutes for most oils (Study Group "Technologies for Industrial Processing of Fats and Oils," 2001). Increasing this period of time may lead to a worsening of the colour, especially at the highest temperature (see Naudet and Drap, 1960b, Figure 2; and Rich, 1964, Figure 9 and Figure 10).

Bleaching under vacuum has the advantage that it reduces the presence of oxygen. This is especially important when acid activated bleaching earths are used because they can cause colour reversion. Applying a vacuum also leads to much lower peroxide values in bleached tallow (see Eicke, 1985, table 4; or Study Group "Technologies for Industrial Processing of Fats and Oils," 2001, Table 2). Table 3.9 (Section 3.7.3.1) shows that the addition of water before applying this vacuum is advantageous. It is also recommended for the removal of residual nickel from hydrogenation products by bleaching earth (Kopp et al., 1989; 1991).

For the use of activated carbon, it is recommended (Patterson, 1993) to add the more expensive carbon after having added the bleaching earth to the oil so that the carbon can then concentrate on contaminants left by the clay, such as heavy PAH; light PAH with up to four condensed aromatic rings will be effectively removed from the oil during deodorisation. On the other hand, it is also mentioned (Study Group "Technologies for Industrial Processing of Fats and Oils," 2001) that bleaching earth and activated carbon do not compete with each other, so that the supply of a ready mixture containing 5 or 10% activated carbon is technologically justified; it also facilitates material handling and may reduce storage requirements. The particle size distribution of activated carbon is not such that the small amounts involved require special filtration measures.

The original batch bleaching process has the advantage that it preserves batch identity. It permits preconstituted blends to be processed provided batch identity can also be preserved during the subsequent deodorisation stage. This is what is now being achieved in the semicontinuous bleachers connected to a downstream stock change deodoriser (see Kellens and De Greyt, 2000, Figure 13.16).

For processing liquid oils in single-stream plants, continuous bleachers feeding continuous deodorisers should be used. They were introduced by Slocum (1938), Hagan (1940), modified (King and Wharton, 1949a), further improved (Sanders, 1951), and provided with a special system to mix the bleaching earth into the oil (Mag and Reid, 1980).

For its removal, the former plate and frame presses (Richter, 1978) have been superseded by pressure leaf-type filters (Arbeitskreis "Technologien der industriellen Gewinnung und Verarbeitung von Speisefetten," 1996). In these filters, woven metal screens in the form of stacked hollow disks provide a support for the filter cake that is gradually built up. Accordingly, a filter cycle comprises the following elements:

- Filling the empty filter with the suspension to be filtered.
- Feeding the filter with more suspension to be filtered while recirculating the filtrate until the filtrate is clear.
- Diverting the filtrate (product) to the intermediate filtrate storage until either the pressure difference exceeds a set value or the filter housing is full.
- Forwarding the liquid present in the filter housing to an intermediate storage.

- Blowing the filter cake with, for example, steam and collecting the recovered oil separately.
- Dumping the filter cake by opening the filter housing and rotating or shaking the disks.

These disks can be mounted horizontally (with their axes vertical) as discussed by Müller (1964) or vertically (Wilke, 1963). Nowadays, the filtration equipment is highly automated and requires little supervision. Often a polishing filter is provided downstream of the main filter to ensure that oil going into the deodoriser is completely free from bleaching earth.

As mentioned above in the theoretical considerations, co-current processes make poor use of the available adsorption capacities of the adsorbents used in "adsorptive cleansing" since spent adsorbent is in equilibrium with bleached product. A more efficient way of using adsorbents is to expose them to oil with a higher adsorbate concentration than fully bleached material. This can be done by adding the adsorbent gradually, allowing the successive portions to reach adsorption equilibrium and removing them before a next portion is added (Harris and Levine, 1972). This procedure makes more sense than gradual addition alone (Baldwin, 1949). However, incorporating multistage filtration into an industrial process is rather cumbersome.

Despite an early failure to demonstrate the effectiveness of counter-current bleaching (James, 1958), several authors/inventors have suggested ways to perform this process (Bailey, 1952; 1953; McMichael et al., 1955; Singleton and McMichael, 1955; Singleton, 1956), but counter-current bleaching was only industrialised after Transfeld introduced the "Öhmi-bleach" process. In this process (Transfeld and Schneider, 1994; Transfeld, 1994), partially bleached oil is brought into contact with a reduced amount of fresh earth, which is subsequently separated from the resulting fully bleached oil by means of a decanter. The bleaching earth slurry leaving this decanter (Transfeld and Börner, 1997; Transfeld et al., 1998) is then used to treat nonbleached oil and the resulting filter cake is discarded. This results in a saving of up to 40% in bleaching earth for strongly coloured oils (Transfeld and Schneider, 1996).

Further savings are claimed by using bleaching earth with finer particles (<10 μm); normal bleaching earth has an average particle size of some 25 μm. To allow these fine particles to be removed from the oil by filtration, they must first be agglomerated by exposing them to an electric field (Transfeld, 1998a; 1998b; Transfeld et al., 2003). No data on oil retention by the fine particles have been published.

Finally, the use of silica hydrogels like Trisyl® and Sorbsil® in adsorptive cleaning will be discussed since these adsorbents are used industrially. It was already known that silica gel or alumina could be used to treat oil miscella before evaporation and bleaching (Strauss et al., 1976). It was discovered in 1986 by Welsh and Parent that silica hydrogels could be used to remove soap, phosphatides, and metal ions from oil. Subsequently, it was discovered that colours could also be removed by silica hydrogel provided it was treated with an acid (Pryor et al., 1988) and that this acid-treated silica also had a greater affinity for phosphatides (Welsh et al., 1990). As pointed out by Chapman (1994), sulfuric acid used for the treatment tends to leach into the oil and then causes the oil colour to increase during the deodorisation stage. This can be counteracted by treating the oil with bleaching earth after the silica treatment and before deodorisation.

The NHP adsorption mechanism is not clear in that the presence of an amount of hydratable phosphatides that exceeds the amount of NHP is felt to be necessary for the removal of NHP to occur (Welsh et al., 1990). Moreover, to explain the observation that soap enhances phosphatide adsorption, it has been suggested that soap and phosphatides form mixed micelles, which become smaller as the soap content increases. The greater solubility of the soap in water would then aid the subsequent inversion of these micelles inside the silica pores (Jalalpoor, 2004). Removal of water by evaporation at reduced pressure also improves phosphatide removal (Van Dalen and Brunia, 1991).

This observation has led to the so-called "Modified Caustic Refining" process (Parker, 1994) employing only a single centrifugal separator. The oil leaving this separator still contains appreciable amounts of soap and some phosphatides, but instead of removing these by water washing, a silica hydrogel is added, the resulting suspension is then sent to a vacuum bleacher and from there either to a filter that has been precoated with bleaching earth or to a bleacher where it is treated with bleaching earth before being sent to a filter (Estes et al., 1995b). Silica hydrogels have also been found to be effective in removing residual nickel from hydrogenated oils (Nock, 1994) and soap from chemically interesterified oils (Leake, 1997a).

3.7.5 Disposal of spent adsorbents

Spent bleaching earth is a waste product or by-product of the edible oil industry. It contains oil and, if this is unsaturated, it can catch fire. As pointed out by Dijkstra (1993), the oil content in spent bleaching earth is commonly determined by Soxhlet extraction, but this does not extract polymerised, oil-derived products. "The proper way to determine how much oil has been lost in the spent earth is by measuring its water content by the Karl Fischer method and its dry clay content by calcination and calculating the oil content as the difference." (see also Section 6.3).

This advice was taken up by Morton (1995), who showed (Table 3.10) that extraction removed only part of

the organics because extracted samples still lost weight when treated in a muffle furnace. The more unsaturated the oil, the higher this loss. Among the samples studied, this loss was never less than some 33%. This corresponds to >50% of the fresh earth mass, which means that statements "that for each 100 kg of fresh earth 25 to 45 kg of oil are lost" (Study Group "Technologies for Industrial Processing of Fats and Oils," 2001) or even that spent earth contains only 4% oil (Röbbeling, 1970) are unrealistically optimistic since they are based on what can be extracted; >50 kg is more likely, even with extensive blowing of the cake.

According to Rich (1960), oil retention by activated clays can vary between 27 and 85% and a relationship exists between the bulk density of the clay and its oil retention. "Activated clay is less compact or more 'fluffy,' contains more air-void space, and thus retains more oil than natural clay after the usual air- and/or steam blowing in the filter press in the plant." The effect of acid activation on oil retention and filtration time is illustrated in Table 3.11 (Fahn and Fenderl, 1974), showing that activation increases oil retention. As a rough guide, it can be stated that oil retention by a neutral clay amounts to one-third of its weight and by an activated clay to one-half of its own weight (Jaillet-Rouyer, 1953). Accordingly, the calorific value of spent bleaching earth is close to that of lignite.

TABLE 3.10 Analysis of bleaching earth filter cake samples

Oil Type	Moisture 169°C [%]	Ign. Loss [%]	Hexane Extract [%]	Ign. Loss After Extraction [%]
Hard fish oil	5.9	49.1	30.9	19.5
Soft fish oil	3.1	53.3	31.3	20.8
Sunflower oil	11.3	43.9	9.2	32.1
Soybean oil	7.4	39.9	8.4	27.3
Rapeseed oil	6.5	41.6	19.2	20.0
Hydrog. rape.	1.6	50.1	44.5	5.2

Source: Morton, R.B. (1995), Oil content of filter cakes, *Paper presented at the 86th AOCS Annual Meeting & Expo,* San Antonio, TX.

TABLE 3.11 Oil retention and filtration time in function of the amount of acid used for activating the bleaching earth

	Oil Retention [%]		Filtration Time [sec]	
Meq. HCl per 100 g Bentonite	Soybean oil	Palm oil	Soybean oil	Palm oil
0	23	25	25	25
280	33	34	25	30
560	40	41	25	30
1000	46	46	25	30
1260	49	50	30	45
1680	54	56	40	45

Source: Fahn, R. and Fenderl, K. (1974), *Oléagineux*, **29**, 193–197.

For disposal purposes, different types of spent adsorbent streams must be distinguished:

1. Spent bleaching earth with possibly small amounts of silica hydrogel originating from the treatment of a single oil.
2. Spent silica hydrogel originating from treating a single oil.
3. Spent bleaching earth with possibly small amounts of silica hydrogel originating from the treatment of different oils and/or fat blends.
4. Spent bleaching earth also containing spent activated carbon.
5. Spent bleaching earth used for bleaching hydrogenated products.

3.7.5.1 Oil recuperation

Given the fairly high oil content, it is not surprising that attention has been paid to recovering this oil after the filter cake has been blown, whereby as little air as possible is used to prevent excessive oxidation (Wittka, 1939), which would impair the quality of the oil to be extracted afterwards. According to Kaufmann and Mukherjee (1967b), who provide many references to a broad range of patents, there are three types of oil recuperation: the oil can be displaced by water or an aqueous solution of surfactants, such as sodium carbonate (Bloemen, 1960), the oil can be saponified and recuperated in the form of soaps, or the oil can be extracted with appropriate solvents (see also Klein, 1986).

In his review, Ong (1983) lists various ways to recover the oil: blowing steam through the filter cake, circulating hot water through the filter cake, circulating hexane through the filter cake, mixing the cake with milled oilseeds or expeller cake and passing it through the extractor, removing the cake from the filter, and sending it to a separate extraction unit that may use hexane or an aqueous extraction process. Because of the rapid deterioration of oil retained in the cake, he favours *in situ* treatment in the filter. He illustrates this rapid deterioration by reporting that a cake from which 28% oil could be extracted 3 hours after it had been removed from the filter only released 4.5% oil on extraction when stored at 70°C for 40 hours.

Aqueous extractions can involve added water-soluble iron, manganese, chromium, and aluminium salts (Slocum, 1938), added silicates (Leue, 1939), ball milling the spent clay in water (Lachle, 1943), sodium carbonate and some alkali (Chaloner and McNicoll, 1955), or a combination of aqueous alkali and sodium sulfate (Hodgson and Red, 1978), to mention just a few of the patents in the field. It can involve the recovery of triglyceride oil followed by the saponification of residual oil (Smith et al., 1980), the saponification of all oil present (Jaynes and Osburn, 1949; Smith et al., 1977; Ouchi

and Saito, 1981), or the total hydrolysis of any ester bond present at an elevated temperature (200 to 250°C) and pressure (Penninger, 1979).

Miscella can be used advantageously to extract oil from spent earth while this is still in the filter (Brücke, 1951), since this utilises some residual bleaching activity (Anon., 1961). The extraction with weak miscella is preferably followed by extraction with pure hexane (Meiners, 1971) and drying the cake *in situ* by blowing it with nitrogen. Liquid propane can also be used as an extraction solvent (Leaders and Argue, 1950; Rothbart, 1997), just as liquid CO_2 (Waldmann and Eggers, 1991), and various alcohols (Lee et al., 2000). Weber (1980) proposed an oil recovery system involving extraction of the spent earth by hexane, which is then displaced by water leading to a wet and easily disposable clay.

After the oil has been recovered from the spent bleaching earth, the extraction residue still has to be disposed of, with landfill being a logical solution. In this case, no distinction needs to be made between the various types of spent adsorbent streams. When choosing the oil recuperation process, types (d) and (e), containing PAH and nickel, respectively, should not be mixed with milled oilseeds or expeller cake for subsequent extraction. It has also been suggested that de-oiled earth could be retorted to produce an activated carbon/clay product for water treatment purposes or for other uses where activated carbon is generally used (Orth Jr., 1981). Heating spent earth up to 450°C under vacuum (Meister, 1945) also generates a product containing activated carbon.

3.7.5.2 Regeneration and reuse

Disposal of the oil-free bleaching earth still constitutes an expense and leads to an interest in regenerating it, which would also substantially reduce the amount of fresh earth to be purchased. Since bleaching earths are also used for mineral oils, early work on regenerating spent bleaching earth stems from the mineral oil industry (Parker and Bent, 1931; Thomopoulos and Liapis, 1962). As tabulated by Kalam et al. (1988b),[23] these early processes always involve a pretreatment to remove most of the oil, followed by a regeneration. An acid wash step to restore the acidity has also been found to be advantageous (Nebergall et al., 1994).

It was noted quite early (Stalmann, 1938) that any heat treatment must be within a narrow temperature range, and Table 3.12 does indeed illustrate this requirement. Several authors report that hardly any bleaching activity is lost during regeneration, which makes this process potentially very attractive. Regenerating the clay surface and pores by burning off what is on it was found to be an effective method in a laboratory muffle furnace, but on an industrial scale, the temperature is difficult to control. Accordingly, various authors reduce the calorific value of the clay by solvent extraction or anaerobic pyrolysis before exposing the material to oxygen.

TABLE 3.12 Recommended activation temperature ranges

Authors	Range [°C]	Remarks
(Parker and Bent, 1931)	400–510	Uses steam to prevent overheating
(Stalmann, 1938)	500–550	Pretreatment: solvent extraction and steaming
(Simpson et al., 1944)	510–595	Carbonisation
(Becht, 1972)	350–550	First extraction, roasting, acid activation
(Staal et al., 1989)	650–850	Synthetic clay, pyrolysis followed by oxidation
(Kuin and Nock, 1992)	700	Synthetic clay, first pyrolysis then oxidation
(Maes and Dijkstra, 1993)	350–700	Fluidised bed, no pretreatment
(Hähn et al., 1995)	500–650	Solvent extraction, roasting, acid treatment
(Ebert et al., 1997)	600–700	Fluidised bed, no pretreatment
(Santos, 1999)	400–485	Solvent extraction, acid activation, thermal polishing
(Seng et al., 2001)	400–600	Best temperature range for surface area

These pretreatments are avoided by using fluidised sand beds and blowing the spent earth through them. In addition, a heat exchanger positioned in the fluidised bed (Maes and Dijkstra, 1993) or above it (Ebert et al., 1997) not only controls the fluidised bed temperature so that the clay particles are not overheated, but it also generates high pressure steam. The process can handle mixtures of clay and activated carbon, the latter being burned off. However, any alkali present in the spent earth originating, for instance, from soaps or phosphatides, causes the earth to vitrify under the regeneration conditions in the fluidised bed, and vitrification leads to loss of surface and of bleaching activity. Consequently, thermal regeneration leads to unpredictable results, which is the main reason why the process has not been adopted.

Another reason is that in Europe, spent bleaching earth is legally considered to be a waste product, and its regeneration by passing it through a fluidised sand bed is regarded as an incineration process. Burning waste may lead to dioxins in the flue gas and dioxins are considered to be very toxic indeed.[24] Accordingly, the authorities

[23] In the table, several first names have been mistaken for surnames, so "Gerhard, S." should read "Stalmann, G.", "Hermann, M." should read "Meister, H.", and "Werner, B." should read "Becht, W."

[24] The fact that Mr. Yushchenko, President of the Ukraine, is still alive after having been exposed to high dioxin levels apparently has no effect on the official view of the toxicity of dioxins, which is based on exposing rats to dioxins. These rodents do not normally include roast meat in their diet and, thus, haven't evolved a tolerance (and taste) for barbecues, as mankind has.

insist that flue gases originating from waste incineration must be subjected to a high temperature treatment before being released into the air. This means that the particle-laden gas stream leaving the fluidised bed would first have to be cooled down to allow it to be filtered to remove the regenerated bleaching earth particles, after which it should be mixed with fuel to permit reheating to the prescribed temperature. Apart from being pointless, this particular operation is also prohibitively expensive.

In addition to the thermal oxidation listed above, wet chemical oxidation has also been attempted. Wet oxidation by molecular oxygen in the presence of water at a temperature range of 125 to 350°C led to 100% regeneration even after the fourth cycle (Kalam and Joshi, 1988a). Acid activation can also be combined with chemical oxidation of the extraction residue by using peracetic acid (Nebergall et al., 1994). However, "all of the currently known procedures are so expensive that fresh bleaching earth is more cost effective" (Zschau, 2000).

3.7.5.3 Disposal of spent adsorbents

Various authors have listed means of disposal of spent bleaching earth as such (Fahn, 1984; Zschau, 1994; Chung and Eidman, 1997; Zschau, 2000; Münch, 2005). These means are compiled below:

- *Animal feed.* Before the BSE scare in the U.K. and the dioxin-crisis in Belgium, spent adsorbents, except those containing nickel (type (e)), were added to the meal sold to the animal feed industry. Nowadays, only type (a) streams can be included in meal, provided the spent adsorbent stays on the same production site and adsorbed oil and meal have the same agricultural origin. For the vertically integrated oil refiner, adding spent adsorbent to his meal has the advantage that he sells his waste stream at meal value. The feed producer buys energy at meal value and chickens thrive on it (Herstad, 1979). Whenever possible, this means of disposal is to be encouraged, but limiting it to vertically integrated operations discriminates against the stand-alone refineries.
- *Landfill.* This is a common way of disposing of spent earth. It has the advantage that it does not require any investment, but instead of providing an income, it incurs an expense.
- *Soil improvement.* In certain circumstances, spent bleaching earth can be spread over agricultural land and it improves the soil quality (Pagès, 1994).
- *Incineration.* Hence, incineration (Goemans, 2004; 2005) is to be preferred. This can be of the spent adsorbent on its own or together with other waste streams having a calorific value (Svensson, 1976). The resulting ash will have to be disposed of, but its mass is reduced by incineration and this process also prevents the subsequent leaching of nickel. Consequently, the ash can go to any type of landfill.
- *Cement kilns, bricks and tiles.* All types including nickel containing type (e) can be used as raw material in cement kilns or brickworks, where the energy content of the spent earth is fully utilised and the inorganics are incorporated into the product. However, cement companies are not keen to work with a self-igniting raw material of variable colour and, being aware of their unique service, charge for its disposal; brick kilns tend to get smelly when firing spent earth. So although this means of disposal is attractive environmentally, it is less so for the refiner.
- *Expanded clay granules.* By using a 1:1 mixture of spent earth and clay and heating it suddenly to 1300°C, expanded clay granules are obtained for use in the construction industry and gardens (Fahn, 1984).
- *Biogas production.* Adding some spent bleaching earth to an anaerobic fermentation of, for instance, pig manure, assists the fermentation in that microbes attach themselves to the clay particles. When they produce methane, the particles are so small that they do not prevent the bubble from rising to the surface, but after the methane has been released, the particle makes the microbe sink again. The particles, thus, act as an agitator, provide a substrate, and thereby facilitate the fermentation.
- *Substrate for edible fungi.* Adding spent bleaching earth to the growth medium for edible fungi increases their yield (Mathiesen, 1996).
- *Riboflavin production.* A higher concentration of riboflavin was observed when spent bleaching earth was added to a culture of *Ashbya gossypii* (Kato and Ming, 2004).

References

Achife, E.C. and Ibemesi, J.A. (1989), Applicability of the Freundlich and Langmuir adsorption isotherms in the bleaching of rubber and melon seed oils, *J. Am. Oil Chem. Soc.,* **66**, 247–252.

Aehnelt, W. (1957), Aktivkohle, in *Ullmanns Encyclopädie der technische Chemie,* Foerst, W., Ed., Urban & Schwarzenberg, München-Berlin, **9**, 800–812.

Andersen, A.J.C. (1962), *Refining of Oils and Fats for Edible Purposes,* Pergamon Press Ltd, Oxford, London, New York, Paris.

Anon. (1947), Blanchîment des corps gras par le chlorite de soude et le bioxide de chlore, *Bull. mens. ITERG,* **2**, 3–6.

Anon. (1961), Procédé pour extraire une terre décolorante contenant de l'huile au moyen de filtre-presses de construction close, avec blanchiment simultané et filtration très fine de la miscella, French Patent 1.276.877, assigned to Öl- und Margarineindustrie Magdeburg.

Arbeitskreis "Technologien der industriellen Gewinnung und Verarbeitung von Speisefetten" (1993), Gemeinschaftsarbeiten der DGF, 135 Mitteilung, Die Bleichung von Speisefetten und -ölen II, *Fat Sci. Technol.,* **95**, 321–325.

Arbeitskreis "Technologien der industriellen Gewinnung und Verarbeitung von Speisefetten" (1996), Gemeinschaftsarbeiten der DGF. Die Bleichung von Speisefetten und -ölen IV, *Fett/Lipid,* **98**, 94–103.

Armstrong, M.R. and Ireland, C.E. (1958), The bleaching of soybean oil. A spectrophotometric evaluation, *J. Am. Oil Chem. Soc.,* **35**, 425–428.

Aschenbrenner, M. (1953), Verfahren zum Bleichen von Wachsen und Fetten, German Patent 894 592, assigned to BASF AG.

Bailey, A.E. (1952), Method for bleaching oil with adsorbent, US Patent 2,618,644, assigned to The Girdler Corporation.

Bailey, A.E. (1953), Improvements in or relating to method of bleaching petroleum or fatty oils, British Patent 700,324, assigned to The Girdler Corporation.

Baldwin, A.R. (1949), Decolorization of edible fats, *J. Am. Oil Chem. Soc.,* **26**, 610–614.

Barsky, G. (1955), Refining and bleaching fatty acid esters, US Patent 2,705,722, assigned to E.F. Drew & Co., Inc.

Bates, R.W. and Endres, J.G. (1967), Color improvement of oils and fats obtained through solvent extraction, US Patent 3,338,932, assigned to Armour and Company.

Bayrak, Y. (2003), Adsorption isotherms in bleaching hazelnut oil, *J. Am. Oil Chem. Soc.,* **80**, 1143–1146.

Becht, W. (1972), Regeneration of bleaching earth, British Patent 1 278 379, assigned to Metallgesellschaft AG.

Blank, E.W. (1942), Chronological list of important dates in the history of the fats and waxes, *Oil & Soap,* **19**, 110–113.

Bloemen, F. (1960), Raffinage des huiles par distillation neutralisante et désodorisation combinées, *Rev. Franç. Corps Gras,* **7**, 255–262.

Boki, K. et al. (1992a), Adsorption isotherms of pigments from alkali-refined vegetable oils with clay minerals, *J. Am. Oil Chem. Soc.,* **69**, 372–378.

Boki, K. et al. (1992b), Bleaching of alkali-refined vegetable oils with clay minerals, *J. Am. Oil Chem. Soc.,* **69**, 232–236.

Boki, K. et al. (1994), Bleaching rapeseed and soybean oils with synthetic adsorbents and attapulgites, *J. Am. Oil Chem. Soc.,* **71**, 595–601.

Bouquet, F. and Faur, L. (1960), Perfectionnement au blanchiment des matières grasses, French Patent 1.225.307, assigned to Unilever.

Brát, J. and Zajíc, J. (1992), Zur Bleichung pflanzlicher Öle II: Kinetik des Bleichprozesses, *Fat Sci. Technol.,* **94**, 312–317.

Brimberg, U.I. (1981), Untersuchungen über die Kinetik des Bleichens mit Bleicherden, *Fette Seifen Anstrichm.,* **83**, 184–190.

Brimberg, U.I. (1982), Kinetics of bleaching of vegetable oils, *J. Am. Oil Chem. Soc.,* **59**, 74–78.

Brinckman Sr., M.J. et al. (1959), Improvements in or relating to the bleaching of palm oil, British Patent 807,600, assigned to Harburger Oelwerke Brinckman & Mergell.

Brooks, D.D. et al. (1992), Oil bleaching method and composition for same, US Patent 5,151,211, assigned to Oil-Dri Corporation of America.

Brown, H.G. and Snyder, H.E. (1985), Adsorption of soy oil phospholipids on silica, *J. Am. Oil Chem. Soc.,* **62**, 753–756.

Brown, H.G. and Snyder, H.E. (1989), Factors influencing phospholipid adsorption on silica from soybean oil miscella, *J. Am. Oil Chem. Soc.,* **66**, 353–355.

Brücke, O. (1951), Verfahren zur Gewinnung von Ölen oder Fetten aus pflanzlichen oder tierischen Stoffen durch Extraktion, German Patent 837 910, assigned to Metallgesellschaft AG.

Chaloner, S.D. and McNicoll, D. (1955), Process for recovering fats and oils, US Patent 2,706,201.

Chapman, D.M. (1994), Benefits and limitations of a novel chlorophyll adsorbent, *J. Am. Oil Chem. Soc.,* **71**, 397–400.

Chapman, D.M. and Pfannkoch, E.A. (1992), Thermodynamic limitations for pigment adsorption during the bleaching of triglyceride oils, *J. Am. Oil Chem. Soc.,* **69**, 1009–1014.

Christenson, R.M. and Harpt, R.E. (1951), Bleaching oils in the presence of steam, US Patent 2,569,124, assigned to Pittsburgh Plate Glass Company.

Chung, C. and Eidman, V. (1997), Economic use of spent bleaching earth, *inform,* **8**, 739–742.

Dalen, J.P. van and Brunia, L. (1991), Process for refining glyceride oil using silica hydrogel, US Patent 5,069,829, assigned to Unilever.

De Greyt, W.F.J. and Kellens, M.J. (2000), Refining practice, in *Edible Oil Processing,* Hamm, W. and Hamilton, R.J., Eds., Sheffield Academic Press, Sheffield, 79–128.

Dijkstra, A.J. (1993), Degumming, refining, washing and drying fats and oils, in *Proceedings of the World Conference on Oilseed Technology and Utilization*, Applewhite, T.H., Ed., AOCS, Champaign, IL, 138–151.

Dijkstra, A.J. (2002), Bleaching revisited, *Technology Award Address presented at the 2nd EuroFedLipid Congress, Strasbourg.*

Drap, C. (1961), *Contribution à l'étude de l'influence de quelques facteurs sur la décoloration des corps gras par les agents d'adsorption,* Ph.D. thesis, Institut des Corps Gras, Laboratoire Nationale des Matières Grasses, France.

Drap, C. et al. (1961), Sur la décoloration des huiles par les agents physiques d'adsorption IV Modification du spectre UV des huiles au cours de la décoloration, *Rev. Franç. Corps Gras,* **8**, 215–221.

Drap, C. and Naudet, M. (1960), Sur la décoloration des huiles par les agents physiques d'adsorption. III Études des isothermes de décoloration, *Rev. Franç. Corps Gras,* **7**, 577–584.

Eaves, P.H. (1967), Bleaching refined cotton oil, US Patent 3,356,701, assigned to USA, Secretary of Agriculture.

Ebert, H. et al. (1997), Verfahren zum Regenerieren von gebrauchten anorganischen Adsorbentien sowie Verwendung der Regenerate, WO Patent 97/12669, assigned to Süd-Chemie AG.

Eicke, H. (1985), Der gegenwärtige Stand der Bleichung im Raffinationsprozess pflanziger Öle und tierischer Fette, *Fette Seifen Anstrichm.,* **87**, 355–358.

Elston, A.A. (1957), Method of bleaching vegetable oils, US Patent 2,795,595, assigned to E.I. du Pont de Nemours and Company.

Erickson, D.R. (1995), Bleaching/adsorption treatment, in *Practical Handboook of Soybean Processing and Utilization,* Erickson, D.R., Ed., AOCS Press and United Soybean Board, Champaign, IL and St. Louis MO, 203–217.

Estes, J. et al. (1995a), Is it necessary to bleach oils to zero peroxide? *Paper presented at the 86th AOCS Annual Meeting & Expo, San Antonio, TX*.

Estes, J. et al. (1995b), Process for the removal of soap from glyceride oils and/or wax esters using an amorphous adsorbent, US Patent 5,449,797, assigned to W.R. Grace & Co.

Fabre, E. (1968), Procédé d'épuration des huiles alimentaires, French Patent 1.535.070.

Fahn, R. (1984), Möglichkeiten der Verwertung gebrauchter Bleicherderückstände, *Fette Seifen Anstrichm.,* **86**, 505–508.

Fahn, R. and Fenderl, K. (1974), Emploi des terres décolorantes activées pour le blanchiment des huiles d'origine tropicale, *Oléagineux*, **29**, 193–197.

Feuge, R.O. and Janssen, H.J. (1951), Bleaching of cottonseed oil in hexane, *J. Am. Oil Chem. Soc.,* **28**, 429432.

Fontana, P. and Colagrande, O. (1962), Decolorazione degli olii vegetali — Nota II, *Riv. Ital. Sostanze Grasse,* **39**, 180–187.

Fontana, P. and Colagrande, O. (1964), Decolorazione degli olii vegetali Nota III. Studio delle isoterme di Langmuir, *Riv. Ital. Sostanze Grasse,* **41**, 246–250.

Fontana, P. and Colagrande, O. (1965), Decolorazione degli olii vegetali, *Riv. Ital. Sostanze Grasse,* **42**, 165–174.

Gingras, L. et al. (1998), Soap, phospholipid and trace metal removal from oil using RiceSil®100 adsorbent, *Paper presented at the 89th AOCS Annual Meeting & Expo, Chicago.*

Goemans, M. (2004), Thermal treatment of spent bleaching earth, filter aids, destillates, gums and soapstock: the HelioSolids® fluidised bed reactor and the Cinclus® thermal oxidizer, *Paper presented at the DGF Symposium on Refining: Handling by-products and minor compounds, Leipzig*.

Goemans, M. (2005), On-site thermal treatment of by-products from the edible oils and fats industry: a renewable energy source, *Paper presented at the 26th ISF World Congress and Exhibition, Prague,* page 8 in the abstracts.

Guillaumin, R. and Pertuisot, J.F. (1967), Influence du mode d'emploi des terres adsorbantes sur certaines caractéristiques des huiles végétales (III), *Rev. Franç. Corps Gras,* **14**, 449–460.

Guillaumin, R. and Pertuisot, J.F. (1968), Nature des triènes formés lors de la décoloration par les terres activées I, *Rev. Franç. Corps Gras,* **15**, 27–33.

Gutfinger, T. and Letan, A. (1978), Pretreatment of soybean oil for physical refining: evaluation of efficiency of various adsorbents in removing phospholipids and pigments, *J. Am. Oil Chem. Soc.,* **55**, 856–859.

Güler, Ç. and Tunç, F. (1992), Chlorophyll adsorption on acid-activated clay, *J. Am. Oil Chem. Soc.,* **69**, 948–950.

Hagan, A.S. (1940), Method of and apparatus for clay treating, US Patent 2,214,671, assigned to Mead Cornell.

Hähn, R. et al. (1995), Verfahren zur Regenerierung von gebrauchten ölhaltigen Bleicherden, WO Patent 95/07137, assigned to Süd-Chemie AG.

Harris, R.D. and Levine, L. (1972), Process for absorbent bleaching of edible oils, US Patent 3,673,228, assigned to The Procter & Gamble Company.

Hassler, J.W. and Hagberg, R.A. (1939), Contact counter current bleaching of vegetable oils, *Oil & Soap,* **16**, 188–191.

Henderson, J.H. (1993), A laboratory study of the press effect in adsorptive bleaching, *J. Am. Oil Chem. Soc.,* **70**, 831–835.

Henderson, J.H. (1997), Maximizing chlorophyll removal with activated bleaching earth, *Paper presented at the 88th AOCS Annual Meeting & Expo, Seattle.*

Henderson, J.H. et al. (1991), Effects of bleaching on peroxide value and anisidine value of soybean oil, *Paper presented at the first international meeting on fats and oils technology, symposium and exhibition, State University of Campinas, Sao Paulo, Brazil*.

Henderson, L.F. and Libby, L.H. (1949a), Bleaching oils and fats, US Patent 2,468,753, assigned to Unilever.

Henderson, L.F. and Libby, L.H. (1949b), Process of bleaching fats and oils, US Patent 2,483,413, assigned to Unilever.

Herstad, O. (1979), Waste fat in fat-containing bleaching earth as feed for chickens, *Acta Agriculturæ Scandinavica,* **26**, 87–93.

Hinners, H.F. et al. (1946), The evaluation of bleaching earths. The adsorptive capacity of some bleaching earths of various pH for chlorophyll in soybean oil, *Oil & Soap,* **23**, 22–25.

Hodgson, A.S. and Red, J.F.P. (1978), Recovery of domestic oil from spent clay, US Patent 4,110,356, assigned to SCM Corporation.

Homberg, E. (1974), Veränderungen der Sterine durch industrielle Verarbeitungsprozesse von Fetten und Ölen I: Einfluss der Raffinationsbedingungen auf der Steringehalt und Sterinzusammensetzung, *Fette Seifen Anstrichm.,* **76**, 433–435.

Homberg, E. (1975), Veränderungen der Sterine durch industrielle Verarbeitungsprozesse von Fetten und Ölen II: Veränderungsprodukte bei der Behandlung von Cholesterin mit Bleicherden, *Fette Seifen Anstrichm.,* **77**, 8–11.

Jacini, G. (1955), Bleichung von Ölen mit Natriumchlorit, *Fette Seifen Anstrichm.,* **57**, 903–905.

Jaillet-Rouyer, B. (1953), Récupération des huiles à partir des terres décolorantes, *Bull. mens. ITERG,* 67–74.

Jalalpoor, M. (2004), Silica adsorption: oil quality, *Paper presented at a EuroFedLipid Practical Short Course on Edible Oil Refining and Processing, Edinburgh, Scotland.*

James, E.M. (1958), The refining and bleaching of vegetable oils, *J. Am. Oil Chem. Soc.,* **35**, 76–83.

Jancik, V. (1958), Variations de l'activité adsorbante des terres décolorantes, *Oléagineux,* **13**, 307–314.

Jaynes, V.H. and Osburn, J.O. (1949), Recovery of fats from press wastes, *J. Am. Oil Chem. Soc.,* **26**, 693–696.

Jespersen, V. (1950), Process for bleaching compounds of fatty acids or the like, US Patent 2,524,056.

Jones, K. et al. (1982), Process for bleaching naturally occurring oils and fats, US Patent 4,325,883, assigned to Unilever.

Kalam, A. and Joshi, J.B. (1988a), Regeneration of spent earth by wet oxidation, *J. Am. Oil Chem. Soc.,* **65**, 1536–1540.

Kalam, A. and Joshi, J.B. (1988b), Regeneration of spent earth in aqueous medium, *J. Am. Oil Chem. Soc.,* **65**, 1917–1921.

Kato, A. and Ming, H. (2004), Utilization of waste activated bleaching earth containing palm oil in riboflavin production by *Ashbya gossypii, J. Am. Oil Chem. Soc.,* **81**, 57–62.

Kaufmann, H.P. and Hamza, Y. (1970), Über die Veränderung der Sterine in Fetten und Ölen bei der industriellen Bearbeitung derselben II, *Fette Seifen Anstrichm.,* **72**, 432–433.

Kaufmann, H.P. and Mukherjee, K.D. (1967a), Neuzeitliche Technologie der Fette und Fettprodukte CXV: Die Raffination der Fette, *Fette Seifen Anstrichm.,* **69**, 128–136.

Kaufmann, H.P. and Mukherjee, K.D. (1967b), Neuzeitliche Technologie der Fette und Fettprodukte CXVI: Die Raffination der Fette, *Fette Seifen Anstrichm.,* **69**, 463–472.

Kaufmann, H.P. et al. (1956), Der Nachweiss der Raffination von Schweineschmalz III: Das UV-Adsorptionsspektrum, *Fette Seifen Anstrichm.,* **58**, 1046–1037.

Kaufmann, H.P. et al. (1970), Über die Veränderung der Sterine in Fetten und Ölen bei der industriellen Bearbeitung derselben I, *Fette Seifen Anstrichm.,* **72**, 242–246.

Kellens, M.J. and De Greyt, W.F.J. (2000), Deodorization, in *Introduction to Fats and Oils Technology*, O'Brien, R.D., Farr, W.E., and Wan, P.J., Eds., AOCS Press, Champaign, IL, 235–268.

Khoo, L.E. et al. (1979), The adsorption of β-carotene I. By bleaching earths, *J. Am. Oil Chem. Soc.,* **56**, 672–675.

King, R.R. et al. (1947), Oil bleaching process and apparatus, US Patent 2.428,082, assigned to Mrs. Tucker's Foods, Incorporated.

King, R.R. and Wharton, F.W. (1949a), Continuous vacuum bleaching of vegetable oils, *J. Am. Oil Chem. Soc.,* **26**, 389–392.

King, R.R. and Wharton, F.W. (1949b), Oxidation effects in adsorption bleaching of vegetable oils, *J. Am. Oil Chem. Soc.,* **26**, 201–207.

Klein, J.-M. (1986), Methods for recovering oil from spent bleaching earth, in *World Conference on Emerging Technologies in the Fats and Oils Industry,* Baldwin, A.R., Ed., American Oil Chemists' Society, Champaign, IL, 169–171.

Kopp, H.-P.J. et al. (1989), Process for preparing refined oil, US Patent 4,857,237, assigned to Unilever.

Kopp, H.-P.J. et al. (1991), Process for preparing refined oil, European Patent 0 259 981, assigned to Unilever.

Kögler, H.P. (1963), Herstellung und Untersuchung von Bleicherden und deren Anwendung in der öl- und fettverarbeitenden Industrie, *Fette Seifen Anstrichm.,* **65**, 834–838.

Kögler, H.P. (1968), Einfluss der Bleicherde auf die Fettsäurebildung während der Bleichung, *Fette Seifen Anstrichm.,* **70**, 394–399.

Kuck, J.C. et al. (1962), Reactivation of alumina used in bleaching of off-colored cottonseed oils, *J. Am. Oil Chem. Soc.,* **39**, 84–86.

Kuin, A.J. and Nock, A. (1992), Application of regenerable adsorbent in edible oil refining, *Proceedings of Precision Process Technology Meeting, Delft Technical University, 27–29 October 1992, The Netherlands.*

Lachamp, M. and Naudet, M. (1963), Sur la décoloration des huiles par les agents physiques d'adsorption VI Influence des phosphatides, *Rev. Franç. Corps Gras*, **10**, 341–346.

Lachle, F.B. (1943), Oil extraction, US Patent 2,325,327, assigned to The Schwarz Engineering Company, Inc.

Langmaack, T. and Eggers, R. (2002), On the bleaching kinetics of vegetable oils — experimental study and mass transfer-based interpretation, *Eur. J. Lipid Sci. Technol.,* **104**, 98–109.

Lanzón, A. et al. (1989), Detección de la presencia de aceite de oliva refinado en el aceite de oliva virgen, *Grasas Aceites,* **40**, 385–388.

Leaders, W.M. and Argue, W. (1950), Treatment of spent bleaching agents, US Patent 2,509,509, assigned to Swift & Company.

Leake, J.R. (1997a), Soap removal from interesterified oils using Sorbsil® silica adsorbents, *Poster presented at the 88th AOCS Annual Meeting & Expo, Seattle.*

Leake, J.R. (1997b), Sorbsil silicas®. The technology, service and their benefits, *Paper presented at the 88th AOCS Annual Meeting & Expo, Seattle.*

Lee, C.G. et al. (2000), Solvent efficiency for oil extraction from spent bleaching clay, *J. Am. Oil Chem. Soc.,* **77**, 1219–1222.

Leue, H. (1939), Verfahren zum Entölen von Bleicherden, German Patent 670 830, assigned to Henkel & Cie. G.m.b.H.

Liebing, H.A. et al. (1975), Über die kontinuierliche Behandlung mit aktivierter Bleicherde bei der Raffination von Aromaten, *Fette Seifen Anstrichm.,* **77**, 338–340.

Liew, K.Y. et al. (1993), Adsorption of carotene from palm oil by acid-treated rice hull ash, *J. Am. Oil Chem. Soc.,* **70**, 539–541.

Maes, P.J. and Dijkstra, A.J. (1993), Process for regenerating spent bleaching earth, European Patent 0 529 555, assigned to N.V. Vandemoortele International.

Mag, T.K. (1990), Bleaching — Theory and practice, in *Edible Fats and Oils Processing: Basic Principles and Modern Practices,* Erickson, D.R., Ed., American Oil Chemists' Society, Champaign, IL, 107–116.

Mag, T.K. and Reid, M.P. (1980), Continuous process for contacting of triglyceride oils with an acid, US Patent 4,240,972, assigned to Canada Packers Limited.

Marmor, R.A. and Moyer, W.W. (1952), Process for decolorizing a soybean oil miscella, US Patent 2,608,566, assigned to A.E. Staley Manufacturing Company.

Mathiesen, N.L. (1996), A method for cultivation of edible fungi, European Patent Application 0 691 072 A1.

McMichael, C.E. et al. (1955), Oil bleaching method, US Patent 2,717,256, assigned to National Cylinder Gas Company.

Meiners, J. (1971), Abtrennung und Extraktion von Bleicherde in geschlossenen Filtern, *Fette Seifen Anstrichm.,* **73**, 370–375.

Meister, H. (1945), Verfahren zur Regenerierung von gebrauchter Bleicherde, Swiss Patent 237380.

Morgan, D.A. et al. (1985), The function of bleaching earths in the processing of palm, palm kernel and coconut oils, *J. Am. Oil Chem. Soc.,* **62**, 292–299.

Morton, R.B. (1995), Oil content of filter cakes, *Paper presented at the 86th AOCS Annual Meeting & Expo, San Antonio, TX.*

Müller, H. (1964), Einsatz der FUNDA-Filter in der Fett-Industrie, *Fette Seifen Anstrichm.,* **66**, 438–446.

Münch, E.-W. (2005), Disposal of used adsorbents — bleaching earth, silica and activated carbon, *Paper presented at the 26th ISF World Congress and Exhibition, Prague.*

Naudet, M. (1965), Alcune riflessioni sulla decolorazione delle sostanza grasse mediante agenti fisici di adsorbimento, *Riv. Ital. Sostanze Grasse,* **42**, 283–288.

Naudet, M. and Drap, C. (1960a), Sur la décoloration des huiles par les agents physiques d'adsorption. I Considérations théoriques du problème, *Rev. Franç. Corps Gras,* **7**, 127–134.

Naudet, M. and Drap, C. (1960b), Über den Einfluss einiger Faktoren auf die Entfärbung von Erdnussöl, *Fette Seifen Anstrichm.,* **62**, 487–489.

Naudet, M. et al. (1961), Sur la décoloration des huiles par les agents physiques d'adsorption V Influence des acides gras libres et des savons, *Rev. Franç. Corps Gras,* **8**, 450–455.

Naudet, M. and Sambuc, E. (1968), Measurement of oil colour, in *Analysis and Characterization of Oils, Fats and Fat Products,* Volume 2, Boekenoogen, H.A., Ed., Interscience Publishers, John Wiley & Sons, New York, 45–98.

Nebergall, R.S. et al. (1994), Process for regenerating spent acid-activated bentonite clays and smectite catalysts, US Patent 5,358,915, assigned to American Colloid Company.

Newby, W. (1947), Soap content of some commercially refined oils; Effect of soap on the bleachability of the oils, *J. Am. Oil Chem. Soc.,* **24**, 375–378.

Ney, K.H. (1964), Einfluss der Bleicherde-Behandlung bei verschiedenen Temperaturen auf die Lage der Doppelbindungen von Fettsäuren, *Fette Seifen Anstrichm.,* **66**, 512–517.

Nock, A. (1994), The removal of nickel from hydrogenated oils using silica hydrogel, *Paper presented at the 85th AOCS Annual Meeting & Expo, Atlanta*.

Nock, A. (1996), Silica adsorbents for edible oil processing and environmental benefits, in *Environmental Issues Facing the Edible Oil Industry,* Allen, D.A. and Kochhar, S.P., Eds., P.J. Barnes & Associates, Bridgwater, U.K., 57–70.

Noder, A. (1941), Method for bleaching oils, fats, and waxes, US Patent 2,250,203, assigned to Buffalo Electro-Chemical Company Inc.

Odeen, H. and Slosson, H.D. (1935), Theory and practice of bleaching fatty oils, *Oil & Soap,* **12**, 211–215.

Okpala, K.O. and Nwoke, M.N. (1989), The palm oil bleaching effect of various Nigerian clay deposits, *Int. J. Food Sci. Technol.,* **24**, 647–651.

Oliveira, C.G. de and Porto, L.M. (2005), A kinetic model for bleaching vegetable oils, *J. Am. Oil Chem. Soc.,* **82**, 537–542.

Ong, J.T.L. and Sinkeldam, E.J. (1983), Recycling and utilization of spent bleaching earth, *Fette Seifen Anstrichm.,* **85**, 304–306.

Orth Jr., G.O. (1981), Method of recovering residual vegetable oil contained in spent bleaching clay and processing the bleaching clay for reuse, US Patent 4,285,832.

Ostrejko, R. (1902), Verfahren zur Gewinnung und Wiederbelebung von Kohle mit großer Entfärbungskraft, German Patent 136,792.

Ottesen, I. (1981), Adsorption effects for some types of bleaching earths, *Paper presented at the 11th Scandinavian Symposium on Lipids, Bergen, Norway,* 185–196.

Ouchi, H. and Saito, N. (1981), Treatment of an oil-containing clay, US Patent 4,283,346, assigned to The Nisshin Oil Mills Ltd.

Pagès, X. (1994), Le traitement des terres décolorantes usagées, *Oléagineux Corps Gras Lipides/OCL,* **1**, 177–178.

Palaniappan, S. and Proctor, A. (1991), Evaluation of soy oil lutein isotherms obtained with selected adsorbents in hexane miscella, *J. Am. Oil Chem. Soc.,* **68**, 79–82.

Pardun, H. (1988), *Die Pflanzenlecithine,* Verlag für chemische Industrie H. Ziolkowsky KG, Augsburg.

Parker, C.A. and Bent, F.A. (1931), Process for revivifying used decolorizing materials, US Patent 1,806,020, assigned to Standard Oil Company of California.

Parker, P.M. (1994), Innovations in refining technology: Silica adsorbents for edible oil, *Oils & Fats Int.,* **10**, 24–27.

Patterson, H.B.W. (1976), Bleaching practices in Europe, *J. Am. Oil Chem. Soc.,* **53**, 339–341.

Patterson, H.B.W. (1993), *Bleaching and Purifying Fats and Oils,* AOCS Press, Champaign. IL.

Penninger, J.M.L. (1979), Extraction of oily matter from spent bleaching earth with water at elevated temperature and pressure, *J. Chem. Tech. Biotechnol.,* **29**, 154–157.

Pons Jr., W.A. et al. (1961), Bleaching of off-colored cottonseed oils, *J. Am. Oil Chem. Soc.,* **38**, 104–107.

Pons Jr., W.A. et al. (1962), Process for bleaching off-color cottonseed oils, US Patent 3,036,102, assigned to USA, Secretary of Agriculture.

Pons Jr., W.A. et al. (1963), Process for bleaching refined cottonseed oil, US Patent 3,087,946, assigned to USA, Secretary of Agriculture.

Proctor, A. et al. (1996), Lipid adsorption on commercial silica hydrogels from hexane and changes in triglyceride complexes with time, *J. Am. Oil Chem. Soc.,* **73**, 693–698.

Proctor, A. and Harris, C.D. (1996), Soy hull carbon as an adsorbent of minor crude soy oil components, *J. Am. Oil Chem. Soc.,* **73**, 527–529.

Proctor, A. and Snyder, H.E. (1988), Adsorption of lutein from soybean oil on silicic acid II. Kinetics, *J. Am. Oil Chem. Soc.,* **65**, 761–763.

Proctor, A. et al. (1992), Phospholipid adsorption onto rice hull ash from soy oil miscellas, *J. Am. Oil Chem. Soc.,* **69**, 1049–1050.

Proctor, A. and Toro-Vazquez, J.F. (1996), The Freundlich isotherm in studying adsorption in oil processing, *J. Am. Oil Chem. Soc.,* **73**, 1627–1633.

Pryor, J.N. et al. (1988), Process for the removal of chlorophyll color bodies from glyceride oils using acid-treated silica adsorbents, US Patent 4,781,864, assigned to W.R. Grace & Co.

Rich, A.D. (1960), Relationship between the apparent bulk density of a bleaching clay and its oil retention, *J. Am. Oil Chem. Soc.,* **37**, 305–307.

Rich, A.D. (1964), Some basic factors in the bleaching of fatty oils, *J. Am. Oil Chem. Soc.,* **41**, 315–321.

Rich, A.D. (1967), Major factors that influence bleaching performance, *J. Am. Oil Chem. Soc.,* **44**, 298A–324A.

Richter, B. (1978), Das Plattenpressfilter, *Fette Seifen Anstrichm.,* **89**, 143–146.

Röbbeling, K.H. (1970), Filtration von Speiseölen und Fetten und deren Automatisierung, *Fette Seifen Anstrichm.,* **72**, 1037–1040.

Rothbart, M.A. (1997), Method for recovering edible oil from adsorbent materials, US Patent 5,670,678, assigned to Hunt-Wesson, Inc.

Sanders, J.H. (1951), Continuous bleaching process, US Patent 2,555,098, assigned to The Procter & Gamble Company.

Sanelli, B. (1980), Le variazioni degli indici spettrofotometrici nell'UV e la rimozione del colore nell'olio d'oliva lampante. Equazione di Freundlich per l'adsorbimento. Nota III: La rimozione dei pigmenti colorati, *Riv. Ital. Sostanze Grasse,* **57**, 295–300.

Santos, B. (1999), Process for regenerating spent clay, US Patent 5,942,457.

Sarier, N. and Güler, Ç. (1988), β-Carotene adsorption on acid-activated montmorillonite, *J. Am. Oil Chem. Soc.,* **65**, 776–779.

Sarier, N. and Güler, Ç. (1989), The mechanism of β-carotene adsorption on activated montmorillonite, *J. Am. Oil Chem. Soc.,* **66**, 917–923.

Scheller, E. (1940), Process for bleaching oils, fats and waxes, US Patent 2,221,559, assigned to Deutsche Gold und Silber Scheideanstalt.

Seng, C.E. et al. (2001), Adsorption of chromium (VI) and nickel (II) ions on acid- and heat-activated deoiled spent clay, *J. Am. Oil Chem. Soc.,* **78**, 831–835.

Simpson, T.P. et al. (1944), Treatment of solvent refined oils with adsorbents, US Patent 2,346,127, assigned to Socony-Vacuum Oil Company Inc.

Sims, R.J. et al. (1959), Method of processing soybean oil to retard flavor reversion, US Patent 2,872,465, assigned to Swift & Company.

Singleton, W.A. (1956), Filtration and bleaching, *J. Am. Oil Chem. Soc.,* **33**, 477–484.

Singleton, W.A. and McMichael, C.E. (1955), Continuous countercurrent vacuum bleaching, *J. Am. Oil Chem. Soc.,* **32**, 1–6.

Slocum, E.M. (1938), Process of recovery of oil from spent bleaching clay, US Patent 2,117,223.

Smith, G.W. et al. (1977), Process for cleansing spent clay, US Patent 4,049,685, assigned to Sylvachem Corporation.

Smith, G.W. et al. (1980), Process for cleansing spent clay from the refining of glyceride esters, US Patent 4,185,026, assigned to Sylvachem Corporation.

Staal, L.H. et al. (1989), Metal-oxide-silica adsorbent for bleaching and refining oil, US Patent 4,812,436, assigned to Unilever.

Stalmann, G. (1938), Improvements in and relating to the regeneration of bleaching clays and earths, British Patent 491,338, assigned to Noblee & Thörl G.m.b.H.

Stillman, R.C. (1955), Bleach and color methods, *J. Am. Oil Chem. Soc.,* **32**, 587–593.

Stout, L.E. et al. (1949), Factors influencing vegetable oil bleaching by adsorption, *J. Am. Oil Chem. Soc.,* **26**, 120–126.

Strauss, H.-J. et al. (1975), Procédé de traitement d'huiles comestibles en vue d'améliorer leur coloration et leurs propriétés d'entreposage, French Patent 2.241.613, assigned to Unilever.

Strauss, H.-J. et al. (1976), Glyceride oil treatment with oxide and bleaching earth, US Patent 3,955,004, assigned to Unilever.

Strauss, H.-J. et al. (1977), Chemical process, British Patent 1 476 307, assigned to Unilever.

Study Group "Technologies for Industrial Processing of Fats and Oils" (2001), Bleaching of edible oils and fats, *Eur. J. Lipid Sci. Technol.,* **103**, 505–550.

Suzuki, K. and Nishioka, A. (1993), Behavior of chlorophyll derivatives in canola oil processing, *J. Am. Oil Chem. Soc.,* **70**, 837–841.

Svensson, C. (1976), Use or disposal of by-products and spent material from the vegetable oil processing industry in Europe, *J. Am. Oil Chem. Soc.,* **53**, 443–445.

Taylor, D.R. (1993), Adsorptive purification, in *Proceedings of the World Conference on Oilseed Technology and Utilization,* Applewhite, T.H., Ed., AOCS Press, Champaign, IL, 152–165.

Taylor, D.R. and Jenkins, D.B. (1988), Acid-activated clays, *Trans. Soc. Mining Eng. AIME,* **282**, 1901–1910.

Taylor, D.R. and Jenkins, D.B. (1990), Factors affecting the pyrophorisity of spent bleaching clay, *J. Am. Oil Chem. Soc.,* **67**, 768–685.

Thomopoulos, C. and Liapis, C. (1962), Méthode de régénération des terres décolorantes, *Rev. Franç. Corps Gras,* **9**, 611–615.

Tollenaar, F.D. and Hoekman, H. (1964), Die Bleichung von Ölen mittels Bleicherden, mit besonderer Berücksichtigung von Palmöl, *Fette Seifen Anstrichm.,* **66**, 430–437.

Topallar, H. (1998), Bleaching kinetics of sunflowerseed oil, *J. Am. Oil Chem. Soc.,* **75**, 531–533.

Transfeld, P. (1986), Modellierung der Entfärbungsisothermen der Bleichung vegetabiler Öle mittels Bleicherde, *Lebensmittelindustrie,* **33**, 125–129.

Transfeld, P. (1994), Method of adsorptive purification of vegetable and/or mineral oils and fats, US Patent 5,342,508, assigned to Öhmi Forschung und Ingenieurtechnik GmbH.

Transfeld, P. (1998a), Countercurrent bleaching with electric filtration technique, *Oléagineux Corps Gras Lipides/OCL,* **5**, 378–380.

Transfeld, P. (1998b), Electrofiltration cuts bleaching earth use, *inform,* **9**, 502–506.

Transfeld, P. and Börner, G. (1997), Verfahren und Vorrichtung zur adsorptiven Reinigung von pflanzlichen und/oder mineralischen Ölen und Fetten, EP Patent 0 808 890, assigned to Öhmi Forschung und Ingenieurtechnik GmbH.

Transfeld, P. et al. (2003), Method for purifying contaminated liquids, US Patent 6,656,364, assigned to Öhmi Forschung und Ingenieurtechnik GmbH.

Transfeld, P. and Schneider, M. (1994), A two-stage countercurrent bleaching process for edible oils and fats, in *Proceedings of the World Conference on Lauric Oils: Sources, Processing, and Applications, Manila,* Applewhite, T.H., Ed., AOCS Press, Champaign, IL, 168–169.

Transfeld, P. and Schneider, M. (1996), Countercurrent system cuts bleaching costs, *inform,* **7**, 756767.

Transfeld, P. et al. (1998), Method and apparatus for adsorptive cleaning of substances, US Patent 5,752,103, assigned to Öhmi Forschung und Ingenieurtechnik GmbH.

Trizis, C. and Uzzan, A. (1958), Application de la technique trichromatique à l'étude de la décoloration par les terres décolorantes des graisses alimentaires. Influence du traitement sur certains caractères des produits décolorés, *Rev. Franç. Corps Gras,* **5**, 499–514.

Venkatarao, P. and Achaya, K.T. (1969), A hypothesis for carotene decolourization by a free radical mechanism in fatty esters and hydrocarbons, *Fette Seifen Anstrichm.,* **71**, 270–272.

Vosmaer, A. (1929), Eenige toepassingen van ozon, *Chem. Weekblad,* 184–186.

Waldmann, C. and Eggers, R. (1991), De-oiling contaminated bleaching clay by high-pressure extraction, *J. Am. Oil Chem. Soc.,* **68**, 922–930.

Weber, K. (1980), Oil recovery from bleaching earth by a three phase extraction and separation column, *Paper presented at the ISF/AOCS World Congress, New York*.

Welsh, W.A. and Bogdanor, J.M. (1986), Phosphorus and trace metal removal with a novel refining material; Trisyl, *Paper presented at the 77th AOCS Annual Meeting & Expo, Honolulu*.

Welsh, W.A. et al. (1990), Silica refining of oils and fats, in *Edible Fats and Oils Processing: Basic Principles and Modern Practices*, Erickson, D.R., Ed., AOCS, Champaign, IL, 189–202.

Welsh, W.A. and Parent, Y.O. (1986b), Method for refining glyceride oils using amorphous silica, European Patent 0 185 182, assigned to W.R. Grace & Co.

Wiedermann, L.H. (1981), Degumming, refining and bleaching of soybean oil, *J. Am. Oil Chem. Soc.,* **58**, 159–166.

Wilke, H. (1963), Probleme der Filtration in der Industrie der Öle und Fette, *Fette Seifen Anstrichm.,* **65**, 470–475.

Wittka, F. (1939), Das Bleichen der Fette und das Entölen der Bleicherden in Zusammenhange mit den bei der Raffination der rohe Öle erreichbaren maximalen Ausbeuten an Speisefetten, *Fette u. Seifen,* **46**, 344–345.

Yatsu, L.Y. et al. (1970), Use of ferric chloride to decolorize cottonseed oil, *J. Am. Oil Chem. Soc.,* **47**, 73–74.

Yatsu, L.Y. et al. (1972), Decolorization of cottonseed oil with ferric chloride, US Patent 3,702,857, assigned to USA, Secretary of Agriculture.

Zschau, W. (1979), Quality control during bleaching, in *Proceedings Quality control seminar in soybean crushing plants and soybean oil processing plants,* American Soybean Association.

Zschau, W. (1987), 80 years activated bleaching earth, *Fette Seifen Anstrichm.,* **89**, 184–189.

Zschau, W. (1994), Spent bleaching earth — practical solutions, *inform,* **5**, 1375–1377.

Zschau, W. (2000), Bleaching, in *Introduction to Fats and Oils Technology*, O'Brien, R.D., Farr, W.E., and Wan, P.J., Eds., AOCS Press, Champaign, IL, 158–178.

3.8 Dewaxing of oils

In the edible oils industry, the terminology concerning physical separation processes leading to two or more fractions is ambiguous. Fractional crystallisation processes leading to two triglyceride fractions in the form of a liquid filtrate (also called olein) and a filter cake (stearin) are called "fractionation" in Europe and Asia. When such fractional crystallisation processes are applied to cottonseed oil or brush-hydrogenated soya bean oil in the U.S., they are referred to as "winterization." However, in Europe, the term "winterisation" is only used for the removal of waxes from liquid oils, such as sunflower seed oil or corn germ oil, and the term "dewaxing" is generally used for the removal of wax from rice bran oil.

Finally, other fractionation processes, such as fractional distillation or fractionation by selective adsorption may also be called fractionation processes. To avoid confusion, the term "fractionation" will be used for the separation of triglycerides into fractions and the term "dewaxing" for wax removal processes. The use of the ambiguous term "winterisation" will be avoided (see also Young, 1976).

The purpose of dewaxing is to ensure that when liquid oils are stored in a refrigerator, they remain clear and brilliant and do not throw a deposit. This means that for oil to remain clear for 8 days at a temperature of 0°C, the wax content has to be reduced to 8 ppm (Turkulov et al., 1986). For salad oils, this is purely cosmetic, but for oils used in mayonnaise, salad dressings, and sauces, dewaxing is a technical necessity in that it prevents these emulsions from breaking (O'Brien, 2000). The various processes and their background are discussed below.

3.8.1 Theoretical considerations

Vegetable oil waxes as they occur in linseed oil, sunflower seed oil, corn germ oil, grape seed oil, safflower oil, and rice bran oil are true waxes, i.e., esters between fatty acids and fatty alcohols. Since these fatty acids and fatty alcohols are predominantly saturated, the melting point of these waxes is high — around 70°C. Although the waxes are completely miscible with hot oil, they tend to precipitate in cold oil (refrigerator temperature). Their miscibility at high temperatures means that oil-milling processes operating at such temperatures also extract the oil-soluble waxes. If sunflower seed oil is obtained by cold pressing, this is reflected in the wax composition of the oil, which will contain less high melting waxes (carbon number 44 to 48) than oil obtained by hot pressing or hexane extraction (Carelli et al., 2002).

The amount of wax present in the raw material processed in the oil mill can vary widely. It depends upon the type of raw material (e.g., sunflower seed vs. corn germs), it depends upon the cultivar (which can have a large effect in the case of sunflower seeds), upon seed storage conditions (Martini and Añón, 2005) and it also depends upon the extent to which the oilseeds have been dehulled before being crushed since waxes occur mainly on the outside of the seed hull where they can protect the seed most effectively; accordingly, washing the seeds with boiling hexane prior to crushing removes most of the waxes (Morrison, 1982).

Since the aim of dewaxing is to provide oil that stays clear at refrigerator temperature, it is not surprising that all dewaxing processes used industrially are based upon the low solubility of the high molecular weight waxes in oil at low temperatures, and, thus, involves cooling the oil to crystallise these waxes and remove them as crystals. Low molecular weight waxes with carbon numbers below 44 have low melting points and will remain in solution when the oil is dewaxed (Carelli et al., 2002).

This also causes wax compositions as reported in the literature to vary more widely than expected; they depend very much on what substrate has been analysed. The crystalline sunflower seed wax fraction isolated by filtration as reviewed by Gracian and Arévalo (1981) gives a very different result from the sunflower seed and olive oil wax isolated by solid phase extraction (Reiter and Lorbeer, 2001). The composition of rice bran waxes has been reported by Yoon and Rhee (1982).

In some dewaxing processes, crystal morphology plays a role and, therefore, merits discussion. When a suspension of small crystals is filtered to obtain a clear filtrate, the ensuing filter cake will have only narrow passageways. These slow down the rate of filtration, which is low anyway because the low temperature has increased the viscosity of the oil. Adding filter aid to the suspension can increase this low filtration rate, but this entails extra expense and the additional oil retained by the more bulky filter cake constitutes a further expense; accordingly, small crystals are preferably avoided.

3.8.2 Dewaxing processes

Wax contents of various oils dewaxed industrially in Europe are listed in Table 3.13 (De Greyt and Kellens, 2000). At 2.0 to 3.5% (Kinsey and Hunnell, 1969), the wax content of rice bran oil is even higher than that of the oils listed in Table 3.13. As pointed out by Denise (1987), the filtration process has the advantage of producing oil with good cold stability and has the disadvantage that its variable costs (filter aid and oil lost in the filter cake) are higher than for other dewaxing processes. However, these other processes leave more wax in the oil than the filtration process, so that the oil has insufficient cold stability.

Accordingly Denise (1987) recommends dewaxing by filtration if the wax content of the nondewaxed oil is below 600 ppm. If its wax content is above this value, two-stage dewaxing is recommended, with the filtration being the final stage and ensuring good cold stability. One of the first stage treatments he mentions comprises cooling the oil to be dewaxed and bubbling nitrogen through the suspension of wax crystals in oil (Zwobada et al., 1980). This creates froth on the oil surface that is heavily enriched in wax crystals. This enrichment (and the foaming) can also be promoted by compounds, such as soap. Unwashed, neutral oil is an appropriate substrate. Residual wax in oil treated according to this process is too high to attain acceptable cold stability and, thus, necessitates subsequent removal by filtration.

TABLE 3.13 Wax content of a number of vegetable oils

Type of Oil	Wax Content [ppm]
Sunflower seedoil (dehulled seeds)	600–800
Sunflower seed oil (nondehulled seeds)	600–3500
Corn germ oil	150–500
Canola	100–200
Olive pomace extraction oil	600–1400
Grapeseed oil[a]	1000–2500

Source: De Greyt, W.F.J. and Kellens, M.J. (2000), in *Edible Oil Processing,* Hamm, W. and Hamilton, R.J., (Eds), Sheffield Academic Press, Sheffield, 79–128.

[a] A value of 8170 ppm is mentioned by Ringers and Segers in their Example XXXVIII (1977)

3.8.2.1 Cooling and crystallisation

The literature is far from unanimous on how to cool and crystallise during the dewaxing process:

- "Dewaxing is rather simple and can be accomplished by rapidly chilling the oil in the presence of filter aid, holding at this temperature for 1 to 2 h, and cold filtration" (O'Brien, 2000).
- "The oil is cooled slowly and kept at a low temperature (5 to 10°C) for a long time (minimum 6 h) to allow the waxes to crystallize" (De Greyt and Kellens, 2000).
- "The feed oil may be passed through a water cooler and mechanical refrigerator to lower the temperature of the oil as desired …" (Kinsey and Hunnell, 1969).
- "The refined and washed oil is then chilled using a standard chilling apparatus to less than 60°F (16°C) and held at these chilled temperatures for longer than 1 hour" (Levine, 1977).
- "The process starts with the bleached oil, which should be at a temperature of 30 to 35°C … . Towards the end of the chilling cycle, which lasts from 48 to 60 h, the rate of crystallisation is sufficiently rapid to cause a slight rise in temperature of 1 to 2°C, which then drops. At this point cooling is stopped, and the oil/crystal mixture is allowed to stand for 12 h at 6°C. It is then ready for filtration" (Neumunz, 1978).
- "At the end of the neutralisation step, the oil is cooled in a plate heat exchanger to 8 to 10°C and sent to maturation tanks … . The chilled oil stays in the first two maturation tanks for a period of 5 hours at a temperature below 10°C" (Gonçalves et al., 1984).
- "After bleaching, the oil is pre-cooled from 60°C to 25 to 30°C in a heat exchanger with the cold oil after filtration. The next cooler cools the oil from 25 to 30°C to 10 to 15°C with brine. The last cooling takes place in the crystallizer where there is an automatic regulation system to ensure the temperature gradient needed" (Leibovitz and Ruckenstein, 1984). After a residence time of 4 hours in the crystalliser, the oil is pumped to maturation vessels, where it stays another 5 to 6 hours before being filtered.
- "Slow cooling according to the invention comprises a first cooling step, in which the oil is quickly cooled to the solubility temperature of the wax present in the oil (about 45°C) and a second cooling step comprising a cooling rate of 6°C per hour … . The oil end temperature was 8°C. The oil/crystallised wax slurry obtained was immediately microfiltered without any additional maturation time" (Asbeck and Segers, 1990).

When waxes crystallise from the triglyceride oil solvent, they first have to form nuclei, which then have to grow into macroscopic crystals (Aquilano and Sgualdino,

2001). Supersaturation encourages nucleation more than crystal growth, so in order to obtain large crystals, crystal growth should be encouraged rather than nucleation. For wax crystals, it is not known to what extent heterogeneous nucleation plays an important role, but according to Rivarola et al. (1985), cooling conditions and wax content in oil determine the morphologies adopted by the crystals. At high cooling rates and low temperatures of the refrigerant, nucleation temperatures are low and this induces the formation of a great number of small crystals.

This means avoiding excessive supersaturation, at least in the early stages of the crystallisation process. There must, of course, be some supersaturation, otherwise no nuclei would be formed, but it should be controlled and maintained at a low degree. In practice, this means slow cooling (e.g., 5°C per hour) from just above the cloud point of the oil to be dewaxed to, say, 20°C below this cloud point (J. De Kock, personal communication). How rapidly the oil is cooled before reaching the cloud point is immaterial and does not affect the size of the wax crystals (Asbeck and Segers, 1990). If this rate of cooling is applied, reasonably large crystals are formed, there is no need for crystal maturation, and the suspension can be filtered as soon as the final temperature has been reached (Dijkstra, 2002). No change in crystal habit is observed, even with residence times of 24 hours (Rivarola et al., 1985), so the Ostwald ripening claimed by various authors who advocate maturation does not occur.

The use of heat exchangers to cool the oil below its cloud point must be avoided, not only because they cool the oil too rapidly and lead to small crystals, but also because they tend to get encrusted with wax deposits (Kehse, 1979; 1980b). Accordingly, most of the processes listed above are to be avoided with the exception of the cooling profile described by Asbeck and Segers (1990). Various authors mention the early addition of filter aid or other adjuvants to ensure the formation of easily filterable crystals. This is not necessary at all.

3.8.2.2 Separation by centrifuge

Wax separation by centrifuge is not limited to the actual dewaxing process, but can also be a side effect of low temperature degumming processes. In the Superdegumming process, for instance (Ringers and Segers, 1977), "the addition of water at a temperature below 40°C presumably converts the phosphatides into a semicrystalline phase that also contains … the waxes." Residual wax contents of 38 to 175 ppm are mentioned in the examples of the patent (Ringers and Segers, 1977), but in these examples, the temperature was not raised just before the centrifugal separation step. For industrial applications, heating to a temperature of 60 to 90°C is recommended to reduce oil viscosity and increase the capacity of the centrifugal separator to normal values.

As demonstrated in Rohdenburg et al., Example 3 (1992), heating the oil can cause the wax to melt and dissolve back into the oil, since the wax contents of the starting oil and the degummed oil (centrifugal separation at 80°C by flash heating) are identical (700 ppm). This is the reason why the S.O.F.T.® degumming process (Jamil et al., 1995; Deffense, 1996) when combined with a dewaxing operation, prescribes heating to only 15°C before the centrifugal separation of gums and waxes (Gibon and Tirtiaux, 1998; 2000) to obtain dewaxed oils with good cold stability, albeit at reduced separator capacity. Another combined degumming/dewaxing process for rice bran oil (Rajam et al., 2005) also comprises a separation at low (20°C) temperature, but even so, still requires an additional dewaxing step.

Predewaxing can also be a separate step in preparing sunflower seed oil for physical refining (Kővári et al., 2000) by using the soap formed *in situ* with the addition of some caustic, as a detergent, in the Lanza fractionation process (Anon., 1907). An update of this process geared to dewaxing employs sodium lauryl sulfate (Seugé and Vinconneau, 1975; Gibble and Rhee, 1976), another uses dilute lye that forms soaps leading to an emulsion that is broken by adding a small amount of phosphoric acid (Young, 1976), or omits the phosphoric acid (Levine, 1977). "An aqueous refining agent capable of producing foots" has also been claimed (Parkin and Walker, 1951).

According to Sullivan (1980) "cold refining, i.e., reacting sodium hydroxide or similar agents with the oil at 5 to 10°C and separating at these temperatures, will produce a well-refined and thoroughly dewaxed oil. The disadvantages can be a reduced capacity through the centrifugal separator and possibly higher refining losses resulting from emulsification at high viscosity."

Tempering the oil at a temperature of between 75°F (24°C) and 120°F (49°C) for 24 hours and holding the tempered oil for 5 hours at a temperature of 40°F (4°C) to 70°F (21°C) and then refining the oil by the addition of caustic at a slightly higher temperature and separating the oil phase from the aqueous phase containing the soaps and the waxes has also been claimed (Beharry, 1981). Similarly, the oil can also be cooled to a lower temperature of between 5 and 15°C and heated to a temperature between 15 and 20°C before being separated by centrifuge (Pallmar and Sarebjörk, 1981). When optimising this kind of process, Baltanás et al. (1991) recommend some 5% water on oil, a maturation temperature of 7.5°C and a soap content of at least 2000 ppm.

Accordingly, the waxes are isolated from the oil as part of a phosphatide mixture or a soapy water mixture, both of which mixtures have to be disposed of. Disposal by addition to oilseed meal may be an option provided the meal drier has sufficient capacity. If a soapstock acidulation unit is available, this can also cope with the wax containing streams. If not, burning together with, for example, bleaching earth may be a satisfactory way to

dispose of the waste stream (Goemans, 2004; 2005). Isolating reasonably pure waxes from such dilute waste streams is probably not economically feasible.

3.8.2.3 Separation by filtration

To obtain good cold stability through a low residual wax content, wax removal by filtration is essential (Denise, 1987). In 1979, Kehse compared various filter types, including a new type of filter that uses a disposable filter band made of a polyester monofilament fleece; it has not been used industrially nor have membrane filters as yet (Mutoh et al., 1985; Asbeck and Segers, 1990).

The most common filter type is still the pressure leaf filter as used for the removal of bleaching earth (Arbeitskreis "Technologien der industriellen Gewinnung und Verarbeitung von Speisefetten", 1996) that is given a precoat of filter aid (Röbbeling, 1970). This type of filter ousted the more labour-intensive plate and frame filter press (Richter, 1978). Various authors (Kehse, 1979; 1980a; 1980b; Denise, 1987) specify types and amount of filter aid to be used to ensure a predictable filter life and smooth industrial operation; this is most important indeed. To operate smoothly, the filtration must be carried out at an assured average rate. When the filter is fresh and the filter cake offers little resistance, the centrifugal pump feeding the filter will maintain a rate that is above this average rate, but as and when the filter cake resistance increases, the rate will gradually drop below this average.

That is acceptable if the filter is more or less full and has to be changed anyway, but if cake resistance has built up rapidly so that flow has decreased to unacceptably low levels well before the filter is full, the parallel filter may not yet be ready, labour to empty and prepare the filter may not be available, time is spent in preparing the filter for the next run and plant capacity may drop and cause running costs to escalate. Filters should only have to be changed when full and the pressure drop over a full filter should not be excessive.

Since a plant operator has to work with the type of filter aid that has been chosen by the the plant's owner, his only means to try and ensure trouble-free operation of the dewaxing process is to vary the amount of filter aid. To be on the safe side, he will tend to use too much, and waste money in doing so. When challenged, he can easily defend this waste by pointing out that using less filter aid might necessitate premature filter changes, etc. To assist the operator, systematic process investigation and development should define the optimal cooling profile leading to the largest wax crystals and a simple test on the raw material should tell him how much filter aid to use.

Washing the filter with hot solvent can regenerate the filter aid and, thus, reduce the damage if too much filter aid is used (Miller, 1983). It is also possible to dewax oil miscella (Morrison and Robertson, 1975) and refine at the same time (Morrison and Thomas, 1976), but these laboratory findings were never applied on an industrial scale.

The filter cake resulting from the dewaxing filtration still contains 50 to 60% oil after the cake has been blown with steam (Denise, 1987), plus the filter aid and some wax. It can constitute a valuable feed ingredient. Having a well-defined and reasonably constant composition, it can also serve as the starting material for the isolation of the wax. This isolation is unexpectedly facilitated when sodium chloride (kitchen salt) is used as filter aid because this retains far less oil than commonly used filter aids, such as diatomaceous earth or sawdust. Of course, the filter cake can also be burned (Goemans, 2004; 2005).

References

Anon. (1907), Verfahren zur Zerlegung des aus Fetten und fetten Ölen gewonnenen Fettsäuregemisches in Ölsäure und feste Fettsäuren, German Patent 191238, assigned to Fratelli Lanza.

Aquilano, D. and Sgualdino, G. (2001), Fundamental aspects of equilibrium and crystallization kinetics, in *Crystallization Processes in Fats and Lipid Systems*, Garti, N. and Sato, K., Eds., Marcel Dekker, New York, 1–51.

Arbeitskreis "Technologien der industriellen Gewinnung und Verarbeitung von Speisefetten" (1996), Gemeinschaftsarbeiten der DGF. Die Bleichung von Speisefetten und -ölen IV, *Fett/Lipid,* **98**, 94–103.

Asbeck, L.S. and Segers, J.C. (1990), Dewaxing of dried oil, European Patent 0 397 233, assigned to Unilever.

Baltanás, M.A. et al. (1991), Descerado de aceite de girasol. Estudio de planta piloto, *Alimentos,* **1**, 9–14.

Beharry, C.R. (1981), Crude edible oil wax removal process, US Patent 4,272,447, assigned to The Procter & Gamble Company.

Carelli, A.A. et al. (2002), Wax composition of sunflower seed oils, *J. Am. Oil Chem. Soc.*, **79**, 763–768.

De Greyt, W.F.J. and Kellens, M.J. (2000), Refining practice, in *Edible Oil Processing*, Hamm, W. and Hamilton, R.J., Eds., Sheffield Academic Press, Sheffield, 79–128.

Deffense, E.M.J. (1996), Soft Degumming, in *Oils-Fats-Lipids 1995*, Castenmiller, W.A.M., Ed., P.J. Barnes & Associates, Bridgewater, U.K., 125–128.

Denise, J. (1987), Le décirage des huiles de tournesol, *Rev. Franç. Corps Gras*, **34**, 133–138.

Dijkstra, A.J. (2002), Hydrogenation and fractionation, in *Fats in Food Technology*, Rajah, K.K., Ed., Sheffield Academic Press, Sheffield, U.K., 123–158.

Gibble, W.P. and Rhee, J.S. (1976), Dewaxing of vegetable oils, US Patent 3,994,943, assigned to Hunt-Wesson Foods Inc.

Gibon, V. and Tirtiaux, A. (1998), Un raffinage S.O.F.T.®, *Oléagineux Corps Gras Lipides/OCL*, **5**, 371–377.

Gibon, V. and Tirtiaux, A. (2000), Removal of gums and waxes — a review, *inform*, **11**, 524–535.

Goemans, M. (2004), Thermal treatment of spent bleaching earth, filter aids, destillates, gums and soapstock: the HelioSolids® fluidised bed reactor and the Cinclus® thermal oxidizer, *Paper presented at the DGF Symposium on Refining: Handling by-products and minor compounds, Leipzig*.

Goemans, M. (2005), On-site thermal treatment of by-products from the edible oils and fats industry: a renewable energy source, *Paper presented at the 26th ISF World Congress and Exhibition, Prague,* page 8 in the abstracts.

Gonçalves, A.C. et al. (1984), Procédé pour l'élimination continue, par centrifugation, des cires des huiles comestibles, French Patent 2 537 155, assigned to Sociedade Nacional de Saboes LDA.

Gracian, J. and Arévalo, G. (1981), Composición de las ceras del aceite de girasol, *Grasas Aceites*, **32**, 1–5.

Jamil, S. et al. (1995), Procédé de dégommage d'un corps gras et corps gras ainsi obtenu, WO Patent 95/00609, assigned to Fractionnement Tirtiaux S.A.

Kehse, W. (1979), Winterisierung von Sonnenblumenöl, *Fette Seifen Anstrichm.*, **81**, 463–466.

Kehse, W. (1980a), Winterisierung von Sonnenblumenöl — Möglichkeiten der Filtration, *Seifen Öle Fette Wachse*, **106**, 69.

Kehse, W. (1980b), Wintérisation de l'huile de tournesol, *Rev. Franç. Corps Gras*, **27**, 567–570.

Kinsey, D.N. and Hunnell, J.W. (1969), Method of dewaxing rice oil, US Patent 3,481,960, assigned to Riviana Foods Inc.

Kővári, K. et al. (2000), Physical refining of sunflower oil, *Oléagineux Corps Gras Lipides/OCL*, **7**, 305–308.

Leibovitz, Z. and Ruckenstein, C. (1984), Winterization of sunflower oil, *J. Am. Oil Chem. Soc.*, **61**, 870–872.

Levine, L. (1977), Dewaxing process for vegetable oils, US Patent 4,035,402, assigned to The Procter & Gamble Company.

Martini, S. and Añón, M.C. (2005), Storage of sunflower-seeds: variation on the wax content of the oil, *Eur. J. Lipid Sci. Technol.*, **107**, 74–79.

Miller, P.A. (1983), Ein Verfahren für die Regenerierung von Filterhilfsmittel und die Rückgewinnung von hochwertige Fetten aus Filtrationsrückständen bei der Winterisierung von Speiseölen und -Fetten, German Patent 32 15 116 A1.

Morrison III, W.H. (1982), A rapid, effective method for the reduction of waxes in sunflower seed oil, *J. Am. Oil Chem. Soc.*, **59**, 519–520.

Morrison III, W.H. and Robertson, J.A. (1975), Solvent winterization of sunflower seed oil, *J. Am. Oil Chem. Soc.*, **52**, 148–150.

Morrison III, W.H. and Thomas, J.K. (1976), Removal of waxes from sunflower seed oil by miscella refining and winterization, *J. Am. Oil Chem. Soc.*, **53**, 485–486.

Mutoh, Y. et al. (1985), Method of dewaxing vegetable oil, US Patent 4,545,940, assigned to Asahi Kasei Kabushiki Kaisha.

Neumunz, G.M. (1978), Old and new in winterizing, *J. Am. Oil Chem. Soc.*, **55**, 396A–398A.

O'Brien, R.D. (2000), Liquid oils technology, in *Introduction to Fats and Oils Technology*, O'Brien, R.D., Farr, W.E., and Wan, P.J., Eds., AOCS Press, Champaign, IL, 463–485.

Pallmar, A.B. and Sarebjörk, K.W.H. (1981), Procédé de traitement des huiles végétales en vue de leur conservation à basse température, French Patent 2 460 996, assigned to Alfa-Laval AB.

Parkin, F.P. and Walker, G.N. (1951), Purified oils and processes of preparing same, US Patent 2,564,407, assigned to Minnesota Paints Inc.

Rajam, L. et al. (2005), A novel process for physically refining rice bran oil through simultaneous degumming and dewaxing, *J. Am. Oil Chem. Soc.*, **82**, 213–220.

Reiter, B. and Lorbeer, E. (2001), Analysis of wax ester fraction of olive oil and sunflower oil by gas chromatography and gas chromatography-mass spectrometry, *J. Am. Oil Chem. Soc.*, **78**, 881–888.

Richter, B. (1978), Das Plattenpressfilter, *Fette Seifen Anstrichm.*, **89**, 143–146.

Ringers, H.J. and Segers, J.C. (1977), Degumming process for triglyceride oils, US Patent 4,049,686, assigned to Unilever.

Rivarola, G. et al. (1985), Crystallization of waxes during sunflowerseed oil refining, *J. Am. Oil Chem. Soc.*, **62**, 1508–1513.

Röbbeling, K.H. (1970), Filtration von Speiseölen und Fetten und deren Automatisierung, *Fette Seifen Anstrichm.*, **72**, 1037–1040.

Rohdenburg, H. et al. (1992), Entschleimungsverfahren, European Patent 0 473 985, assigned to Krupp Maschinentechnik GmbH.

Seugé, J.P. and Vinconneau, H.F. (1975), Le procédé Lipofrac Alfa-Laval pour le fractionnement et la frigélisation en continu des graisses comestibles, *Oléagineux*, **30**, 25–30.

Sullivan, F.E. (1980), Sunflower oil processing from crude to salad oil, *J. Am. Oil Chem. Soc.*, **57**, 845A–847A.

Turkulov, J. et al. (1986), The effect of temperature and wax content on the appearance of turbidity in sunflowerseed oil, *J. Am. Oil Chem. Soc.*, **63**, 1360–1363.

Yoon, S.H. and Rhee, J.S. (1982), Composition of waxes from crude rice bran oil, *J. Am. Oil Chem. Soc.*, **59**, 561563.

Young, H.T. (1976), Simultaneous refining and dewaxing of crude vegetable oil, US Patent 3,943,155, assigned to The Procter & Gamble Company.

Zwobada, F. et al. (1980), Procédé de traitement des huiles végétales pour l'amélioration de leur stabilité à froid, French Patent 2 482 976, assigned to Lesieur-Cotelles & Associés.

3.9 Vacuum stripping of oils and fats

The above title encompasses the deodorisation process as well as the physical refining process, both of which lead to fully refined oil as illustrated in Figure 3.8. Various authors refer to these processes as steam distillation processes, but this is not correct[25]. In a steam distillation process, as used to purify a water-immiscible organic compound, there will be three separate phases (*P*): liquid water, the liquid organic compound, and the vapour phase. The number of components (*C*) is only two (water

[25] The existing confusion is well illustrated by the following quotation (Balchen et al., 1999): "A stripping process carried out as a batch process using steam is normally called a 'steam distillation,' while stripping carried out in a counter-current manner in multistage towers is called a 'desorption.' The special case of batch 'stripping' in which no stripping gas is used, is analogous to a one-stage 'flash distillation' or just 'flashing.' 'Stripping' with no use of stripping gas, etc."

and the organic compound), so according to the Phase Rule of Gibbs, the number of the degrees of freedom (F) equals:

$$F = C - P + 2 = 2 - 3 + 2 = 1 \qquad (3.17)$$

There being only one degree of freedom means that if the pressure of a steam distillation is chosen (e.g., atmospheric), its temperature can no longer be freely chosen. It is determined by the properties of the components involved.

In a steam stripping process, the situation is fundamentally different. There the only phases are the liquid being stripped and the vapour phase ($P = 2$). There are still two components ($C = 2$), but because of the fewer phases, the number of degrees of freedom (F) has increased to two. Both the stripping temperature and the stripping pressure can be freely chosen, without the choice of the one affecting or determining the other.

Early attempts to improve the odour of edible oils and fats involved boiling a mixture of oil and water. Subsequently, H. Eckstein of N.K. Fairbank Company of Chicago (the man who also introduced fuller's earth into edible oil processing), started to blow a current of live steam through the oil to be deodorised in 1891 (Blank, 1942). Then in 1891 (Lee and King, Jr., 1937) or 1892 (Mérat, 1954), Edouard Bataille (1924)[26] in France developed the vacuum deodorisation process that was subsequently and independently developed in 1900 by Wesson in the U.S. (Morris, 1949). These were batch processes and the various operations (heating, deaerating, steam stripping, and cooling) are performed one after the other in the same *batch deodoriser* vessel.

The next development was to use separate vessels (trays) that are superimposed inside the same shell (Bailey, 1949; 1954). The oil cascades down intermittently from these trays, each of which is provided with its own stripping medium sparging system and connected directly to the vacuum system. This kind of deodoriser is called a *semicontinuous cross-flow deodoriser*, and it can be used to deodorise consecutive batches with little cross-contamination. When the oil to be deodorised is allowed to flow downwards continuously from tray to tray (Kehse, 1961) or from one deodoriser cylinder to the next (Kehse, 1965; Tirtiaux and Gibon, 1997), the equipment is called a *continuous cross-flow deodoriser* since each tray or cylinder is still connected directly to the vacuum system.

As will be explained below, stripping medium requirements of the cross-flow system can be improved upon, and one way of doing so is by ensuring that the stripping medium leaving the deodoriser has been in contact with nondeodorised oil. This is realised in the *continuous counter-current deodoriser* (Sergueev, 1955; Stenberg and Sjöberg, 1996).

In yet another system, oil and water are mixed and sent as an aerosol to a separator to recuperate the deodorised oil so that the stripping medium leaving the deodoriser is more or less in equilibrium with deodorised oil; the equipment concerned is a *continuous co-current deodoriser* (Rowan, Jr., 1975). Finally, there are mixed systems comprised of a counter-current section and a cross-flow section (Raffaetá, 1961).

3.9.1 Physical aspects of vacuum stripping

Vacuum stripping processes are predominantly physical, but since they are usually carried out at elevated temperatures, some chemical reactions may be involved as well.

3.9.1.1 Stripping medium requirements

For the batch and the cross-flow deodorisation processes, the stripping steam requirement has been derived by Bailey (1941),[27] but subsequent authors (Szabó Sarkadi, 1956; 1958b; Bloemen, 1966; Martinenghi, 1971; Bloemen, 1975; Lau, 1978; 1995a; Deffense, 1995b; De Greyt, 1998) have pointed out that for the physical refining process, the simplifications introduced by Bailey are not justified. The full, nonsimplified equation should be used:

$$S = \frac{PO}{EP_v} \ln \frac{V_s}{V_e} + \frac{P}{EP_v}(V_s - V_e) - (V_s - V_e) \qquad (3.18)$$

In this equation,

S = amount of stripping steam required [mol]
P = total pressure in the system [pressure units]
O = amount of oil being processed [mol]
E = vaporisation efficiency [-]
P_v = vapour pressure of pure volatile compound [same pressure units]
V_s = amount of volatiles before deodorisation [mol]
V_e = amount of volatiles after deodorisation [mol]

In Equation 3.18, the first term on the right-hand side corresponds to the Bailey equation. It indicates that in a steam stripping process, the amount of steam required for a given degree of deodorisation is proportional to the amount of oil being deodorised. It is also proportional to the system pressure (P), but since the steam volume is inversely proportional to the system pressure, it follows that a fixed volume of steam is required for the removal of a set volatile fraction from a certain

[26] Like Wesson, Bataille also started by keeping his process a secret, but eventually, in 1914, Bataille was granted French Patent 474 474, whereas Wesson never sought patent protection.

[27] According to Möller (1964b) Equation 3.18 had already been derived by W. Adriani in 1920, but kept confidential as an internal document. Garber and Lerman on the other hand (1943) credit a publication by Carey in *Chemical Engineers Handbook,* New York, McGraw-Hill, 1941.

amount of oil in a deodoriser with a given vaporisation efficiency.

The Bailey equation also shows that a given volume of steam reduces the volatile content of the oil being deodorised by a certain fraction. Accordingly, the absolute amount of volatiles being removed per unit of steam volume decreases in the course of the process, as the amount present decreases. It also means that deodorisation will never remove all the volatiles.

The second term in Equation 3.18 is a relatively minor correction to the equation put forward by Bailey, who assumed the amount of volatiles (V) to be negligible in comparison with the amount of oil (O) and, therefore, simplified the equation by substituting O for ($O + V$). The third term on the right-hand side of Equation 3.18 can be quite significant since it originates from the partial pressure of the volatiles. During deodorisation, this partial pressure will be low in comparison to the system pressure, but when high acidity oils are physically refined, this partial pressure can equal the system pressure and the simplification made by setting the system pressure equal to the steam pressure is no longer warranted.

For the continuous counter-current steam stripping process, several authors (Garber and Lerman, 1943; Bates, 1949; Martinenghi, 1971; Gavin, 1978; Brekke, 1980; Stage, 1981; Stage, 1982; Møller and Balchen, 1994; Young, 1994; Balchen et al., 1999) provide some form of equation intended to be indicative of the stripping medium requirement, but it was only in 1999 that Dijkstra derived this requirement mathematically by using the approach suggested by Bloemen (1966) and in doing so arrived at the following equation:

$$V_e = V_s \frac{(PO)^n (P_v S - PO)}{(P_v S)^{n+1} - (PO)^{n+1}} \qquad (3.19)$$

The variables in the above equation have the same meaning as in Equation 3.18 with the additional variable n standing for the number of theoretical transfer units (Bloemen, 1966). Such a unit is defined as the deodorisation column segment that ensures that the oil leaving this segment at the bottom is in physical equilibrium with the vapour leaving this segment at the top. Its actual height will depend upon the construction of the deodorisation column, the residence time of the oil, etc.

Although Equation 3.19 pertaining to the continuous counter-current deodoriser looks quite different from the extended Bailey Equation 3.18 pertaining to the cross-flow deodoriser, it nevertheless has quite a lot in common. As in the Bailey equation, it is only the ratio V_e/V_s of the volatiles that is determined by the other variables and not their absolute magnitudes. Similarly, the steam requirement (S) is proportional to the system pressure (P) and inversely proportional to the vapour pressure (P_v) of the pure compound (for the detailed derivation, see Dijkstra, 1999).

The third type of deodoriser mentioned is the continuous co-current deodoriser. Several inventors/authors (Clayton, 1949; Ballestra, 1954; 1960; Graham and Webb Jr., 1963; Martinenghi, 1965; Rowan, Jr., 1975; Schumacher, 1976; Zehnder, 1976; 1976) have described co-current deodorisation processes and, according to one author (Hrushowy, 1978), such a process (supplied by the Parkson Corporation) has been used industrially for the physical refining of palm oil.

The stripping medium requirements of this type of deodoriser can be derived by assuming the vapour phase and the liquid phase to be in equilibrium after the oil/water mixture has been released *in vacuo*. By defining p_v as the partial pressure of the volatiles in the vapour phase, this variable can be expressed according to Raoult's law as:

$$p_v = P_v \frac{V_e}{O + V_e} \qquad (3.20)$$

This partial pressure p_v can also be written as a fraction of the total pressure (P) in the system, which is made up by the steam (S) and the volatiles that evaporated (V_s - V_e) according to:

$$p_v = P \frac{V_s - V_e}{S + (V_s - V_e)} \qquad (3.21)$$

Combining Equation 3.20 and Equation 3.21, eliminating p_v and solving for S gives:

$$S = (V_s - V_e) \frac{PO - (P_v - P)V_e}{P_v V_e} \approx (V_s - V_e) \frac{PO}{P_v V_e} \qquad (3.22)$$

The simplification is fully permissible since $O >> V_s$. Accordingly, an Equation 3.22 for S results that could have also been worked out by substituting $n = 1$ in Equation 3.19 which pertains to the continuous counter-current system. In other words, a co-current system performs as a counter-current system with a single transfer unit.

In Figure 3.18, stripping medium usage has been plotted for various deodoriser types in function of the residual volatile content after assuming a system pressure P of 4 mbar and a vapour pressure P_v of the pure volatile compound of 44 mbar. The figure shows that the co-current deodoriser requires the most stripping medium. Deodorisers of this type have been described in patents (Clayton, 1949; Graham and Webb Jr., 1963; Rowan, Jr., 1975) and have been welcomed as a worthwhile innovation (Schumacher, 1976; Zehnder, 1976), but given its high stripping medium usage, it is not surprising that subsequent alkali refining is necessary (Clayton, 1949) or that working in two consecutive co-current stages has been advocated in a patent subclaim (Graham and Webb Jr., 1963).

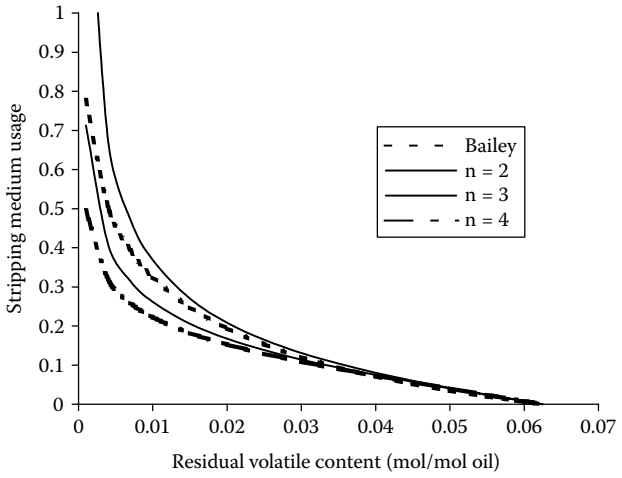


FIGURE 3.18 Stripping medium usage for different deodorizer types. (From Dijkstra, A.J. (1999), *J. Am. Oil Chem. Soc.*, **76**, 989–993. With permission.)

Co-current deodorisation (or "flash devolatilization") has also been used to treat a cocoa butter emulsion containing 2% water (Bergman and Nauman, 1986). Butyric acid was reduced to 14% of its initial value during the first pass, while it was reduced to 10% and 5% of its initial value after the second and third passes, respectively.

The stripping medium usage for the batch or cross-flow system has been indicated as "Bailey" in Figure 3.18. This is quite close to that of a counter-current deodoriser with only two transfer units. As the number of transfer units increases, the stripping medium requirement decreases, but there are some practical difficulties to be considered. A counter-current column will present resistance to the upward flow of the stripping medium. Consequently, there will be a pressure drop over the column that will be proportional to the length of the column or the number of transfer units. This means that the pressure underneath the column may become unduly high and ineffective for deodorisation. In industrial practice, a low pressure above deodorisation trays below the column can be realised by introducing a pump (steam ejector) in between these trays and the column (Kellens and de Suraÿ, 2005).

Since the equation for the counter-current deodoriser has been derived while assuming the same pressure throughout the system, deviations will arise as and when the number of transfer units increases. Nevertheless, Figure 3.18 clearly illustrates that operating counter-currently has clear advantages as far as stripping medium requirements are concerned.

3.9.1.2 Fatty acid vapour pressure

Most equations above comprise the variable P_v, which has been defined as the vapour pressure of the pure volatile. In practice, there will be a whole range of volatiles, each with its own vapour pressure. The various volatile compounds act independently of each other and a high volatility and a high molar fraction favour volatilisation during vacuum stripping. However, sfatty acids, the main type of compound being volatilised during the physical refining process, exhibit a more complex behaviour in that they can form dimers (Szabó Sarkadi, 1956; 1958b).

The concentration of these dimers is relatively large in pure fatty acids, but when they are diluted, more and more dimers dissociate. Consequently, the vapour pressure of pure fatty acids is strongly affected (depressed) by the presence of these dimers, whereas under deodorisation conditions, dimer formation has a smaller effect upon the fatty acid vapour pressure. Since *Pv,* the vapour pressure of the pure compound, is measured on a liquid containing dimers, it is lower than the value that should be filled in in Equation 3.18, Equation 3.19 and Equation 3.22 given above. The result is what has been called "a positive deviation from Raoult's law" (Holló and Lengyel, 1960).

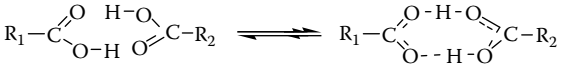


FIGURE 3.19 Fatty acid dimerisation.

In fact, the situation is even more complicated in that fatty acids also form a complex with triglycerides. This lowers their volatility in comparison with a solution of fatty acids in paraffin. According to Szabó Sarkadi (1958b), stearic acid monomer has a vapour pressure at 180°C of 3.9 mmHg, whereas the dimer has a vapour pressure of only 0.015 mmHg. The value reported in the literature for the vapour pressure of pure stearic acid at this temperature is 0.80 mmHg and for stearic acid at infinite dilution in peanut oil, a pressure of 1.2 mmHg can be calculated. Accordingly, about 30% of the acid is present as free acid and 70% as a triglyceride complex at this temperature. By studying the dimerisation equilibrium at different temperatures, Szabó Sarkadi was able to calculate the enthalpy difference involved as 13.4 kcal/mol (56.1 kJ/mol) and to note that it does not depend upon the length of the fatty acid chain.

3.9.1.3 Vaporisation efficiency

The "positive deviation from Raoult's law" mentioned above can manifest itself in a vaporisation efficiency $E > 1$, whereas by definition, this efficiency should be ≤ 1. It only reaches unity when a gas bubble injected below the oil surface, rising rapidly while expanding, and then bursting through the surface has a composition fully reflecting the fatty acid content of the oil. In fact, several mainly German authors (Siemes and Mechler, 1963; Liebing, 1970; Fedeli and Tiscornia, 1979; Stage, 1985; Arbeitskreis "Technologien der industriellen Gewinnung und Verarbeitung von Speisefetten," 1988) go so far as to suggest using a kind of average pressure (P_D) by

calculating the average "oleostatic" pressure and adding this to the gas pressure (P), according to:

$$P_D = P + \rho_{oil} g(h:2) \quad (3.23)$$

with g being the gravitation constant and h the height of the oil column. There is no need to do so. See also Kehse (1960).

After having constructed a laboratory deodoriser, Szabó Sarkadi (1958a) tested this piece of equipment by using a material (mercury) with known vapour pressure. At different steam rates, he found the product ($E\ P_v$) of the vaporisation efficiency and the vapour pressure of mercury to be equal to values published in the literature for the vapour pressure of mercury, so that he concluded that his laboratory deodoriser had a vaporisation efficiency equal to unity. Much later, doubt was expressed whether an equilibrium between the gas phase and the liquid phase could be reached when operating under reduced pressure (Coelho Pinheiro and Guedes de Carvalho, 1994). However, the same authors also concluded that the mass transfer efficiency was independent of the depth of the liquid phase.

For a laboratory deodoriser, Bailey (1949) first observed vaporisation efficiencies between 0.40 and 0.45, but after fitting appropriate baffles, he observed them to increase to 0.80 to 0.90. According to Pardun (1988), even higher values were observed, which made Bailey conclude that Raoult's law was not fully applicable. It is, but the fatty acid dimerisation equilibrium should be taken into account.

3.9.1.4 Steam or nitrogen?

According to Equations 3.18, Equation 3.19, and Equation 3.22 given above, the chemical identity of the stripping medium is immaterial. Any cheap, food-grade, inert gas that is easy to handle can, in principle, be used in the vacuum stripping process of edible oils. Moreover, all of these gases should be equally effective as stripping medium on a molar basis. However, some authors maintain that this is not the case. It has been claimed that aliphatic aldehydes having a boiling point below 200°C are especially effective in removing "the undesirable tastes and odors of fatty substances" (Buxton and Colman, 1941).

Cheng et al. (1992), for instance, not only provide an incomprehensible equation for "the amount of process steam theoretically necessary to maximize stripping," they also give examples that supposedly demonstrate the superiority of nitrogen over steam. In several examples, the amount of nitrogen (in moles) leading to a certain deodorisation result is indeed far less than the amount of steam used in deodorisation experiments leading to similar results.

Other authors (Graciani Constante et al., 1991; 1994; Ruiz Méndez et al., 1996) also argue that using nitrogen instead of steam leads to a higher efficiency. This superiority of nitrogen could perhaps be explained by assuming the steam to cause hydrolysis (Huesa Lope and Dobarganes García, 1991). According to Pagès (1990), nitrogen is used industrially as a stripping medium in conjunction with mechanical pumps.

Bates (1949) mentions that some hydrolysis occurs and that the extent of this hydrolysis is proportional to the system pressure. Subsequent authors (Mérat, 1954; Podbielnak, 1964; Kaufmann and Mukherjee, 1968a; Sjöberg, 1991; Maza et al., 1992; Zehnder, 1995) also mention triglyceride hydrolysis as being partially responsible for yield losses during deodorisation, but do not quantify this loss. On the other hand, Kehse (1963) measured the amount of FFA in his raw material and distillate and concluded that hydrolysis corresponded to 0.05% of the oil being deodorised.

On a laboratory scale, a very similar figure was determined (Deffense, 1995a), and Szabó Sarkadi (1959) concludes that the hydrolysis is catalysed by FFA; this conclusion is disputed by Jeberg (1979), who considers the hydrolysis to be proportional to the amount of steam passed through the oil. Matters could be further complicated by the possibility that FFA will react with partial glycerides, whereby the FFAs act as acid catalyst. This is an equilibrium reaction whereby the ester formation is favoured by a high concentration of free hydroxyl and carboxyl groups (high acidity oil) and a low water concentration (deodorisation conditions).

Accordingly, several authors specifically investigated possible differences between steam and nitrogen and concluded that they were nonsignificant for counter-current deodorisation (Balchen and Adler-Nissen, 1998) and batch deodorisation (Decap et al., 2004). The latter authors quote a paper quantifying neutral oil loss (Petrauskaitè et al., 2000) and mention hydrolysis, whereas the original authors only report neutral oil loss due to the volatility of medium chain partial glycerides.

3.9.2 Chemical aspects of vacuum stripping

The temperatures involved during the vacuum stripping process of edible oils tend to be quite high (<< 200°C). Since the period of time during which the oil is held at such temperatures may be several hours, there is ample opportunity for thermally induced reactions to occur (Arbeitskreis "Technologien der industriellen Gewinnung und Verarbeitung von Speisefetten," 1992; Billek, 1992). Some of these reactions are considered to be desirable with respect to oil quality, whereas others are preferably minimised. Since each of these reactions will have its own kinetics and the stripping effect also depends upon time (amount of steam) and temperature, a set of process conditions has to be sought that ensures proper deodorisation, i.e., a bland taste and cost-efficient operation of the equipment, as well as meeting one or more other quality criteria.

3.9.2.1 Breakdown of flavour precursors

Vacuum stripping of oils and fats aims at removing their volatile constituents, especially their FFA and malodorous compounds. Accordingly, the duration of the process could equal the time needed to pass the correct amount of steam through the oil that will ensure that little of these malodorous compounds is left and that the oil meets quality standards; this approach favours counter-current stripping. According to Mérat (1954), this is the approach also taken by Bailey, but Mérat also reports that certain refiners maintain that proper deodorisation requires a minimum of time to prevent subsequent flavour development, which is also referred to as "flavour reversion."

Looking at what happens to deep frying fats may assist in resolving this controversy. Deep-frying fat used for the first time doesn't smell; only repeated use leads to the development of the typical smell associated with deep fat frying. However, this typical smell is complex, in that it has an oil/fat constituent and also another constituent that is specific for the agricultural origin of the oil or fat. That is why some people prefer their chips (French fries) to be prepared in tallow olein, whereas others have a strong preference for palm oil or even coconut oil. Each oil or fat gives its own flavour to the chips, but not when the oil or fat has just been deodorised. It has to age somewhat at elevated temperature, as during deep frying, before this flavour develops.

It is therefore possible that edible oils contain one or more thermally instable compounds that are to some extent oil-specific. Their breakdown products then give the oil or fat its typical smell. Preventing this smell from developing requires the breaking down of these compounds prior to use, for instance, during the high-temperature deodorisation treatment, or the hydrogenation process. According to Andersen (1962), hydrogenation products are less prone to flavour reversion and this could be because some of the flavour precursors present in the crude oil were broken down thermally during hydrogenation and some others did not survive deodorisation. So hydrogenated and subsequently deodorised oil contains fewer residual flavour precursors than oil that has only been deodorised and, therefore, is less prone to flavour reversion. However, such oil may develop a so-called "hydrogenation flavour." Deodorising the oil before its hydrogenation diminishes that kind of flavour (Merker and Brown, 1956). Could it be that a hydrogenated flavour precursor generates the typical hydrogenation flavour?

Literature does not provide experimental evidence for the above hypothesis, but only circumstantial evidence and plenty of opinions. "Heat stabilisation" at 230 to 300°C (or rather 255 to 260°C) for 1 hour to 1 1/2 hours before deodorisation at 185°C provides soya bean oil with improved flavour stability (Jakobsen, 1949). Thermally controlled reactions that are required to obtain a stable oil take at least 3 hours at 200°C (Möller, 1964a). There is the "established opinion" (Kaufmann and Mukherjee, 1968c) that certain odoriferous compounds are formed during deodorisation and that a minimum deodorisation time is necessary to destroy their precursors. If this minimum time is not respected, some precursors will be left and cause poor shelf life, even if the freshly deodorised oil is fully acceptable.

"Experience has shown that certain reactions within the oil itself, and not related to FFA removal, are necessary to provide a stable oil after deodorisation." (Gavin, 1977). In a similar vein, Athanassiadis (1988) confirms that, in practice, perfect deodorisation of an oil is more complex and takes longer than deacidification by steam stripping and mentions that, moreover, other odoriferous compounds may arise during the deodorisation process and these also must be eliminated. When discussing a laboratory deodoriser, Pardun (1988) also includes a comparison between industrially deodorised oil and oil treated in the laboratory for different periods of time. He concludes that the industrially treated oil (8 to 10 hours at 165 to 175°C) has a superior keeping quality compared with the laboratory treated oil, especially when the latter treatment was fast and the oil was rather unsaturated.

On the other hand, it has also been stated that, given the thermal and oxidative sensitivity of the natural oil stabilisers, the residence time should be as short as possible (Schumacher, 1976; Stage, 1976). In a falling film evaporator (Shadiakhy, 1984), the residence time is short, but, nevertheless, very low FFA levels and peroxide values are attained. According to the *Schaal* oven test, oil so produced is even more stable (Bitner et al., 1986). There is no need to use a downstream sparged holding vessel (Shadiakhy, 1984). For the short residence SoftColumn® (Stenberg, 1996; Stenberg, 1997), on the other hand, this was found to be essential. Accordingly, a three-tray retention section was incorporated below the packed column, so that the residence time of 5 min in the column could be increased by 0 to 90 min (Ahrens, 1998).

Accordingly, there are indications that oil quality, expressed as the tendency to develop oil-specific flavours on prolonged heating as, for instance, during deep frying, can profit from a heat treatment provided as part of the steam stripping process. Although not stated specifically, this is in all likelihood the reason why a minimum deodorisation time (in function of deodorisation temperature) is prescribed by a major purchaser of refined oils (Van Duijn, 2003). It is not known to what extent such a heat treatment affects other quality indicators.

3.9.2.2 Heat bleaching

Whereas the thermal breakdown of flavour precursors is somewhat speculative since nobody has given a clue about the chemical identity of these precursors, the thermal

breakdown of colouring compounds (heat bleaching) is there for all to see and the breakdown of peroxides can be quantified analytically. With respect to peroxides, opinions differ. Should they be totally broken down during bleaching (Wiedermann, 1981) or can they be left to decompose thermally during deodorisation (Estes et al., 1995)? The experimental evidence in favour of the latter shows that peroxide values of deodorised oils are invariably zero. The mechanism involved has not been explained.

When discussing the bleaching of palm oil (Loncin, 1962), the author shows that heat bleaching starts above 180°C and that its rate depends strongly upon the temperature, but also that the presence of bleaching earth greatly accelerates heat bleaching (see also Tollenaar and Hoekman, 1964). Like Baldwin (1948), Loncin (1962) also concludes that the temperature is the only process parameter affecting the rate of heat bleaching; pressure has no effect. Sunflower seed oil can also be bleached thermally and yield a satisfactory finished product (Ostrić-Matijasević et al., 1980).

In a more recent review article (Arbeitskreis "Technologien der industriellen Gewinnung und Verarbeitung von Speisefetten," 1992), it is mentioned about palm oil that at 210°C, carotene breakdown takes a few hours and at 270°C a couple of minutes. It has also been suggested to preheat the oil for a few minutes to 240 to 260°C and bleach subsequently (Martinenghi, 1973). But even with heat exchange this looks like an expensive process. Various break-down products have been identified (Hinnekens et al., 1976).

The counter-current deodoriser described by Lowrey (1970) incorporates an annular heat-bleaching chamber. Accordingly, the oil is heat-bleached before being deodorised. The reverse order is also possible, provided that the oil being heat bleached is sparged at the same time to remove breakdown products.

3.9.2.3 Isomerisation

During deodorisation, two kinds of thermally induced isomerisation reactions have been observed, both of them affecting product quality. One of these reactions can be described as an interesterification, which may affect the crystallisation behaviour of, for instance, palm oil (Willems and Padley, 1985). The other reaction concerns the isomerisation of unsaturated fatty acids and leads to the formation of *trans* isomers. In addition, dimeric or even oligomeric glycerides may be formed, but below 240°C, this reaction is insignificant (Eder, 1982).

When cocoa butter is deodorised at 260 to 264°C, it interesterifies to such an extent that it is no longer suitable for chocolate manufacture (Stage, 1984). Deodorising a mixture of hydrogenated and liquid sunflower seed oil causes its differential thermal analysis (DTA) curves to change in function of deodorisation temperature and duration; the crystallisation behaviour also changes, as shown by microscopy (Zajíc et al., 1968). Quantitative data on the rate of thermally induced interesterification have been provided by Rossell et al. (1981), who physically refined soya bean oil for various lengths of time and at various temperatures up to 280°C for 2 hours.

This has implications for fats whose crystallisation behaviour is of paramount importance, such as cocoa butter equivalents (CBEs). Such fats usually contain a palm fraction obtained from palm oil that may have been physically refined and thereby have undergone some interesterification that is not obvious from fatty acid analysis, but shows itself in the crystallisation behaviour. It also means that the final deodorisation of CBEs should be carried out in such a way that interesterification is prevented. For cocoa butter, this is even more important.

The isomerisation of unsaturated fatty acids during deodorisation can manifest itself by a poor cold stability of otherwise stable oils, such as soya bean oil (Billek, 1992). The advent of capillary GLC (gas-liquid-chromatography) allowed a distinction between geometrical and positional isomers and it was concluded (Ackman et al., 1974) that thermally induced isomerisation is geometrical only. The authors also conclude that among the common unsaturated fatty acids, linolenic acid is primarily responsible for the formation of *trans* isomers that predominantly comprise the *cis*-9, *cis*-12, *trans-15,* and the *trans*-9, *cis*-12, *cis*-15 isomers of linolenic acid; this was subsequently confirmed (Devinat et al., 1980; Grandgirard et al., 1984). For a proposed reaction scheme, see Wolff et al. (1996).

Wolff (1993) used linseed oil and heated this in sealed ampoules at a temperature range of 190 to 260°C for up to 16 hours. This approach also allowed him to detect isomers containing two double bonds in the *trans*-configuration, and he also confirmed the absence of positional isomers. In addition, he calculated the rate constants of the rate of disappearance of linolenic acid after he had found this to be first order in linolenic acid. Another report claims that the formation of *trans* isomers depends on the temperature but less on time (Denecke and Pudel, 1996), (Pudel and Denecke, 1997), but this is unlikely given the internal consistency of the data from Wolff (1993). His data have been reproduced in Table 3.14 and the data from this table have been plotted in Figure 3.20 to illustrate the good linearity of the data that allow an activation energy of 148 kJ/mol to be calculated.

TABLE 3.14 First order isomerisation rate constants of linolenic acid

Temperature [°C]	Slope [h^{-1}]	1000/T [K^{-1}]	Log Slope
190	0.00093	2.1598	–3.03152
220	0.00724	2.0284	–2.14026
145	0.3504	1.9305	–1.45544
260	0.09270	1.8762	–1.03292

Source: Wolff, R.L. (1993), *J. Am. Oil Chem. Soc.*, **70**, 425–430.

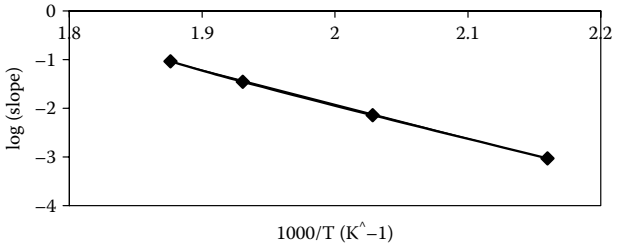


FIGURE 3.20 Arrhenius plot of isomerisation rate constants.

This value is quite close to the values of 146 kJ/mol reported by O'Keele (1993) for the formation of *c,c,t*-18:3 isomers and of 144 kJ/mol reported by Kővári et al.(1997); however, these values are substantially higher than the earlier values of 88 kJ/mol (Rossell et al., 1981) and 82 kJ/mol for the disappearance of 18:3 (O'Keele et al., 1993). The rate constants published by Hénon et al. (1997) also allow an activation energy of some 136 kJ/mol to be calculated, but the data reported by Bertoli et al. (1996) are insufficient for this purpose.

The insight gained by these kinetic studies has made it possible to build a mathematical model that predicts the extent of isomerisation as a function of operating parameters (Hénon et al., 1999; Hénon, 2000). These predictions can then be combined with data on the loss of tocopherols (De Greyt, 1998; De Greyt et al., 1999; Verleyen et al., 2002) to arrive at process conditions that ensure that the intended product properties are actually attained.

3.9.2.4 When to sparge

Vacuum stripping implies sparging the oil with stripping medium, but does it have to be sparged all the time? As illustrated in Figure 3.21, the oil is heated in two stages. First by heat exchange with oil, which has been processed, and then by using an external heat source to bring it to deodorisation temperature. When this second stage is executed at the topmost tray of the deodoriser, this tray must be sparged with stripping medium to provide the agitation that is necessary for heat transfer.

As pointed out by Bailey (1949), this sparging costs steam and this expense can be avoided by heating the oil to final deodorisation temperature outside the deodoriser shell in a closed system (White, 1956; Kehse, 1963; Dell'Acqua and Perozzi, 1969). Provided the oil has been well deaerated, the use of such a closed system does not affect oil quality and when direct-fired tubular heat exchangers are used (Kuroda and Young, 1989; Kuroda, 1998), "the inside surface of the tube is always clean" and a unit used for the deodorisation of rice bran oil since 1982 had not needed decoking right up publication of the paper (Kuroda and Young, 1989). Direct-fired heat exchangers are a viable and cheaper alternative for high-pressure steam heat exchangers when replacing heat exchangers using thermal oil (Meershoek and Reeves, 1998).

Adequate degassing of the oil, therefore, is important. In the U.S., it is not uncommon to add some citric acid

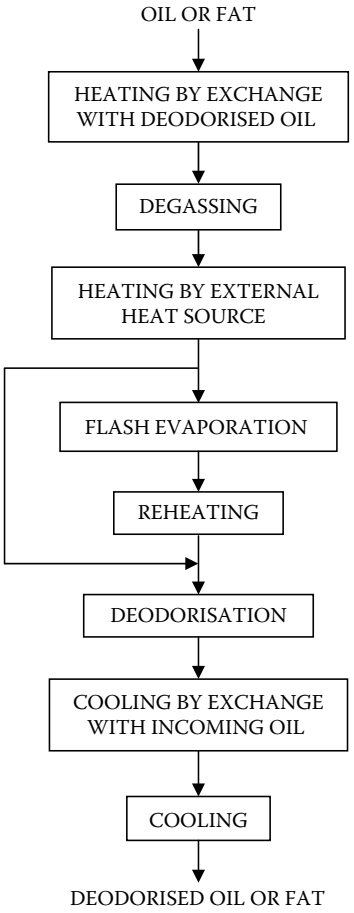


FIGURE 3.21 Flow diagram of vacuum stripping process.

(0.01% as a 20% solution) to the oil prior to heating the oil (Dutton et al., 1947; White, 1956). This addition was developed in Germany before WW II by Taussky (Pardun, 1981) and introduced in the U.S. after a fact-finding tour just after the war (Goss, 1946).

As pointed out (Dudrow, 1983), citric acid is broken down at temperatures above 150°C (Sjöberg, 1991), which means that it cannot be active at deodorisation temperatures (Miyakoschi, 1978). A possible reason for its apparent efficacy could be that the water added as part of the citric acid solution evaporates during degassing and, thus, acts as stripping medium for oxygen, just as steam stripping has been suggested as a means to remove oxygen (Baker and Edwards, 1970).

On the other hand, adding some citric acid to the oil after it has been deodorised and cooled to below 150°C (Gimeno Villacampa, 1957b) or when the temperature has dropped to 100 to 120°C (Gimeno Villacampa, 1957a) may well protect oil against oxidation. Similarly, saturation with nitrogen or storage under a nitrogen blanket (Gavin, 1981) serves the same purpose. However, oil that has been saturated with nitrogen after deodorisation will still attract oxygen from the air, but more slowly than when no nitrogen had been dissolved in the oil. It must continue to be nitrogen blanketed to prevent oxygen ingress.

Figure 3.21 also shows a flash evaporation unit as suggested quite early on by Thurman (1956) and subsequently by *i.a.* Athanassiadis (1988), but as the by-pass shows, this need not be an integral part of the deodoriser. This unit could be a falling film (Thurman, 1956) or a packed column. During the deodorisation proper, the trays should be sparged with stripping medium (steam). The sparging rate should be equal for all trays and close to the design rate to make maximum use of the equipment.

Sparging during cooling is recommended (Carlson, 1988) and supported by subsequent experimental evidence (Deffense, 1995a) showing the headspace of oil cooled under vacuum while being sparged to contain less than half as much pentane as the reference sample. There may also be an explanation for the recommendation. When oil is heated to deodorisation temperatures, it is not only the flavour precursors that start to break down thermally, but the triglyceride oil itself also breaks down and generates breakdown products that are removed by the vacuum stripping operation. A dynamic equilibrium results, since the rate of formation of these products only depends upon the temperature whereas their rate of disappearance also depends upon their concentration and the rate of sparging steam supply. Since the rate of formation is more temperature-dependent than the vapour pressure of the pure breakdown products, the equilibrium concentration decreases when the temperature is lowered. Since a bland taste is synonymous with the absence (or at least a low concentration) of odoriferous compounds, a low-temperature equilibrium must be aimed for (Dijkstra, 2001).

This implies lowering the high-temperature equilibrium concentration by vacuum stripping until a temperature below 150°C has been reached (Zehnder, 1995) and the low-temperature equilibrium has been established. A series of separate vessels, each of which is kept at a lower temperature than the previous one, has been suggested for this purpose (Brücke, 1942). Similarly, a high temperature treatment at 240 to 275°C for deacidification can be followed by deodorisation at a lower temperature of some 185°C (Jakobsen, 1950). The oil can also be treated in a cross-flow deodoriser comprising separate vessels with heaters and then coolers in between these vessels (Thurman, 1952a; 1952b).

3.9.3 Vacuum stripping processes

The various deodoriser types in industrial use and/or described in the literature (batch and continuous, cross-flow, counter-current flow, and co-current flow) have been already listed in Section 3.9. The present section will focus on the various unit operations that together constitute a vacuum stripping process.

3.9.3.1 Deaeration

The oil to be deodorised or refined physically will have been bleached (Figure 3.8) and the bleaching process involves drying the oil *in vacuo*. Accordingly, the air or rather oxygen content of the oil will usually be already quite low. However, to guard against the few instances where air has dissolved in the oil after bleaching, oil to be deodorised is invariably deaerated before being heated to deodorisation temperature.

In the batch deodoriser, this deaeration is easily achieved by evacuating the vessel and then drawing the oil into the vessel through an inlet pipe near the top of the vessel (Andersen, 1962). Blowing some stripping steam through the oil while this is being heated serves the double purpose of agitating the oil, which improves heat transfer, and stripping residual oxygen out of the oil.

Continuous and semicontinuous deodorisers always comprise a separate deaeration unit that is supplied with warm bleached oil sprayed at the top of the unit and collected by a pump at the lowest point of the unit. The unit is evacuated and means are provided to prevent oil entrainment into the vacuum system. As explained above, the addition of a small amount of water (rather than a citric acid solution), causes residual oxygen levels to be reduced.

3.9.3.2 Heating and cooling

With the batch process, a preheating stage is impractical (Möller, 1964a), which is why the energy requirements of this process are much higher than for continuous deodorisers. In the latter, the deaerated oil can be pumped through coils that are situated in the penultimate tray (the precooling tray) before being pumped to the top tray where it is further heated indirectly by high-pressure steam (Gavin and Berger, 1973; De Greyt and Kellens, 2000).

By introducing an additional preheating tray ahead of the tray where the oil is brought to its final deodorisation temperature and fitting this preheating tray with heating coils, it is also possible to use the coils in the precooling tray to generate medium pressure steam and allow this steam to enter the coils in the preheating tray. That being the coldest spot, the steam will condense there according to *Watt's* principle and liberate its latent heat. The condensate then flows back to the coils in the precooling tray where it will evaporate again; this is commonly referred to as a thermosiphon. By combining such an internal thermosiphon with further steam usage and generation (see Kellens and De Greyt, 2000, Figure 13.10), heat recovery amounts to 75%.

Heating the oil to deodorisation temperature can be performed in a sparged tray comprising heating coils supplied with high-pressure steam. It is customary to have a dedicated high-pressure steam generator next to the deodoriser. Older installations, which still use a thermal oil or Dowtherm®/Thermex® (the 26.5 to 73.5 eutectic mixture of diphenyl and diphenyloxide) certainly have dedicated heating units, which may be the reason why the high-pressure steam generators are treated similarly.

If the oil is to be heated to its final temperature by direct-fired tubular heat exchangers (Kuroda and Young,

1989; Kuroda, 1998), these will be outside the deodoriser shell. This final heating system, therefore, cannot be combined with preheating the oil by generation and condensation of steam in a closed system, but its combination with the preheating system that involves pumping the oil to be preheated through heat exchange coils in the precooling tray presents no difficulties whatsoever.

In continuous and semicontinuous deodorisers, the deodorised oil is invariably precooled by an exchange of heat with ingoing oil. Final cooling to storage temperature is by cooling water. This can be in the last tray, but it is more usual to employ a heat exchanger that is located outside the deodoriser for this purpose because the oil then no longer requires sparging during this final cooling stage.

3.9.3.3 Sparging

Care must be taken that the stripping medium contains as little oxygen as possible (Bataille, 1952; De Smet, 1965). Although superheating has been recommended (Bataille, 1952) to avoid cooling the oil (White, 1951), its temperature is not critical since the stripping medium will quickly assume the temperature of the oil and its heat capacity is so low that it will hardly affect the oil temperature.

Early steam "distributors" were based on the devices used in fractional distillation (Rocca, 1902), such as bubble caps (Lee and King, Jr., 1945; Suriano, 1957) or various types of packings (McCubbin and Monical, 1949). However, these entail a hydrostatic resistance with the result that the pressure below the tray, and especially below a series of trays, is much higher than above the tray or topmost tray, which high pressure is not conducive to efficient deodorisation. These drawbacks were overcome by the introduction of the cross-flow system, which ensures the same pressure above each tray.

Various mechanical constructions have been developed and are in use to ensure good contact between the stripping medium and the oil, leading to high vaporisation efficiencies. Bailey noted (1949) that appropriate baffling doubled this efficiency. A distinction can be made between deep bed and shallow bed deodorisation systems and there are numerous proprietary constructions. The deep bed systems often employ so-called steam lift pumps or mammoth pumps (Lineberry and Dudrow, 1973; see Zehnder, 1995, Figure 14.2). Shallow beds may just comprise a tube with holes (Tirtiaux and Gibon, 1997). Trays may comprise spiral paths to ensure a narrow residence time distribution (Kehse, 1961; Gianazza, 1968).

In the case of counter-current vacuum stripping equipment, the stripping medium is introduced as a gas and contact between this gas and the oil is assured by various means, such as repeatedly collecting the oil trickling down a packing and atomising it with steam (Bollmann, 1930), wiping the oil as a film over the surface of a vertical cylindrical tube (Bloemen, 1960), using a specified packing material (Baker and Edwards, 1970) or just packing material (Lowrey and Durchholz, 1970), structured packing (Hillström and Sjöberg, 1999), and other means.

3.9.3.4 Scrubbing

Early deodorisers did not have scrubbers as we know them nowadays. The vacuum unit, comprising a series of steam-fed boosters and direct water condensers, was connected directly to the deodoriser shell. Consequently, all vapours leaving the deodoriser passed through the first booster. Originally, surface condensers downstream of the first steam ejector (the booster or augmentor) were used to isolate the organics and reduce the gas volume. They had the disadvantage that the cooling water temperature strongly affected the vacuum level in the deodoriser, that they needed frequent cleaning, and involved high maintenance costs (Kaufmann and Mukherjee, 1968b). Subsequently, the surface condensers were omitted and the vapours were mostly condensed in the first barometric condenser, with the organics ending up floating in the hotwell (Kehse, 1967).

In these systems, the pressure at which the steam can be condensed depends on the temperature of the cooling water (Gehring, 1985; 1987) and may be subject to seasonal fluctuations. Accordingly, the use of chilled water has been suggested since this permits condensation at lower pressure and saves on motive steam. On the other hand, barometric condensers have the disadvantage that when the cooling water fed to the condensers has passed through a cooling tower, it will be saturated with air at atmospheric pressure. As pointed out by Weineck (1940), this air will leave the cooling water when the latter is exposed to a reduced pressure, and the downstream ejectors will also have to remove this additional air from the system.

To prevent excessive fouling, scrubbers were introduced and originally they were located downstream of the first steam ejector (Sullivan, 1976; Zehnder, 1976), just like the earlier surface condensers. For a number of reasons listed below, the scrubber is nowadays positioned between the deodoriser and the booster (Gavin et al., 1977; Gavin, 1977; Aalrust, 1978; Athanassiadis, 1980; Gavin, 1981; Posschelle, 1981; Tandy and McPherson, 1984):

- A booster atomises splash oil and, in practice, this cannot be retained by a wire mesh demister and so it will contaminate the cooling water.
- Fatty acids passing through the booster can cause severe corrosion.
- Positioning the scrubber between the deodoriser and the booster reduces the load on the booster by partial condensation of the vapours.
- It further reduces the load by reducing the volume of the vapours that have not been condensed because the scrubber lowers their temperature. This also saves on motive steam.

- Besides, the pressure drop over a scrubber is only 0.2 to 0.4 mbar.

One of the first scrubbers (Fiala, 1959; 1962; Brion and Fiala, 1966) is still located downstream of the booster so it can use water to condense the deodoriser vapours. This water is contained in a closed circuit and its temperature is maintained by evaporation. This allows the distillate to be recovered. An early scrubber that is connected directly to the deodoriser has been described by Klingerman and West (1965). It circulates the condensate via an indirect cooling section to a sprayer (see also Jackson and Reynolds, 1968) and differs only from more modern scrubbers in that it does not have a demister.

Such a demister has been described by Gilbert and Tandy quite some time later (1979) and it is interesting to note that they still position the scrubber downstream of the booster. Modern scrubbers when outside the deodoriser shell, often comprise a co-current spraying of cooled condensate, perhaps a small packed bed through which cooled condensate and vapours flow counter-currently and finally a demister. If the scrubber is inside the deodoriser shell, a higher packed bed ensures condensation of most organics in the vapour stream (see De Greyt and Kellens, 2000, for figures) The scrubber can also be located at the lowest part inside the deodoriser shell, which has constructional advantages (Kellens and Harper, 2005a; 2005b).

Using two scrubbers that operate at different temperatures and are positioned in series, allows some fractionation of the distillate. The first scrubber that operates at the highest temperature will only condense the least volatile constituents out of the vapour stream, whereas the downstream scrubber, operating at the lowest temperature, will condense all remaining organics. The fractionation is not perfect, but suffices to generate a first condensate that is enriched in tocopherols and, thus, can be sold at a premium (Kellens and De Greyt, 2000).

3.9.3.5 Ice-condensing process

The ice-condensing process is also referred to as "freeze condensing" (Lines, 2001) or "dry condensing" (see De Greyt and Kellens, 2000, Figure 4.16; or Kellens and De Greyt, 2000, Figure 13.14). The idea of condensing all vapours leaving the deodoriser on a surface condenser held at a temperature at which water also solidifies dates from the 1960s (Martinenghi, 1965; 1969) and was soon after mentioned in connection with the physical refining of animal fats (Droste, 1972), but its industrial realisation took quite a while (Stadel Nielsen, 1986). No scrubber is used in this process and the original Martinenghi patents already mention two surface condensers in parallel. When one of them is used to condense the vapours, the other can be cleaned by thawing. This means frequent temperature changes and demanding conditions for joints, which must remain vacuum tight (Jellema and Nijdam, 2003).

Since both the organic vapours and the stripping steam condenses on the surface condenser, this leaves only the noncondensables (essentially air) to be handled by the vacuum system. This, therefore, can be much smaller than when it also had to handle the stripping steam. Steam consumption of the ejector system is much reduced. Electrical power consumption is higher because of the refrigeration required and the investment is also higher than with scrubber systems, but, in general, ice condensation is the cheapest method for deodorisers exceeding a certain location-dependent size.

3.9.4 Deodorisation vs. steam refining

The vacuum stripping process ensures the removal of volatiles and can be used for both the deodorisation of neutral, bleached oil, and for the steam refining of degummed, bleached oil; so far, hardly any distinction has been made between these two applications of the vacuum stripping process, but there are differences. This is clearly illustrated by the composition of the distillates as given in Table 3.15.

The condensed distillates resulting from the steam refining treatments show a much higher FFA content, so that the other constituents cannot but show a lower concentration in the distillates. In absolute terms, these other constituents may amount to the same when expressed at a percentage of the oil. In fact, they may even be higher in the case of physical refining because, all other things being equal, this process requires more stripping medium since more FFA have to be removed.

The idea of deacidifying edible oils by vacuum stripping has already been put forward by Wecker in 1923[28] (see also Young, 1990). Unilever acquired patents for the steam refining process quite early (Craig, 1924; Craig and Shawfield, 1925) and it continued to be suggested by Baroni (1958). However, effective degumming processes had not yet been developed and,

TABLE 3.15 Typical composition ranges of vacuum stripping condensates

	Soybean Oil		Corn Oil	Sunflower Seed Oil	
Component	**Alkali**	**Steam**	**Steam**	**Alkali**	**Steam**
Neutral oil	5–6	4	1–2	2–3	1–2
FFA	33	75	77–81	39	70
Insaponifiables:					
– squalene	1–2	0.5	0.5–10	0.5	0.5
– tocopherols	16–20	5–7	2–4	5–7	1–2
– sterols	19–23	11	3–6	12–14	4–5

Source: De Greyt, W.F.J., *Practical Short Course on Edible Oil Refining and Processing*, Edinburgh, September 2004.

[28] Sullivan (1976), when quoting Andersen (1962), mentions a certain Hefter as the author of a paper published in 1905 suggesting steam refining. However, in the Andersen bibliography, the number of the reference mentions Wecker as the author.

consequently, many seed oils obtained by solvent extraction could not yet be physically refined to produce high-quality oil. Consequently, physical refining was limited for quite some time to "easy" oils like palm oil (Gavin et al., 1977; Aalrust, 1978; MacLellan, 1983) and lauric oils (Forster and Harper, 1983) as well as animal fats, such as lard and tallow (Dijkstra, 2001); groundnut oil, olive oil, and shea butter were also mentioned (Dell'Acqua and Perozzi, 1969) because of their FFA content of more than 2%.

Physical refining has also been used as a method of treating high acidity oils with less loss of neutral oil than alkali refining. Steam refining would ensure the removal of most FFA, subsequent alkali refining would remove nearly all residual FFA (Clayton, 1949) and the final deodorisation would take care of what was left and also the malodorous compounds (Bloemen, 1967).

With the advent of effective degumming processes (Dijkstra, 1998), physical refining became possible for all oils and fats and it has been widely adopted since the 1950s, especially in Europe, (Athanassiadis, 1977) for the following reasons (Graner, 1965; Balicer et al., 1984; Kővári, 2004):

- The inherent neutral oil loss (NOL) is lower with physical refining (1.1 x FFA + 0.2%) than with alkali refining (1.25 × TL + 0.3%) with TL being the "theoretical loss," defined as the sum of the FFA, phosphatides, moisture, and a term of 0.3. As explained in Section 3.5.1, the weight percent of the FFA has to be adjusted for the average molecular weight of the fatty acids.
- From the above, it is clear that the difference in NOL increases with an increase in FFA. Accordingly, high acidity oils like palm oil and, subsequently, canola and sunflower seed oil, were prime candidates for switching to physical refining. It is only later that this process also became attractive for soya bean oil.
- Steam refining eliminates a process step, as illustrated by Figure 3.8.
- There being no soapstock, steam refining obviates any soapstock treatment and, therefore, does not lead to high BOD effluent, nor does it require any chemicals to produce the acid oils and treat said effluent.
- Both distillates (steam refining and deodorisation following alkali refining) can be sold as such, whereby the deodorisation distillate has the advantage of having a higher tocopherol content, but the disadvantage of possibly containing more pesticide residues, low molecular weight PAHs, and the like (Arbeitskreis "Technologien der industriellen Gewinnung und Verarbeitung von Speisefetten," 1990). As mentioned at the end of Section 3.9.3.4, scrubbing at two different temperatures leads to a fractionation of the distillate and a concentration of the tocopherols.
- The fatty acid distillate has a higher quality than the acid oils (Leibovitz and Ruckenstein, 1981).

On the other hand, there are also some aspects that can make physical refining less attractive than alkali refining:

- Because the alkali refining process can remove some colouring compounds, the physical refining process may require more bleaching earth.
- Using more bleaching earth entails a higher oil loss that may not be offset by the lower oil loss of physical refining in the case of low FFA oils, such as soya bean oil.
- Although acid refined oils can be hydrogenated as such (Cleenewerck and Dijkstra, 1992; Drigalla and Pudel, 1993; Drigalla and Krause, 1995), many refiners prefer to hydrogenate neutral oils. Abandoning alkali refining would entail the steam refining of the hydrogenation feedstock and the subsequent deodorisation of the hydrogenated product. On quality grounds, this might actually have advantages since Merker and Brown (1956) report "high flavor stability of hydrogenated soya bean oil, which was deodorized and protected from autoxidation prior to hydrogenation."
- Sodium methanolate catalysed interesterification requires a neutral oil, so there again, two vacuum stripping steps may be needed (Grothues, 1981).
- Pesticide residues will be concentrated in the distillates (Addison et al., 1978), but the acid oils resulting from soapstock acidulation will contain very little of these residues.

However, the adoption of physical refining should not entail using an existing deodoriser for steam refining. That would decrease its capacity because the scrubber system has not been designed for the increased amount of FFA resulting from steam refining. Besides, the optimal steam refining process may require a different temperature profile from what has been used satisfactorily for deodorisation.

In Europe, there is a tendency to prescribe a maximum *trans* isomer content and a minimum tocopherol content for liquid RBD oils as tabulated in Table 3.16. This prescription affects steam stripping process conditions and may affect stripping equipment design. It excludes prolonged high temperature treatment, but may comprise a short time at a high temperature followed by a longer period at a somewhat reduced temperature (Thurman, 1952b; Copeland and Belcher, 2001); this may require physical cooling, but the oil may also cool down by FFA evaporation. Steam stripping at temperatures that are increased step-by-step has also been described (Thurman, 1952a).

TABLE 3.16 Refined oil specifications

Oil Property	Linolenic Acid Oils (Soybean, Rapeseed)	Linoleic Acid Oils (Sunflower Seed, Corn Germ)	Monounsaturated Oils (Palm Oil)
trans-content [%]	< 1.0	< 0.5	< 0.5
tocopherols [ppm]	> 500 (U.S.) > 1000 (Europe)	800–1000	
tocotrienols [ppm]			> 250

Source: De Greyt, W.F.J., *Practical Short Course on Edible Oil Refining and Processing*, Edinburgh, September 2004.

References

Aalrust, E. (1978), Physikalische Raffination von Palmöl, *Fette Seifen Anstrichm.*, **80**, 342–344.

Ackman, R.G. et al. (1974), Linolenic acid artifacts from the deodorization of oils, *J. Am. Oil Chem. Soc.*, **51**, 42–49.

Addison, R.F. et al. (1978), Behavior of DDT, polychlorinated biphenyls (PCBs) and Dieldrin at various stages of refining of marine oils for edible use, *J. Am. Oil Chem. Soc.*, **55**, 391–394.

Ahrens, D. (1998), Comparison of tray, thin-film deodorization, *inform*, **9**, 566–576.

Andersen, A.J.C. (1962), *Refining of Oils and Fats for Edible Purposes*, Pergamon Press Ltd., Oxford, UK.

Arbeitskreis "Technologien der industriellen Gewinnung und Verarbeitung von Speisefetten" (1988), Gemeinschaftsarbeiten der DGF, 102 Mitteilung. Die Dämpfung von Speisefetten und -ölen zur Desodorisierung und Entsäuerung II, *Fat Sci. Technol.*, **90**, 207–210.

Arbeitskreis "Technologien der industriellen Gewinnung und Verarbeitung von Speisefetten" (1990), Gemeinschaftsarbeiten der DGF, 117 Mitteilung. Die Dämpfung von Speisefetten und -ölen zur Desodorisierung und Entsäuerung VII, *Fat Sci. Technol.*, **92**, 459–463.

Arbeitskreis "Technologien der industriellen Gewinnung und Verarbeitung von Speisefetten" (1992), Gemeinschaftsarbeiten der DGF, 125 Mitteilung Die Dämpfung von Speisefetten und -ölen zur Desodorierung und Entsäuerung VIII, *Fat Sci. Technol.*, **94**, 1–8.

Athanassiadis, A. (1977), Refining and segregation of palm oil, *Oléagineux*, **32**, 173–177.

Athanassiadis, A. (1980), Considerations on energy saving in vegetable oil refining, *Fette Seifen Anstrichm.*, **82**, 219–224.

Athanassiadis, A. (1988), The deacidification of vegetable oils by distillation during deodorization, *Fat Sci. Technol.*, **90**, 522–526.

Bailey, A.E. (1941), Steam deodorization of edible fats and oils. Theory and practice, *Ind. Eng. Chem.*, **33**, 404–408.

Bailey, A.E. (1949), The semicontinuous deodorization of fats, *J. Am. Oil Chem. Soc.*, **26**, 166–170.

Bailey, A.E. (1954), Method and apparatus for deodorizing oils, US Patent 2,691,665, assigned to National Cylinder Gas Company.

Baker, J.S. and Edwards, J.B. (1970), Continuous high temperature steam deodorization of edible oils, US Patent 3,506,696, assigned to The Procter & Gamble Company.

Balchen, S. and Adler-Nissen, J. (1998), Application of non-condensable stripping gases in counter-current packed columns for deodorisation/deacidification, *Poster presented at the 89th AOCS Annual Meeting & Expo, Chicago.*

Balchen, S. et al. (1999), Deodorization principles, *inform*, **10**, 245–262.

Baldwin, A.R. (1948), Effects of deodorization on the stability of vegetable oils, *J. Am. Oil Chem. Soc.*, **25**, 33–35.

Balicer, Z. et al. (1984), Physical (steam) refining — advantages and limits for soft oils, *Fette Seifen Anstrichm.*, **86**, 537–540.

Ballestra, M. (1954), Nouvelle méthode pour la désodorisation des huiles en continu, *Rev. Franç. Corps Gras*, **1**, 562–564.

Ballestra, M. (1960), Apparatus for the distillation of liquid material, US Patent 2,938,838.

Baroni, L. (1958), La neutralisation des corps gras par distillation des acides gras, *Oléagineux*, **13**, 65–66.

Bataille, E. (1924), Vapor-removing plant, US Patent 1,489,109.

Bataille, R. (1952), Trois réflexions sur la façon de désodoriser les huiles, *Bull. mens. ITERG*, 361–363.

Bates, R.W. (1949), Fundamentals of deodorization, *J. Am. Oil Chem. Soc.*, **26**, 601–606.

Bergman, T.J. and Nauman, E.B. (1986), The flash devolatilization of cocoa butter, *J. Am. Oil Chem. Soc.*, **63**, 1469–1472.

Bertoli, C. et al. (1996), Formation of trans fatty acids during deodorization of low erucic acid rapeseed oil, *Poster presented during the World Conference on Oilseed and Edible Oil Processing, Istanbul.*

Billek, G. (1992), Die Veränderungen von Nahrungsfetten bei höheren Temperaturen, *Fat Sci. Technol.*, **94**, 161–172.

Bitner, E.D. et al. (1986), Laboratory continuous deodorizer for vegetable oils, *J. Am. Oil Chem. Soc.*, **63**, 338–340.

Blank, E.W. (1942), Chronological list of important dates in the history of the fats and waxes, *Oil & Soap*, **19**, 110–113.

Bloemen, F. (1960), Appareil pour la désodorisation et la neutralisation par distillation des acides gras non combinés, des corps gras végétaux et animaux, ainsi que pour la distillation simple ou fractionnée des acides gras de ces corps, French Patent 1.238.792, assigned to Bloemen-Jarry, L.S.

Bloemen, F. (1966), Nouvelle colonne pour la distillation neutralisante et la désodorisation des corps gras et pour la distillation simple ou fractionnée des acides gras, *Rev. Franç. Corps Gras*, **13**, 247–259.

Bloemen, F. (1967), Combinaison rationelle et économique de la distillation neutralisante avec le raffinage classique aux alkalis, *Rev. Franç. Corps Gras*, **14**, 183–185.

Bloemen, F. (1975), Considérations sur les économies de vapeur, réalisables dans la désodorisation et la distillation neutralisante des corps gras., *Rev. Franç. Corps Gras*, **22**, 537–542.

Bollmann, H. (1930), Method of deodorizing fats and fatty oils, US Patent 1,754,598.

Brekke, O.L. (1980), Deodorization, in *Handbook of Soy Oil Processing and Utilization*, Erickson, D.R., Pride, E.H., Brekke, O.L., Mounts, T.L., and Falb, R.A., Eds., American Soybean Association, St. Louis, MO.

Brion, D.J. and Fiala, R.J. (1966), Process for deodorizing fats and oils by distillation with steam under vacuum conditions, US Patent 3,239,547, assigned to A.E. Staley Manufacturing Company.

Brücke, O. (1942), Verfahren zur kontinuierlichen Entfernung von Geruchs – und Geschmackstoffen, Fettsäuren und anderen fluchtigen Beimengungen aus pflanzlichen oder tierische Ölen und Fetten, German Patent 723 436, assigned to Metallgesellschaft AG.

Buxton, L.O. and Colman, H.B. (1941), Process for refining fatty substances, US Patent 2,258,672, assigned to National Oil Products Company.

Carlson, K.F. (1988), Deodorization options and trends, *J. Am. Oil Chem. Soc.*, **65**, 306–313.

Cheng, A.T.Y. et al. (1992), Deodorizing edible oil and/or fat with non-condensible inert gas and recovering a high quality fatty acid distillate, European Patent 0 513 739, assigned to Union Carbide Industrial Gases Technology Corporation.

Clayton, B. (1949), Process of refining glyceride oils, US Patent 2,478,089, assigned to Benjamin Clayton (Refining Unincorporated).

Cleenewerck, B. and Dijkstra, A.J. (1992), The total degumming process — theory and industrial application in refining and hydrogenation, *Fat Sci. Technol.*, **94**, 317–322.

Coelho Pinheiro, M.N. and Guedes de Carvalho, J.R.F. (1994), Stripping in a bubbling pool under vacuum, *Chem. Eng. Sci.*, **49**, 2689–2698.

Copeland, R. and Belcher, W.M. (2001), Methods for refining vegetable oils and byproducts thereof, US Patent 6,172,248 B1, assigned to IP Holdings, L. L. C.

Craig, R. (1924), Improvements in the refining of oils and fats, British Patent 224,928, assigned to Unilever.

Craig, R. and Shawfield, C.E.C. (1925), Improvements in and relating to plant for the removal of free fatty acids and other impurities from oils, fats and the like, British Patent 242,316, assigned to Unilever.

Decap, P. et al. (2004), Comparison of steam and nitrogen in the physical deacidification of soybean oil, *J. Am. Oil Chem. Soc.*, **81**, 611–617.

Deffense, E.M.J. (1995a), Theorie und Praxis der physikalischen Raffination, *Fette Seifen Anstrichm.*, **97**, 481–484.

Deffense, E.M.J. (1995b), Usage of steam in physical refining, *Oils & Fats International*, **11**, 2428.

De Greyt, W.F.J. (1998), *Effect of physical refining on selected minor components in vegetable oils*, Ph.D. thesis, Universiteit Gent, Belgium.

De Greyt, W.F.J., *Practical Short Course on Edible Oil Refining and Processing*, Edinburgh, September 2004.

De Greyt, W.F.J. and Kellens, M.J. (2000), Refining practice, in *Edible Oil Processing*, Hamm, W. and Hamilton, R.J., (Eds.), Sheffield Academic Press, Sheffield, U.K., 79–128.

De Greyt, W.F.J. et al. (1999), Effect of physical refining on selected minor components in vegetable oils, *Fett/Lipid*, **101**, 428–432.

Dell'Acqua, G. and Perozzi, G. (1969), La désacidification physique des huiles et graisses par distillation des acides gras libres comme méthode moderne et économique de raffinage, *Rev. Franç. Corps Gras*, **16**, 37–47.

Denecke, P. and Pudel, F. (1996), Formation of trans-fatty acids during deodorization, in *Oils-Fats-Lipids* 1995, Castenmiller, W.A.M., Ed., P.J. Barnes & Associates, Bridgewater, U.K., 163–166.

De Smet, J.A. (1965), Pratique de la désodorisation, *Oléagineux*, **20**, 43–45.

Devinat, G. et al. (1980), Isomérisation de l'acide linolénique durant la désodorisation des huiles de colza et de soja, *Rev. Franç. Corps Gras*, **27**, 283–287.

Dijkstra, A.J. (1998), Degumming revisited, *Oléagineux Corps Gras Lipides/OCL*, **5**, 367–370.

Dijkstra, A.J. (1999), Stripping medium requirements in continuous countercurrent deodorization, *J. Am. Oil Chem. Soc.*, **76**, 989–993.

Dijkstra, A.J. (2001), Refining and processing for shortening manufacture and other uses, in *Animal Carcass Fats*, Rossell, J.B., (Ed.), LFRA Limited, Leatherhead, U.K., 51–75.

Drigalla, P. and Krause, Th. (1995), Direkte Hydrierung von TOP-Rapsöl in Anwesenheit eines Adsorbens, *Fat Sci. Technol.*, **97**, 280–284.

Drigalla, P. and Pudel, F. (1993), Untersuchungen zur direkten Hydrierung von TOP-Rapsöl, *Fat Sci. Technol.*, **95**, 241–248.

Droste, P.D. (1972), Quelques apects de l'évolution du raffinage des corps gras animaux, *Rev. Franç. Corps Gras*, **19**, 575–586.

Dudrow, F.A. (1983), Deodorization of edible oil, *J. Am. Oil Chem. Soc.*, **60**, 272–274.

Duijn, G. van (2003), Quality control in oil processing, *Paper presented at the 25th ISF World Congress, Bordeaux,* page 61 of the abstracts.

Dutton, H.J. et al. (1947), The flavor problem of soybean oil I. A test of the water washing-citric acid refining technique, *J. Am. Oil Chem. Soc.*, **24**, 261–264.

Eder, S.R. (1982), Über die Bildung von Artefakten bei der Dämpfung von Speiseölen und — fetten, *Fette Seifen Anstrichm.*, **84**, 136–141.

Estes, J. et al. (1995), Is it necessary to bleach oils to zero peroxide? *Paper presented at the 86th AOCS Annual Meeting & Expo, San Antonio, TX*.

Fedeli, E. and Tiscornia, E. (1979), Processi di distilazione in corrente di vapore nella tecnologia delle sostanze grasse. Nota 1: Deodorazione, *Riv. Ital. Sostanze Grasse*, **56**, 218–224.

Fiala, R.J. (1959), A commercial process for the recovery of deodorizer distillates, *J. Am. Oil Chem. Soc.*, **36**, 375–379.

Fiala, R.J. (1962), Method for recovering vegetable oil distillates, US Patent 3,061,622, assigned to A.E. Staley Manufacturing Company.

Forster, A. and Harper, A.J. (1983), Physical refining, *J. Am. Oil Chem. Soc.*, **60**, 265–271.

Garber, H.J. and Lerman, F. (1943), Principles of stripping operations, *Transac. Am. Inst. Chem. Eng.*, **39**, 113–131.

Gavin, A.M. (1977), Edible oil deodorizing systems, *J. Am. Oil Chem. Soc.,* **54**, 528–532.

Gavin, A.M. (1978), Edible oil deodorization, *J. Am. Oil Chem. Soc*., **55**, 783–791.

Gavin, A.M. (1981), Deodorization and finished oil handling, *J. Am. Oil Chem. Soc*., **58**, 175–184.

Gavin, A.M. and Berger, R.W. (1973), Deodorizing system modification for heat recovery and steam refining of palm oil, *J. Am. Oil Chem. Soc*., **50**, 466A–472A.

Gavin, A.M. et al. (1977), Steam (physical) refining deodorizer for Malaysian palm oil, *J. Am. Oil Chem. Soc.*, **54**, 321A–315A.

Gehring, H. (1985), Vakuumpumpen — notwendiges Übel oder integrierte Verfahrensstufe? *Fette Seifen Anstrichm*., **87**, 512–516.

Gehring, H. (1987), Vakuumpumpsysteme für Speiseöl-Anlagen — Einflussgrössen und Auslegung, *Fat Sci. Technol*., **89**, 493–495.

Gianazza, A. (1968), Raffinage des corps gras végétaux; raffinage de l'huile de palme par distillation neutralisante au moyen du Physitron, *Oléagineux*, **23**, 259–261.

Gilbert, W.J. and Tandy, D.C. (1979), Odor control in edible oil processing, *J. Am. Oil Chem. Soc*., **56**, 654A–658A.

Gimeno Villacampa, M. (1957a), Estabilización de los aceites refinados de soja, oliva y algodón con ácido cítrico, *Grasas Aceites*, **8**, 162–165.

Gimeno Villacampa, M. (1957b), Stabilisation à l'acide citrique des huiles raffinées de soja, d'olive et de coton, *Rev. Franç. Corps Gras,* **4**, 559–559.

Goss, W.H. (1946), Processing oilseeds and oils in Germany, *Oil & Soap*, **23**, 241–244.

Graciani Constante, E. et al. (1994), Physical refining of edible oils using nitrogen as stripping gas. Process optimization, *Grasas Aceites,* **45**, 132–146.

Graciani Constante, E. et al. (1991), Deacidification by distillation using nitrogen as stripper. Possible application to the refining of edible oils, *Grasas Aceites*, **42**, 286–292.

Graham, W.A. and Webb Jr., O. (1963), Apparatus for continuous edible oil refining, US Patent 3,102,062, assigned to Stratford Engineering Company.

Grandgirard, A. et al. (1984), Geometrical isomerization of linolenic acid during heat treatment of vegetable oils, *J. Am. Oil Chem. Soc*., **61**, 1563–1568.

Graner, J. (1965), Neutralisation-superdésodorisation combinée, *Rev. Franç. Corps Gras*, **12**, 218–220.

Grothues, B.G.M. (1981), Physical refining of soy oil, in *Proceedings of the second A.S.A. symposium on soybean processing. Refining of soy oil, caustic or physical*? Leysen, R.J.F., Ed., American Soybean Association, Brussels, Belgium.

Hénon, G. (2000), Modèle prédictif de l'isomérisation trans des acides gras polyinsaturés au cours de la désodorisation industrielle des huiles végétales, *Oléagineux Corps Gras Lipides/OCL*, **7**, 91–94.

Hénon, G. et al. (1997), Degradation of α-linolenic acid during heating, *J. Am. Oil Chem. Soc.*, **74**, 1615–1617.

Hénon, G. et al. (1999), Deodorization of vegetable oils, Part I: Modelling the geometrical isomerization of polyunsaturated fatty acids, *J. Am. Oil Chem. Soc*., **76**, 73–81.

Hillström, A. and Sjöberg, P. (1999), Deodorisation plant for fatty oils, US Patent 6,001,220, assigned to Alfa-Laval AB.

Hinnekens, H. et al. (1976), Dégradation thermique du β-carotène dans l'huile de palme, *Rev. Franç. Corps Gras*, **23**, 609–616.

Holló, J. and Lengyel, T. (1960), Dampf-Flüssigkeits-Gleichgewicht von Fettsäuren im Vakuum, *Fette Seifen Anstrichm*., **62**, 913–918.

Hrushowy, R.H. (1978), Palm oil processing, *J. Am. Oil Chem. Soc*., **55**, 190A–191A.

Huesa Lope, J. and Dobarganes García, C. (1991), A process for deodorizing oils and fats, European Patent 0 405 601 A2, assigned to Sociedad Espanola de Carburos Metalicos S.A. and Consejo Superior de Investigaciones Cientificas.

Jackson, D.H. and Reynolds, J.T. (1968), Method for the recovery of deodorization distillate, US Patent 3,369,344, assigned to Croll-Reynolds Company Inc.

Jakobsen, J.L. (1949), Purification of a fatty oil, US Patent 2,465,969, assigned to General Mills, Inc.

Jakobsen, J.L. (1950), Soybean oil, US Patent 2,508,919, assigned to General Mills, Inc.

Jeberg, B.V. (1979), Methematical model for removal of fatty acids during deodorization, *Paper presented at the 10th Scandinavian Symposium on Lipids, Nyborg, Denmark,* D-159-D-164.

Jellema, P. and Nijdam, J.L. (2003), Apparatus for subliming or condensing a water-containing fluid in a closed space, US Patent 2003/0097842 A1, assigned to Solutherm.

Kaufmann, H.P. and Mukherjee, K.D. (1968a), Neuzeitliche Technologie de Fette und Fettprodukte CXVII: Die Raffination der Fette, *Fette Seifen Anstrichm*., **70**, 197–203.

Kaufmann, H.P. and Mukherjee, K.D. (1968b), Neuzeitliche Technologie der Fette und Fettprodukte CXIX: Die Raffination der Fette, *Fette Seifen Anstrichm*., **70**, 589–596.

Kaufmann, H.P. and Mukherjee, K.D. (1968c), Neuzeitliche Technologie der Fette und Fettprodukte CXVIII: Die Raffination der Fette, *Fette Seifen Anstrichm*., **70**, 370–375.

Kehse, W. (1960), Über den Einfluss der Ölschichthöhe bei einem Konti-Deso, *Fette Seifen Anstrichm*., **62**, 937–938.

Kehse, W. (1961), Procédé de désodorisation continue de graisses et huiles végétales et animales au moyen de vapeur passant travers ces graises et huiles, French Patent 1.272.228, assigned to Pintsch-Bamag A.G.

Kehse, W. (1963), Über das Verweilzeitspektrum und die Anwendung eines Fettsäure-Kondensators bei einem kontinuierlichen Desodorisierer., *Fette Seifen Anstrichm*., **65**, 217–221.

Kehse, W. (1965), Deodorizing apparatus, US Patent 3,172,922, assigned to Pintsch-Bamag A.G.

Kehse, W. (1967), Abwasserreinhaltung und Verwertung von Desodorierdestillaten, Fette Seifen Anstrichm., **69**, 868–870.

Kellens, M.J. and De Greyt, W.F.J. (2000), Deodorization, in *Introduction to Fats and Oils Technology*, O'Brien, R.D., Farr, W.E., and Wan, P.J., Eds., AOCS Press, Champaign, IL, 235–268.

Kellens, M.J. and de Suraÿ, D. (2005), Method and apparatus for vacuum stripping, European Patent 1 505 145 A1, assigned to De Smet Engineering N.V.

Kellens, M.J. and Harper, A.J. (2005a), Equipment and process for physical refining and/or deodorisation of edible oils and fats, European Patent 1 258 524 B1, assigned to De Smet Engineering N.V.

Kellens, M.J. and Harper, T. (2005b), Equipment for physical refining and deodorization of edible oils and fats, US Patent 6,953,499 B2, assigned to De Smet Engineering N.V.

Klingerman, R.H. and West, R.W. (1965), A simple and efficient method for the recovery of deodorizer distillates, *J. Am. Oil Chem. Soc*., **42**, 176A–188A.

Kővári, K. (2004), Recent developments, new trends in seed crushing and oil refining, *Chevreul Award Address presented at the 3rd EuroFedLipid Congress, Edinburgh,* page 4 in the abstracts.

Kővári, K. et al. (1997), Kinetic of trans isomer fatty acid formation during heating, *Paper presented at the 22nd ISF World Congress*, *Kuala Lumpur.*

Kuroda, Z. (1998), Using a tubular furnace for direct heating, *inform*, **9**, 1086–1088.

Kuroda, Z. and Young, C. (1989), An edible oil deodorizer with a direct-fired heater, *J. Am. Oil Chem. Soc*., **66**, 1781–1783.

Lau, J. (1978), Ölveredelung: Produkt-, Energie-, Prozeß- und Umweltdaten, Ergebnisse von Grossanlagen, *Fette Seifen Anstrichm*., **80**, 355–357.

Lee, A.P. and King Jr., W.G., (1937), Edible oil deodorizing equipment and methods: a short historical sketch, *Oil & Soap*, **14**, 263–269.

Lee, A.P. and King, Jr., W.G., (1945), Purification and deodorization process, US Patent 2,368,669.

Leibovitz, Z. and Ruckenstein, C. (1981), New processes in degumming, bleaching, deacidifcation-deodorization and winterizing of edible oils, *Rev. Franç. Corps Gras*, **28**, 303–308.

Liebing, H.A. (1970), Über die destillative Entsäuerung von Fetten und Ölen, *Fette Seifen Anstrichm*., **72**, 640–650.

Lineberry, D.D. and Dudrow, F.A. (1973), Apparatus and method for deodorizing oils, British Patent 1 321 299, assigned to Chemetron Corporation.

Lines, J.R. (2001), Freeze-condensing systems for deodorization, *inform*, **12**, 263–268.

Loncin, M. (1962), Palmöl und seine Verarbeitung für die Margarine-Industrie, *Fette Seifen Anstrichm*., **64**, 531–536.

Lowrey, E.R. and Durchholz, R.F. (1970), Apparatus and process for continuous heat-bleaching and high temperature steam deodorization of edible oils, US Patent 3,542,653, assigned to The Procter & Gamble Company.

MacLellan, M. (1983), Palm oil, *J. Am. Oil Chem. Soc*., **60**, 368–373.

Martinenghi, G.B. (1965), Procédé et dispositifs pour le raffinage d'huiles ou de graisses de glycérides, French Patent 1.412.173.

Martinenghi, G.B. (1969), Refining process comprising deacidification and deodorization of glyceride oils and equipment for said process, US Patent 3,455,975.

Martinenghi, G.B. (1971*), Physical Refining of Fats and Oils*, Corbella, Milan.

Martinenghi, G.B. (1973), Traitements de l'huile de palme III. Le raffinage physique, *Oléagineux*, **28**, 189–191.

Maza, A. et al. (1992), Effects of deodorization and steam-refining parameters on finished oil quality, *J. Am. Oil Chem. Soc*., **69**, 1003–1008.

McCubbin, K. and Monical, C.E. (1949), Deodorization of oils, US Patent 2,461,694, assigned to A.E. Staley Manufacturing Company.

Meershoek, C.J.M. and Reeves, R.M. (1998), Thermal heating fluids — a hot issue, *inform*, **9**, 542–551.

Mérat, P. (1954), La désodorisation des corps gras, *Rev. Franç. Corps Gras*, **1**, 396–414.

Merker, D.R. and Brown, L.C. (1956), The effect of deodorization prior to hydrogenation on the development of hydrogenation odor in fats, *J. Am. Oil Chem. Soc.,* **33**, 141–143.

Miyakoschi, K. (1978), Influence des conditions de désodorisation et de chauffage sur le taux d'acide citrique et de ses produits de décomposition dans les huiles végétales, *Rev. Franç. Corps Gras*, **25**, 571.

Möller, O. (1964a), Fragen der Wirtschaftlichkeit beim Desodorieren von Ölen und Fetten, *Fette Seifen Anstrichm*., **66**, 517–523.

Möller, O. (1964b), Fragen der Wirtschaftlichkeit beim Desodorieren von Ölen und Fetten, *Fette Seifen Anstrichm*., **66**, 446–455.

Møller, H. and Balchen, S. (1994), A continuous packed column for deodorization of edible oils, *Poster presented at DGF meeting.*

Morris, C.E. (1949), Mechanics of deodorization, *J. Am. Oil Chem. Soc*., **26**, 607–610.

O'Keele, S.F. et al. (1993), Effect of temperature on linolenic acid loss and 18:3 delta 9-cis, delta 12-cis, delta 15- trans formation in soybean oil, *J. Am. Oil Chem. Soc*., **70**, 915–917.

Ostrić-Matijasević, B. et al. (1980), Quality of sunflower oil bleached during deodorization, *J. Am. Oil Chem. Soc*., **57**, 323–325.

Pagès, X. (1990), Désodorisation d'huiles végétales à l'azote, *Rev. Franç. Corps Gras*, **37**, 186.

Pardun, H. (1981), Die Stabilisierung von Sojaöl durch Behandlung mit Zitronensäure — Zur Erinnerung an Julius D. Taussky, *Fette Seifen Anstrichm*., **83**, 76–78.

Pardun, H. (1988), Desodorierung von Speiseölen im Laboratoriummassstab, *Fat Sci. Technol*., **90**, 5–14.

Petrauskaitè, V. et al. (2000), Physical refining of coconut oil: effect of crude oil quality and deodorization conditions on neutral oil loss, *J. Am. Oil Chem. Soc*., **77**, 581–586.

Podbielnak, W.J. (1964), Procédé et appareil de désodorisation, French Patent 1.373.723, assigned to Dresser Industries Inc.

Posschelle, G.L. (1981), De Smet process for physical refining of soybean oil, *J. Am. Oil Chem. Soc*., **58**, 203–205.

Pudel, F. and Denecke, P. (1997), Influences on the formation of trans fatty acids during deodorization of rapeseed oil, *Oléagineux Corps Gras Lipides/OCL*, **4**, 58–61.

Raffaetá, R. (1961), Process for deodorizing edible liquid fats, US Patent 2,991,298.

Rocca, É. (1902), Process of refining oil, US Patent 699,571.

Rossell, J.B. et al. (1981), Chemical changes in soy oil during high temperature processing, in *Proceedings of the Second ASA Symposium on Soybean Processing*, Leysen, R.J.F., Ed., American Soybean Association, Brussels, Belgium.

Rowan Jr., C.M. (1975), Verfahren zur Desodorierung und Dampfraffination von Fetten und Ölen, German Patent 24 42 217, assigned to Parkson Corp.

Rowan Jr., C.M. (1976), Process for deodorization and steam refining of fats and oils, British Patent 1 446 831, assigned to Parkson Corp.

Ruiz Méndez, V. et al. (1996), Comparative performance of steam and nitrogen as stripping gas in physical refining of edible oils, *J. Am. Oil Chem. Soc*., **73**, 1641–1645.

Schumacher, H.O. (1976), Ein neues Verfahren zur Entsäuerung und Desodorierung von Ölen und Fetten, *Fette Seifen Anstrichm*., **78**, 192–196.

Sergueev, A.G. (1955), Désodoriseur continu SLET, *Rev. Franç. Corps Gras*, **2**, 338–339.
Shadiakhy, A. (1984), Praktische Erfahrungen mit der Fallfilmdesodorier-Anlage, *Fette Seifen Anstrichm.*, **86**, 225–228.
Siemes, W. and Mechler, K. (1963), Die Verweilzeit des Öles in einem kontinuierlichen Kreutzstrom-Desodorisierer, *Fette Seifen Anstrichm.*, **65**, 289–294.
Sjöberg, P. (1991), Deodorization technology, *Lipid Technol.*, **3**, 52–57.
Stadel Nielsen, K. (1986), Trockenkondensierung — Ein neues Verfahren in der Fettsäuretechnologie, *Fette Seifen Anstrichm.*, **88**, 96–97.
Stage, H. (1976), Gegenstrom-Fallfim-Treibdampdestillation mittels eines von aussen aufgezwungenen Temperaturfeldes zur wirksamen Desodorierung von Fettsäuren und Speiseölen, *Fette Seifen Anstrichm.*, **78**, 457–463.
Stage, H. (1981), Physical refining of vegetable oils with special emphasis on soy oil today and tomorrow, in *Proceedings of the Second ASA Symposium on Soybean Processing*, Leysen, R.J.F., Ed., American Soybean Association, Brussels, Belgium.
Stage, H. (1982), Physical refining and deodorization of food oils I, *Fette Seifen Anstrichm.*, **84**, 377–395.
Stage, H. (1984), Entsäuerung und Desodorierung von Speiseölen und Fetten in ein- oder mehrstufig betriebenen Gegenstrom-Filmanlagen - Grundlagen und Wirtschaftlichkeit, *Fette Seifen Anstrichm.*, **86**, 255–264.
Stage, H. (1985), The physical refining process, *J. Am. Oil Chem. Soc.*, **62**, 299–308.
Stenberg, O. (1996), Improved deodorizing technology from Alfa Laval, *Lipid Technol.*, **8**, 105–106.
Stenberg, O. (1997), Refining with SoftColumn™ technology, *Paper presented at the Lipidex Conference, Antwerp, Belgium*.
Stenberg, O. and Sjöberg, P. (1996), Thin-film deodorizing of edible oils, *inform*, **7**, 1296–1304.
Sullivan, F.E. (1976), Steam refining, *J. Am. Oil Chem. Soc.*, **53**, 358–360.
Suriano, J.F. (1957), Method and apparatus for deodorizing oils and fats, US Patent 2,804,427.
Szabó Sarkadi, D. (1956), *Associatie en dampspanning van stearinezuur en andere vetzuren*, Ph.D. thesis, Technische Hogeschool Delft, The Netherlands.
Szabó Sarkadi, D. (1958a), Laboratory deodorizer with a vaporization efficiency of unity, *J. Am. Oil Chem. Soc.*, **35**, 472–475.
Szabó Sarkadi, D. (1958b), Vapor pressure equilibrium of stearic acid in triglyceride and in high paraffin solutions, *J. Am. Oil Chem. Soc.*, **35**, 479–481.
Szabó Sarkadi, D. (1959), Hydrolysis during deodorization of fatty oils. Catalytic action of fatty acids, *J. Am. Oil Chem. Soc.*, **36**, 143–145.
Tandy, D.C. and McPherson, W.J. (1984), Physical refining of edible oil, *J. Am. Oil Chem. Soc.*, **61**, 1253–1258.
Thurman, B.H. (1952a), Method of deodorizing glyceride oils, US Patent 2,621,196, assigned to Kraft Foods Company.
Thurman, B.H. (1952b), Purification of glyceride oil, US Patent 2,621,197, assigned to Kraft Foods Company.
Thurman, B.H. (1956), Process for deodorization of glyceride oils, US Patent 2,759,883, assigned to Kraft Foods Company.
Tirtiaux, A. and Gibon, V. (1997), La désodorisation ... à la carte, *Oléagineux Corps Gras Lipides/OCL*, **4**, 45–51.
Tollenaar, F.D. and Hoekman, H. (1964), Die Bleichung von Ölen mittels Bleicherden, mit besonderer Berücksichtigung von Palmöl, *Fette Seifen Anstrichm.*, **66**, 430–437.
Verleyen, T. et al. (2002), Oxidation at elevated temperatures: competition between α-tocopherol and unsaturated triacylglycerols, *Eur. J. Lipid Sci. Technol.*, **104**, 228–233.
Wecker, E. (1923), Verfahren zur Abtrennung flüchtiger Stoffe von schwer-oder nichtflüchtigen, German Patent 397 332.
Weineck, P. (1940), Vakuum durch Strahlsäuger, *Fette u. Seifen*, **47**, 65–72.
White, F.B. (1951), Better oil from new or old deodorizers, *J. Am. Oil Chem. Soc.*, **28**, 438–440.
White, F.B. (1956), Deodorization, *J. Am. Oil Chem. Soc.*, **33**, 495–506.
Wiedermann, L.H. (1981), Degumming, refining and bleaching of soybean oil, *J. Am. Oil Chem. Soc.*, **58**, 159–166.
Willems, M.A.G. and Padley, F.B. (1985), Palm oil: quality requirements from a customer's point of view, *J. Am. Oil Chem. Soc.*, **62**, 454–459.
Wolff, R.L. (1993), Heat-induced geometrical isomerization of α-linolenic acid: effect of temperature and heating time on the appearance of individual isomers, *J. Am. Oil Chem. Soc.*, **70**, 425–430.
Wolff, R.L. et al. (1996), Participation of the cis-12 ethylenic bond to cis-trans isomerization of the cis-9 and cis-15 ethylenic bonds in heated α-linolenic acid, *J. Am. Oil Chem. Soc.*, **73**, 327–332.
Young, F.V.K. (1990), Physical refining, in *Edible Fats and Oils Processing: Basic Principles and Modern Practices*, Erickson, D.R., Ed., American Oils Chemists' Society, Champaign, IL, 124–135.
Young, F.V.K. (1994), Modification processes, in *The Lipid Handbook*, Gunstone, F.D., Harwood, J.L., and Padley, F.B., Eds., Chapman & Hall, London, 277–285.
Zajíc, J. et al. (1968), Änderung verschiedener Eigenschaften von Fetten während der Desodorierung, *Fette Seifen Anstrichm.*, **70**, 860–863.
Zehnder, C.T. (1976), Deodorization 1975, *J. Am. Oil Chem. Soc.*, **53**, 364–369.
Zehnder, C.T. (1995), Deodorization, in *Practical Handbook of Soybean Processing and Utilization*, Erickson, D.R., Ed., AOCS Press and United Soybean Board, Champaign, IL and St. Louis, MO, 239–257.

3.10 HACCP for oils and fats supply chains

The oils and fats industry has been very successful over the last two decades in producing ever-increasing volumes at lower and lower costs. Economies of scale in both primary production and processing have contributed much to this. However, this successful system proved to be sensitive to serious, large-scale incidents as, for example, the toxic olive oil syndrome in Spain (Fondo de Investigaciones Sanitarias, 1990) and the dioxin contamination of feed fats in Belgium. Many of these widely publicised incidents had their origin in contamination of

raw materials for feed and/or food production and led to substantial material and immaterial losses.

Consequently, the EU authorities were forced to take a broader approach to food safety, which traditionally focused on the slaughterhouses (Woodgate and Veen, 2004) and the catering sector, and they realised that the globalisation of the food and feed chains required an international approach. The basis for this approach is the Sanitary and Phytosanitary Measures Agreement (the SPS Agreement) reached by the World Trade Organisation in 1994. This agreement requires member states to base their food safety measures on internationally adopted food safety standards and more specifically on those listed in the Codex Alimentarius.

The food safety system of the Codex Alimentarius is based on scientific principles and, in particular, on risk assessment of all potential hazards throughout the entire food chain from primary production to final consumption, highlighting key hygiene controls at each stage.

The system of the Codex originates from the NASA space programme in the 1960s, when the Pillsbury Company in the U.S. was asked to produce food for use by the NASA space missions that was not contaminated by bacteria, toxins, or viruses. This led to the development of a system that provides a structured, systematic approach to the identification of hazards and methods to control them. The Pillsbury Company presented this system in 1971 under the name of HACCP (Hazard Analysis Critical Control Points) at the first American National Conference for food protection. Since then, the HACCP system was further developed in the food processing industry to eliminate or reduce food-borne hazards. The positive experience with this system in the food processing industry led to an internationally agreed common approach. The system was reviewed by the UN's Food and Agriculture Organization (FAO) and World Health Organization (WHO) in 1995 and endorsed by the Codex Alimentarius Food Hygiene Commission in 1996.

The HACCP system is used now worldwide. In the EU, all food and feed companies are required to implement the HACCP system to ensure that all potential hazards are identified and controlled (EU, 2004; EU, 2005b).

3.10.1 Principles of the HACCP system

The HACCP system consists of the following seven basic principles as outlined by the Codex Alimentarius Commission of the joint FAO/WHO Food Standards Programme (Codex, 1969):

1. Conduct a hazard analysis.
2. Identify the Critical Control Points (CCPs).
3. Establish critical limits.
4. Establish a system to monitor the control of the CCP.
5. Establish the corrective action to be taken when monitoring indicates that a particular CCP is not under control.
6. Establish procedures to verify that the HACCP system is working effectively.
7. Establish documentation concerning all procedures and records appropriate to these principles and their application.

3.10.2 Application of the HACCP principles

The first step of a HACCP study is the definition of the scope of the study. It should describe the food product and which segment of the food chain is involved. When defining this, one should remember that the food industry is responsible for the safety of the end product as sold. This means in practice that the last segments in the supply chain should have access to all the required safety information, to be able to judge whether the preceding steps have controlled all the hazards, so that they can take the required measures themselves if this is not the case. For the oils and fats supply chains, which consist of many steps from farm to table, it is nearly always necessary to extend the study to include primary production, storage, and raw product processing because quite a number of potential food safety hazards originate from these initial stages in the supply chain.

Further, before a HACCP study is set up, it is important to identify and describe the specific codes of good working practices in each segment and to establish whether these practices are in place. The General Principles of Food Hygiene of the Codex Alimentarius (Codex, 1969) provide a baseline structure for these codes. The specific codes of good working practice for a certain segment are mostly set up and agreed by branch organisations for that segment, such as the EC Seed Crushers and Oil Processors Federation (FEDIOL, Féderation Européenne des Industries Oléagineuses) for the European oil and protein meal industry. Their codes of good working practice can be found on the website: www.fediol.be.

When the scope of the HACCP study and the relevant working practices have been identified and verified, one can start on a rigorous hazard analysis and risk assessment. The aim of this analysis is to identify which hazards are of such a nature that their elimination or reduction to acceptable levels is essential to the production of a safe food. Risk analysis and assessment consists basically of the following steps:

- Preparation of a flow chart that covers all the elements of the supply chain from farm to the end customer, whereby the end customer may be the final oil or food processor that supplies the product to the retailer.
- Description of the food safety hazards that may occur in the various segments of the supply chain. A food safety hazard, as defined by the Codex Alimentarius, is a biological, chemical, or physical agent in a food product, or a condition of a food product, with the potential to cause an adverse health effect

- The risk assessment of the hazards, which is determined according to the probability of their occurrence and the severity of their adverse health effects.

The probability of the hazard occurring or its risk level can be assessed by experts as very low, low, medium, or high. FEDIOL quantifies these risk levels in their risk analysis of oilseed supply chains as published on their website as follows:

- Very low: the hazard has never occurred, but may occur.
- Low: the hazard occurs on average every 5 years.
- Medium: the hazard occurs on average once a year.
- High: the hazard occurs more often than once a year.

The assessment of the severity of the adverse health effects or "seriousness" of a hazard should be guided by toxicological and legal expertise. FEDIOL classifies the adverse health effects as low, medium, or high.

The risk level and the "seriousness" of each hazard together give the risk classification of the hazard. FEDIOL recognises four risk classes: 1, 2, 3, and 4. For example, hazards that occur more than once a year and that have been classified as having medium or severe adverse health effects, are classified as risk class 4.

The risk class of a hazard then determines which control measure must be taken:

Risk class 1:	No control measure needed.
Risk class 2:	No control measure needed, but periodical evaluation is required.
Risk class 3:	Risk to be controlled by verifiable measures, such as Good Manufacturing Practice (GMP).
Risk class 4:	Risk to be controlled by a measure that is specifically designed to control the risk (CCP).

Further general guidelines for the application of HACCP principles can be found in the Codex Alimentarius. It also describes a logical sequence of 12 steps for the application of HACCP.

3.10.3 Hazards in the coconut oil supply chain

A risk assessment study of coconut oil is discussed below as an example of hazards in the supply chain.

The EU annually imports an average of over 700,000 tonnes of crude coconut oil for the production of fully refined coconut oil. The crude oil mainly originates from the Philippines and Indonesia, where it is extracted from locally produced copra.

Most coconuts are produced by smallholders with less then five hectares of land (Ranasinghe, 1999). The farmers prepare copra from the nuts by cracking the nut and drying of the wet copra. It is important to reduce the moisture content of the kernel of the freshly opened nut from the original 50% to 6% as fast as practically possible. The high moisture content and the presence of protein and sugar make the fresh kernel very susceptible to attack by fungi, enzymatic hydrolysis, and oxidation. This can lead to the formation of aflatoxin and free fatty acids, and the development of rancidity.

In most parts of the Philippines and Indonesia, the smallholders use a dryer or kiln with direct heat and smoke generated from burning the husks, shells, and firewood available near the kiln at little cost. The copra produced by this method generally has moisture levels between 8 and 15%. The safe moisture level for hot air dried copra is below 8%, and for smoke dried copra it is below 11%. The higher moisture level tolerable for smoke-dried copra is due to smoke particles inhibiting mould growth (Ranasinghe, 1999).

Before milling, the dried copra is stored in copra warehouses to provide a buffer stock for the mills. During storage, the copra is further dried and blended to ensure a moisture content that is acceptable to the mills. During storage, copra must be protected from attack by pests. After transport to the mill, the oil is extracted from the copra by either a full press extraction, giving a cake with 6 to 10% oil content, or by prepressing and solvent extraction. The oil is then transported to the storage facility at the port of shipment, transported by sea to Europe, unloaded and stored at the port of arrival, then transported to a refinery. The refinery processes the oil by either alkaline or physical refining, and it is then transported to the food processor. (The flow chart of the coconut oil supply chain for food use is shown in Figure 3.22.)

On the basis of this flow chart, the HACCP team should now draw up a list of all possible hazards for each step of the flow chart, followed by a risk assessment for each hazard. This gives the following overview of the main hazards of the coconut oil supply chain and their risk assessment.

3.10.3.1 Polycyclic aromatic hydrocarbons (PAHs)

The smoke generated by the combustion of husks and firewood inhibits mould growth on the copra at moisture contents of up to 11%, but it also contains a whole range of polycyclic aromatic hydrocarbons (PAHs), which will dissolve into the coconut oil on milling (Ranasinghe, 1999).

There are two groups of PAHs: light PAHs, as illustrated in Figure 3.23, and heavy PAHs, shown in Figure 3.24. The latter, of which benzo[a]pyrene is an example, are considered to be carcinogenic. According to the Scientific Committee on Food, benzo[a]pyrene can be used as a marker for the occurrence and effect of carcinogenic PAHs in Food (Regulation (EC) no.208/2005) (EU, 2005a). The EU legislation has set a limit of max 2 ppb for benzo[a]pyrene in oils and fats for direct human consumption (Regulation (EC) no.208/2005). Since most

crude coconut oils from the Philippines and Indonesia exceed this limit, the likelihood that the crude oil contains an excessive amount of a hazardous material is very high.

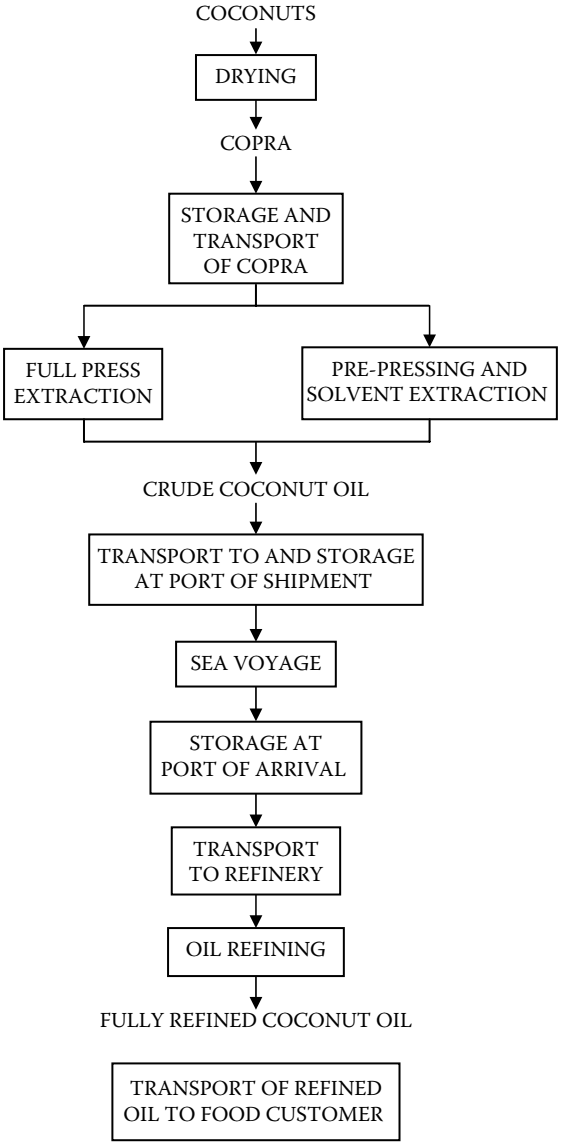


FIGURE 3.22 Flow chart for coconut oil for food purposes.

PAHs in crude coconut oil have to be classified as a serious hazard, which occurs frequently (Risk class 4). Therefore, a critical control point has to be set up to eliminate the heavy PAHs. It is not likely that the smallholders will be able to avoid the contamination of the copra with PAHs, which means that the PAHs must be removed further downstream in the supply chain.

Heavy PAHs can be removed in the refining operation by treatment with activated carbon. The light PAHs can normally be removed to a large extent by deodorisation (Biernoth and Rost, 1968). For a proper control of the PAHs, it is recommended to measure the PAHs in the crude oil and remove them by means of active carbon adsorption and deodorisation under validated process conditions. For both the active carbon treatment and the deodorisation, it is essential to maintain the process conditions within the critical limits which have been established during the validation process.

3.10.3.2 Dioxins

The smallholders' drying process also entails the use of waste material under uncontrolled conditions; this can lead to the formation of dioxins, which will dissolve into the oil during the milling operation (FEDIOL, 2006). During refining, activated bleaching earth is used which may contain dioxin as well, which will also dissolve into the oil during the bleaching step. EU legislation has set limits for the maximum concentrations of dioxin in vegetable oils. Regulation 466/2001/EC (EU, 2001a) limits the dioxin content in oils for direct human consumption to a maximum value of 0.75 ng WHO-PCDD/F-TEQ/kg.

Dioxins in crude coconut oil have to be considered as a serious hazard, but the likelihood of its occurrence is low. This hazard, therefore, is controlled by verifiable measures, such as the application of codes of practice (Risk class 3).

FEDIOL has developed a code of practice on the purchasing specifications of fresh bleaching earth for oil refining, which include a maximum of 1 pg WHO-TEQ/g. It is recommended to source fresh bleaching earth from suppliers that meet the FEDIOL specifications for fresh bleaching earth.

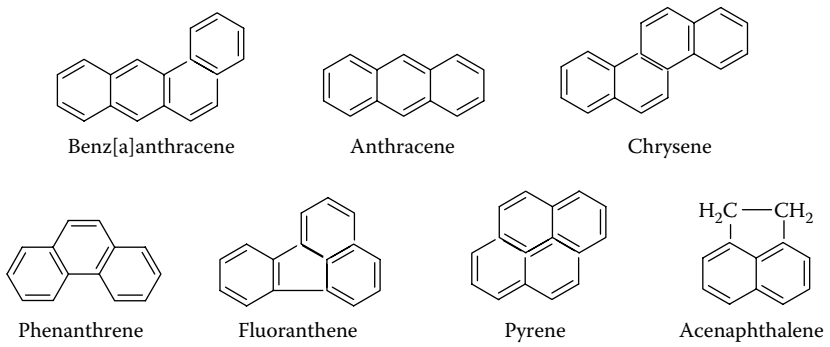


FIGURE 3.23 Structural formulae of light PAHs.

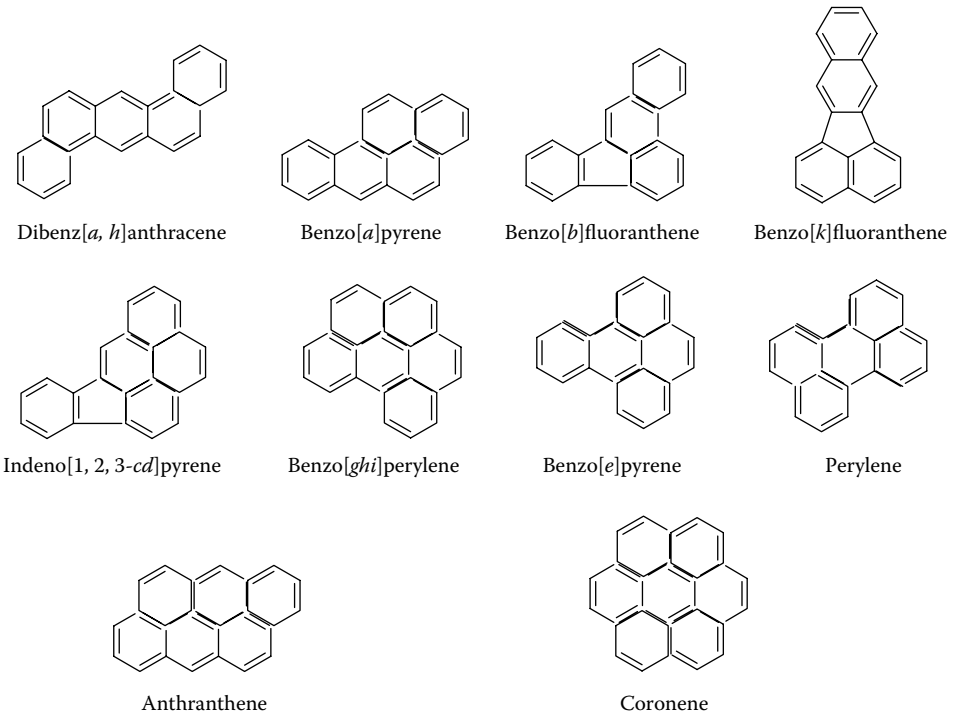


FIGURE 3.24 Structural formulae of heavy PAHs.

Monitoring dioxins in crude coconut oil by the Dutch Commodity Board for Margarine, Fats and Oils has shown that the dioxin content was lower than a specified maximum value of 0.22 ng WHO PCCD/F –TEQ/kg. The likelihood of the dioxin content of the crude coconut oil being higher than the maximum permitted under the EU legislation is considered to be very low. However, it is recommended to evaluate this periodically. Dioxins present in the crude oil can be removed during the deodorisation step under validated process conditions (Rohrbeck, 2005).

3.10.3.3 Aflatoxins

If poorly dried copra is stored for longer periods, or if it is not protected from humidity during transport and further storage, there is a possibility that aflatoxins will form. Aflatoxins are a group of toxic chemicals produced by the *Aspergillis* mould. Aflatoxins found in the copra are B1, B2, G1, and G2. Aflatoxin B1, which is the most abundant, is extremely poisonous. When contaminated copra is milled for oil, the aflatoxin passes partially into the oil (Ranasinghe, 1999). Aflatoxin B1 in crude coconut oil must be seen as a serious hazard, with a low likelihood of occurring (Risk class 3) This may not be the case when the copra originates from another country than the Philippines. In that case, aflatoxin may fall into Risk class 4 (thus, a CCP). The hazard in Risk class 3 must be controlled by verifiable measures such as good refining practice.

Aflatoxins are soluble in water, and chemical refining followed by bleaching and deodorisation remove all aflatoxins from the oil (Parker and Melnick, 1966). As physical refining normally does not contain a water-washing step, it is not certain that all aflatoxin is removed by this refining process. This means that the physical refining process as performed at the specific site must be examined to assess its ability to remove different levels of aflatoxin B1 completely.

3.10.3.4 Pesticide residues

During storage of copra, losses due to attack by insect pests and rats have to be avoided. Warehouses and returnable empty bags are fumigated. However, details of the type of insecticides and rodenticides are often not known (Ranasinghe, 1999). The countries exporting coconut oil, like the Philippines and Indonesia, work with positive lists for the use of pesticides during cultivation and storage, which may be in conflict with European legislation with respect to certain substances. In the EU, some pesticides are banned and others are only allowed if specified residue levels are not exceeded, Directive 642/1990 (EU, 1990). Pesticides in crude coconut oil can be a serious hazard, but the likelihood of its occurrence is low or very low. The Risk class is, thus, either 3 or 2.

When it is not known which pesticides were used during the cultivation and the storage, it will be difficult to eliminate this hazard since over 1000 active substances are used worldwide. Various strategies can be employed: FEDIOL members share analytical results on pesticide contamination of imported raw materials, while others monitor the oil with a pesticide screen and/or monitor the agricultural practices in the producing countries.

The European Legislation sets Maximum Residue Levels (MRLs) for raw agricultural commodities; they have been tabulated for a number of oilseeds and corn germ in Table 3.17. MRLs for processed products, such as crude vegetable oil, have to be derived from the former by taking into account the concentration or dilution factor that results from the processing. A certain concentration of fat-soluble pesticides in the oil, thereby, is allowed, but the magnitude of the transfer factors is usually not known. Pesticides that are not listed in Annex 1 to Directive 91/414 (EU, 1991) may not be used and products containing residues of such pesticides may not be processed. Pesticides that have not been mentioned in the EC Directives have not yet been regulated on an EU level.

As a preventive measure, the oil must be monitored periodically for the presence of pesticides. Most pesticides can be removed by refining (Gooding, 1966; Thomas, 1976), but it is very important to have the refining operations validated on site for the removal of any pesticides found.

3.10.3.5 Mineral oils

Copra is sometimes dried by spreading it out on a road and, thus, may pick up spilled mineral oil, which during milling will be concentrated in the crude oil (FEDIOL, 2006). Faulty oil mill equipment and transshipment of the oil may lead to contamination of the product with hydraulic oils or lubricants. Contamination with mineral oil is a serious hazard, but the likelihood of its occurrence is very low to low (Risk class 2 to 3).

As a preventive measure, the consumption of hydraulic oils and lubricants should be monitored closely and deviations from agreed standards should be investigated immediately. Food grade hydraulic oils and lubricants should be used in the oil mills and in the hydraulic systems of the portable pumps sometimes used for loading and unloading of ships. However, it is not always easy to verify whether all these measures are in place. In that case, it is recommended to check all incoming crude oil at the port of arrival for the presence of mineral oil; the port is then a CCP.

3.10.3.6 Previous cargoes

The storage tank farms in the ports of shipment and the ports of arrival are not always dedicated to edible oils and fats, which can lead to contamination of the edible oils by previous cargoes that may not be suitable for consumption. The tanks should be dedicated to the storage of edible oils or at least to foodstuffs. The tanks should be properly cleaned between cargoes, unless quality control personnel deem cleaning to be unnecessary.

Bulk transport of edible oils by sea is mostly by parcel tankers, which transport not only edible oils, but also a whole range of other products, including many chemicals. This can lead to unacceptable contaminations. Sea transport of edible oils from Asia to Europe is carried out under FOSFA (Federation of Oils, Seeds and Fats Associations) contracts. These contracts prescribe a whole system of control measures for the previous cargoes. For import into Europe, only foodstuffs that are named in an EU list of acceptable previous cargoes are permitted as the last cargo, Regulation 852/2004 (EU, 2004). The second and third last cargoes have not been regulated for sea transport, but the master of the ship must keep records of these cargoes, which have to be checked by accredited inspection bodies in both the port of loading and the port of shipment. In some cases and certainly if products on a list of banned previous cargoes have been transported as the second and the third previous cargoes, the cargo must be checked for the presence of these banned materials as a preventive measure.

FEDIOL has also drawn up a code of practice for the transport in bulk of oils and fats into or within the EU. This code gives more detailed rules for the transport of oils and fats than Regulation 852/2004 (EU, 2004) and basically requires that transport in bulk by road or rail tankers, by barge and in drums must be fully dedicated to transport of foodstuffs. The FEDIOL code also sets rules for bulk transport by short sea voyage into or within the territories of the EU and for transport in bulk by ocean going vessels.

The transport of coconut oil by road tankers in the countries of origin is not regulated, so there is a risk that the tankers are also used for transport of hazardous liquids that can lead to contamination. As control measure, the transport tankers should be sealed and dedicated to vegetable oils.

3.10.3.7 Adjacent cargoes

The use of bulking stations and parcel tankers for simultaneous transport and storage of edible oils and hazardous liquids can lead to contamination through the use of common pipelines during loading and unloading and as a result of leakage from adjacent tanks on the ship during transport.

The risk of contamination via the pipelines should be controlled by the use of dedicated pipelines for vegetable oils during transfer of the oils. The owner of the oil should check the stowage list for adjacent loads in the port of arrival, and if hazardous liquids are present, they should arrange an analytical control of the oil for the presence of those hazardous liquids.

3.10.3.8 Thermal heating fluids

During storage, transport by sea vessel, and processing, it is possible that a system failure leads to the accidental contamination of the vegetable oil by a thermal heating fluid (THF), most of which are not food grade. Contamination with thermal heating fluid is a serious hazard, but the likelihood of it occurring is considered to be a medium risk (Risk class 4). Therefore, a critical control point should be set up to reduce the risk that coconut oil is contaminated with THF.

TABLE 3.17 European Union's maximum residue levels in oilseeds and corn germ expressed as mg/kg, version of 2 February 2006

Pesticide	Codex Nr	CN	CS	Gs	GN	LS	MG	MS	RS	SA	SB	SU
mesotrione		0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
abamectine	177	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02
acephate	95	0.02	0.05	0.05	0.05	0.05	0.02	0.05	0.05	0.05	0.05	0.05
aldicarb	117	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
aldrin	1	0.01	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02
amitraz	122	0.05	1	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
amitrole[P]	79	0.01	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02
aramite		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
atrazine		0.1	0.1	0.1	0.1	0.1		0.1	0.1	0.1	0.1	0.1
azimsulfuron[P]		0.02	0.1	0.1	0.1	0.1	0.02	0.1	0.1	0.1	0.1	0.1
azinphos-ethyl	68	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
azocyclotin	129	0.1	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
azoxystrobin		0.1	0.05	0.05	0.05	0.05	0.05	0.05	0.5	0.05	0.5	0.05
barban		0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
benalaxyl	155	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
benfuracarb		0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
benomyl	69	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1
bentazone[P]	172	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
bifenthrin	178	0.05	0.1	0.1	0.1	0.1	0.05	0.1	0.1	0.1	0.1	0.1
binapacryl	3	0.05	0.05	0.05	0.05	0.05	0.01	0.05	0.05	0.05	0.05	0.05
bitertanol	144	0.1	0.1	0.1	0.1	0.1	0.05	0.1	0.1	0.1	0.1	0.1
bromide inorganic	47						50					
bromophos-ethyl	5	0.05	0.05	0.05	0.05	0.05		0.05	0.05	0.05	0.05	0.05
bromopropylate	70	0.05	0.1	0.1	0.1	0.1	0.05	0.1	0.1	0.1	0.1	0.1
bromoxynil		0.05	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
camphechlor (toxaphene)	71	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
captafol	6	0.02	0.02	0.02	0.02	0.02	0.05	0.02	0.02	0.02	0.02	0.02
carbaryl	8						0.5					0.1
carbendazim	72	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1
carbofuran	96	0.02	0.1	0.1	0.1	0.1	0.02	0.1	0.1	0.1	0.1	0.05
carbon disulfide	9						0.1					
carbon tetrachloride	10						0.1					
carbosulfan	145	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
carfentrazone-ethyl		0.01	0.02	0.02	0.02	0.02	0.05	0.02	0.02	0.02	0.02	0.02
chlorbenside		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
chlorbufam		0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
chlordane	12	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
chlorfenson		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
chlormequat (chloride)	15	0.1	0.1	0.1	0.1	0.1	0.05	0.1	0.1	0.1	0.1	0.1
chlorobenzilate	16	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
chlorothalonil	81	0.01	0.01	0.01	0.05	0.01	0.01	0.01	0.01	0.01	0.01	0.01
chloroxuron		0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
chlorpropham	201	0.05	0.1	0.1	0.1	0.1	0.02	0.1	0.1	0.1	0.1	0.1
chlorpyrifos	17	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
chlorpyrifos-methyl	90	0.05	0.05	0.05	0.05	0.05	3	0.05	0.05	0.05	0.05	0.05
chlozolinate		0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
cinidon-ethyl[P]			0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
clofentezine	156	0.05	0.05	0.05	0.05	0.05		0.05	0.05	0.05	0.05	0.05
cyazofamid[P]		0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
cyclanilida[P]			0.2	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
cyfluthrin	157	0.02	0.02	0.02	0.02	0.02	0.05	0.02	0.05	0.02	0.02	0.02
Cyhalofop-butyl[P]			0.05	0.05	0.05	0.05	0.02	0.05	0.05	0.05	0.05	0.05
cyhexatin	67	0.1	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
cypermethrin	118	0.05	0.2	0.05	0.05	0.2	0.05	0.05	0.2	0.05	0.05	0.2
cyromazine	169	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
2,4-D[P]	20	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
daminozide	104	0.05	0.05	0.05	0.05	0.05	0.02	0.05	0.05	0.05	0.05	0.05
DDT	21	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
deltamethrin	135	0.05	0.05	0.05	0.05	0.05	1	0.05	0.1	0.05	0.05	0.05
diallate		0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
diazinon	22	0.05	0.05	0.05	0.05	0.05	0.02	0.05	0.05	0.05	0.05	0.05

(Continued)

TABLE 3.17 Continued

Pesticide	Codex Nr	CN	CS	Gs	GN	LS	MG	MS	RS	SA	SB	SU
1,2-dibromomethane	23	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
1,1-dichloro-2,2-bis(4-ethyl-phenylalanine)		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
1,2-dichloroethane	24	0.01	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02
dichlorprop		0.05	0.05	0.05	0.05	0.05		0.05	0.05	0.05	0.05	0.05
dichlorvos	25						2					
dicofol	26	0.05	0.1	0.05	0.05	0.05	0.02	0.05	0.05	0.05	0.05	0.05
dieldrin		0.01	0.02	0.02	0.02	0.02		0.02	0.02	0.02	0.02	0.02
dimethenamide		0.01	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02
dimethoate	27	0.05	0.05	0.05	0.05	0.05	0.02	0.05	0.05	0.05	0.05	0.05
dinoseb		0.05	0.05	0.05	0.05	0.05	0.01	0.05	0.05	0.05	0.05	0.05
dinoterb		0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
dioxathion	28	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
diphenylamine	30	0.05	0.05	0.05	0.05	0.05		0.05	0.05	0.05	0.05	0.05
diquat (dibromide)[P]	31		0.1	0.1	0.1	5	1	0.5	2	0.1	0.2	1
disulfoton	74	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
DNOC		0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
endosulfan	32	0.1	0.3	0.1	0.1	0.1	0.05	0.1	0.1	0.1	0.5	0.1
endrin	33	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
esfenvalerate		0.02	0.05	0.05	0.05	0.05	0.02	0.05	0.05	0.05	0.05	0.05
RR + SS isomers												
esfenvalerate		0.02	0.05	0.05	0.05	0.05	0.02	0.05	0.05	0.05	0.05	0.05
RS + SR isomers												
ethephon	106	0.1	2	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
ethofumesate[P]			0.1	0.1	0.1	0.1	0.05	0.1	0.1	0.1	0.1	0.1
ethoxysulfuron		0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
ethylene oxide		0.1	0.2	0.2	0.2	0.2	0.02	0.2	0.2	0.2	0.2	0.2
famoxadone[P]		0.02	0.05	0.05	0.05	0.05	0.02	0.05	0.05	0.05	0.05	0.05
fenamidone		0.02	0.05	0.05	0.05	0.05	0.02	0.05	0.05	0.05	0.05	0.05
fenamiphos	85	0.02	0.05	0.05	0.05	0.05	0.02	0.05	0.05	0.05	0.05	0.05
fenarimol	192	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
fenbutatin oxide	109	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
fenchlorphos	36	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
fenhexamid[P]		0.05	0.1	0.1	0.1	0.1	0.05	0.1	0.1	0.1	0.1	0.1
fenpropimorph	188	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
fentin	40	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
fenvalerate	119	0.02	0.05	0.05	0.05	0.05	0.02	0.05	0.05	0.05	0.05	0.05
flazasulfuron		0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
florasulam[P]		0.1	0.1	0.1	0.1	0.1	0.01	0.1	0.1	0.1	0.1	0.1
flucythrinate	152	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
flufenacet		0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
flupursulfuron-methyl[P]		0.02	0.05	0.05	0.05	0.05	0.02	0.05	0.05	0.05	0.05	0.05
fluroxypyr[P]		0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
flurtamone		0.02	0.05	0.05	0.05	0.05	0.02	0.05	0.05	0.05	0.05	0.05
foramsulfuron[P]		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
formothion	42	0.05	0.05	0.05	0.05	0.05	0.02	0.05	0.05	0.05	0.05	0.05
fosthiazate		0.02	0.05	0.05	0.05	0.05	0.02	0.05	0.05	0.05	0.05	0.05
furathiocarb		0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
glyphosate	158	0.1	10	0.1	0.1	10	0.1	10	10	0.1	20	0.1
alpha-HCH		0.01	0.02	0.02	0.02	0.02		0.02	0.02	0.02	0.02	0.02
beta-HCH		0.01	0.02	0.02	0.02	0.02		0.02	0.02	0.02	0.02	0.02
gamma-HCH		0.01	0.02	0.02	0.02	0.02		0.02	0.02	0.02	0.02	0.02
HCH (hexachloro-cyclohexane)		0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
heptachlor	43	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01
hexachlorobenzene	44	0.01	0.02	0.02	0.02	0.02		0.02	0.02	0.02	0.02	0.02
hexaconazole	170	0.05	0.05	0.05	0.05	0.05	0.02	0.05	0.05	0.05	0.05	0.05
hydrogen cyanide	45						15					
hydrogen phosphide	46						0.1					
imazalil	110	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
imazamox[P]		0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
iodosulfuron-methyl		0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02

(Continued)

TABLE 3.17 Continued

Pesticide	Codex Nr	CN	CS	Gs	GN	LS	MG	MS	RS	SA	SB	SU
ioxynil		0.05	0.1	0.1	0.1	0.1	0.05	0.1	0.1	0.1	0.1	0.1
iprodione	111	0.02	0.02	0.02	0.02	0.5	0.02	0.02	0.5	0.02	0.02	0.5
iprovalicarb[P]		0.05	0.1	0.1	0.1	0.1		0.1	0.1	0.1	0.1	0.1
isoproturon[P]			0.1	0.1	0.1	0.1	0.05	0.1	0.1	0.1	0.1	0.1
isoxaflutole		0.05	0.1	0.1	0.1	0.1	0.05	0.1	0.1	0.1	0.1	0.1
kresoxim-methyl[P]		0.1	0.1	0.1	0.1	0.1	0.05	0.1	0.1	0.1	0.1	0.1
lambda-cyhalothrin		0.05	0.02	0.02	0.02	0.02		0.02	0.02	0.02	0.02	0.02
lindane	48	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
linuron[P]		0.05	0.1	0.1	0.1	0.1	0.05	0.1	0.1	0.1	0.1	0.1
malathion	49						8					
maleic hydrazide	102	0.2	0.5	0.5	0.5	0.5	0.2	0.5	0.5	0.5	0.5	0.5
maneb		0.1	0.1	0.1	0.1	0.1	0.05	0.1	0.5	0.1	0.1	0.1
mecarbam	124	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
mecoprop		0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
mepanipyrim		0.01	0.05	0.05	0.05	0.05	0.01	0.05	0.05	0.05	0.05	0.05
mercury compounds		0.01	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02
metalaxyl[P]	138	0.05	0.1	0.1	0.1	0.1	0.02	0.1	0.1	0.1	0.1	0.1
methacrifos	125	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
metamidophos	100	0.01	0.1	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
methidathion	51	0.05	0.02	0.02	0.02	0.02	0.02	0.02	0.05	0.02	0.02	0.02
methomyl	94	0.05	0.1	0.05	0.1	0.05	0.05	0.05	0.05	0.05	0.1	0.05
methoxychlor		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
methyl bromide			0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
metsulfuron[P]		0.05	0.1	0.1	0.1	0.1	0.05	0.1	0.1	0.1	0.1	0.1
molinate		0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
monolinuron		0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
myclobutanil	181	0.05	0.05	0.05	0.05	0.05		0.05	0.05	0.05	0.05	0.05
nitrofen	140	0.01	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02
oxadiargyl[P]		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
oxasulfuron[P]		0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
oxydemeton-methyl	166	0.02	0.05	0.05	0.05	0.05	0.02	0.05	0.05	0.05	0.05	0.05
paraquat	57	0.05	0.05	0.05	0.05	0.05		0.05	0.05	0.05	0.05	0.05
parathion	58	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
parathion-methyl	59	0.02	0.05	0.05	0.05	0.05	0.02	0.05	0.05	0.05	0.05	0.05
penconazole	182	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
pendimethalin[P]		0.05	0.1	0.1	0.1	0.1	0.05	0.1	0.1	0.1	0.1	0.1
permethrin	120	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
phorate	112	0.05	0.05	0.05	0.1	0.05	0.05	0.05	0.05	0.05	0.05	0.05
phosphamidon	61						0.05					
picoxystrobin		0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
pirimiphos-methyl	86	0.05	0.05	0.05	0.05	0.05	5	0.05	0.05	0.05	0.05	0.05
prochloraz	142						0.05					
procymidone	136	0.05	0.05	0.05	0.05	0.05	0.02	0.05	1	0.05	1	1
profenofos	171	0.05	2	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
prohexadione[P]		0.05	0.1	0.1	0.1	0.1	0.05	0.1	0.1	0.1	0.1	0.1
propham	183	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
propiconazole	160	0.05	0.1	0.1	0.2	0.1	0.05	0.1	0.1	0.1	0.1	0.1
propoxur	75	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
propoxycarbazone		0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
propyzamide		0.02	0.05	0.05	0.05	0.05		0.05	0.05	0.05	0.05	0.05
prosulfuron[P]			0.1	0.1	0.1	0.1	0.02	0.1	0.1	0.1	0.1	0.1
pymetrozine[P]		0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
pyraclostrobine		0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
pyrazophos	153	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
pyrethrins	63						3					
pyridate[P]		0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
quinalphos		0.05	0.05	0.05	0.05	0.05		0.05	0.05	0.05	0.05	0.05
quinoxyfen		0.02	0.05	0.05	0.05	0.05	0.02	0.05	0.05	0.05	0.05	0.05
quintozene	64	0.02	0.02	0.02	0.05	0.02	0.02	0.02	0.02	0.02	0.02	0.02
resmethrin		0.2	0.2	0.2	0.2	0.2	0.05	0.2	0.2	0.2	0.2	0.2
silthiofam		0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05

(Continued)

TABLE 3.17 Continued

Pesticide	Codex Nr	CN	CS	Gs	GN	LS	MG	MS	RS	SA	SB	SU
spiroxamine[P]		0.05	0.05	0.05	0.05	0.1	0.05	0.05	0.05	0.05	0.05	0.05
sulfosulfuron[P]			0.1	0.1	0.1	0.05	0.05	0.1	0.1	0.1	0.1	0.1
2,4,5-T	121	0.05	0.05	0.05	0.05	0.05		0.05	0.05	0.05	0.05	0.05
tecnazene	115	0.05	0.05	0.05	0.05	0.01	0.05	0.05	0.05	0.05	0.05	0.05
TEPP		0.01	0.01	0.01	0.01	0.05		0.01	0.01	0.01	0.01	0.01
thiabendazole	65	0.1	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
thifensulfuron-methyl		0.05	0.05	0.05	0.05	0.2	0.05	0.05	0.05	0.05	0.05	0.05
triademefon	133	0.2	0.2	0.2	0.2	0.05	0.1	0.2	0.2	0.2	0.2	0.2
triasulfuron		0.05	0.05	0.05	0.05	0.02	0.05	0.05	0.05	0.05	0.05	0.05
triazophos	143	0.02	0.02	0.02	0.02	0.1	0.02	0.02	0.02	0.02	0.02	0.02
tridemorph		0.1	0.1	0.05	0.1	0.05	0.05	0.1	0.1	0.1	0.1	0.1
trifloxystrobin		0.02	0.05	0.05	0.05	0.05	0.02	0.05	0.05	0.05	0.05	0.05
triforine	116	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
trimethyl-sulfonium		0.02	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	10	0.05
vinclozolin	159	0.05	1	0.05	0.05	0.05	0.05	0.05	1	0.05	0.05	0.05
zoxamide		0.02	0.05		0.05		0.02	0.05	0.05	0.05	0.05	0.05

Note: CN, coconuts; CS, cottonseed; GS, grape seed; GN, groundnuts; LS, linseed; MG, corn germ; MS, mustard seed; RS, rapeseed; SA, safflower seed; SB, soya beans; SU, sunflower seed; [P], provisional.
Source: Courtesy Productschap Margarine, Vetten en Oliën, www.mvo.nl.

Since 1997, steam is the only heating medium used by FEDIOL members in their seed crushing and oil processing plants (see FEDIOL's thermal heating oils code of practice). FOSFA contracts for sea transport also exclude the use of thermal heating oils. The buyer of the oil, however, should check the possible use of thermal heating oils in the port of loading and/or on the sea vessel and impound the cargo if THF has been used; this is a CCP.

3.10.3.9 Cleaning agents

The road tankers used for transport of coconut oil in bulk, both in the EU and in the countries of origin, should be properly cleaned between two cargoes. If wet cleaning is applied, there is a risk that nonfood-grade cleaning agents are used, especially when the cleaning station is not dedicated to vegetable oils. The same is true for the tanks of sea vessels.

Contamination with cleaning agents is rated as a medium hazard, and the probability of this occurring is also medium (Risk class 3). Contamination must be controlled by means of verifiable preventive measures. The cleaning of road tankers should be carried out in food grade-approved cleaning stations. The cleanliness of ship tanks should be checked by accredited inspection bodies before loading (FOSFA procedure).

3.10.3.10 Processing aids

All processing aids that come directly or indirectly into contact with the oil during milling and refining must be food grade quality. Contamination of oil with nonfood-grade processing aids is rated as a medium hazard, and the likelihood of this happening is also medium (Risk class 3). Possible contamination of this type must be controlled by means of verifiable preventive measures.

The purchasing contracts of these processing aids require a validated and verified food grade certificate (see also FEDIOL code of practice for manufacturing of feed materials from oilseed crushing and refining).

3.10.4 Critical control points for the coconut oil supply chain

After analysis, as described in the preceding section, a list can be made of the hazards that must be controlled by means of verifiable preventive measures. The hazards, which call for a CCP, are the possible use of thermal heating oils, previous/adjacent cargoes, and mineral oils. These can best be controlled by inspections and appropriate sampling, and taking any necessary action in the port of shipment and/or the port of arrival.

A number of the contaminants, such as PAHs, dioxins, aflatoxins, and postharvest insecticides arise from the production and storage of copra by smallholders and are unavoidable (Ranasinghe, 1999). These hazards must be identified by checking the levels of these contaminants in the incoming oil of the oil processing plant so that appropriate treatments can be applied during the refining of the coconut oil.

The second CCP is needed for the removal of heavy PAH by a treatment with active carbon. A third CCP is needed to control the removal of pesticides, aflatoxin (if present), and light PAHs by deodorisation under validated process conditions. Last, but not least, there is the bulk loading of refined coconut oil. A high care zone must be set up to prevent contamination of the oil lot with foreign bodies (glass, insects, birds, etc).

3.10.5 Validation and verification of the processing steps for a CCP

Some of the treatments for removal of hazardous components have been investigated extensively as, for

example, the removal of residues of chlorinated pesticides by high temperature deodorisation (Gooding, 1966), removal of dioxin from fish oil with active carbon and deodorisation (Rohrbeck, 2005), and removal of benzo[a]pyrene with active carbon (Biernoth and Rost, 1968). What has to be done to remove these compounds is clear in principle, but the refinery must also validate its own process conditions, since most published literature is based on laboratory trials.

For example, high temperature deodorisation can be a CCP for the removal of chlorinated pesticide residues. The HACCP system then stipulates that critical limits are defined and validated for the relevant process conditions of the deodorisation process, such as temperature, headspace pressure, and the amount of stripping steam. These process condition limits should be controlled and recorded, preferably with an automated process control system. The measuring equipment used to confirm that the process conditions are within the critical limits must be calibrated at intervals not exceeding 12 months.

Little has been published on the removal of mycotoxins (Parker and Melnick, 1966), residues of organo-phosphorus pesticides (Ten Brink et al., 2002) and pyretheroids currently in use. The proliferation of EU legislation concerning contaminants in foods including lists of banned pesticides and lists of maximum residue levels (MRL) will require effective preventive control measures for these hazards.

3.10.6 Analysis of contaminants

The General Food Law (GFL) of the EU has been the start of much EU legislation to ensure a high level of consumer protection with regard to food and feed safety. The European Food Safety Authority (EFSA) was also set up in 2002 and in close cooperation with national authorities and in open consultation with the relevant stakeholders, it provides objective scientific advice on all food and feed safety matters. The risk assessment activities of the EFSA provide the legislators with a sound scientific basis for the EU legislation relating to food and feed safety.

The GFL lays down the basic principles of European food legislation, upon which other European food safety legislation concerning contaminants, pesticides, hygiene, and control is based. Basic principles regarding contaminants in food are regulated in Food Contaminants Regulation 315/93/EEC. The Food Contaminants Regulation 466/200/EC gives specific maximum levels for contaminants in foodstuffs, and these play an important role in each hazard analysis.

The EU legislation on pesticides residues in food is gradually replacing national laws. The EU has now approved MRLs for about 150 active substances in primary agricultural products. These MRLs are based on an assessment of the highest residues that can be found in field trials using GAPs (Good Agricultural Practices) according to the ALARA principle (As Low As Reasonably Achievable). All other pesticides currently in use in the EU will be reassessed, Council Directive 91/414/EEC (EU, 1991). The Banned Pesticides Directive 1979/117/EEC (EU, 1979) gives a list of pesticides that may not be used in the EU.

The MRL is set to prevent illegal and excessive use of the pesticide, and this limit is mostly far below the toxicological limit. However, if an MRL is exceeded, experts must be consulted on how to handle the case. In some countries, the authorities take measures if the MRLs are exceeded by, for example, 50%, and these measures range from notifying the EU RAPID ALERT SYSTEM to recalls and fines. In other countries, the acute exposure is compared to 100% ArfD (acute reference dose).

The sampling methods and methods of analysis used to determine contaminants in foodstuffs must be appropriate for the foodstuffs tested. For most contaminants, the EU has set minimum criteria for the method of analysis. See, for example, Directive 2002/69/EC for dioxin and dioxin-like PCBs (EU, 2002a) and Directive 2001/22/EC for lead, cadmium, mercury, and 3-MCPD (EU, 2001a).

It is important to select the testing laboratories carefully, when their results are to be used to confirm that certain products and/or processes are within the defined critical limits of the HACCP system. The testing laboratories must be approved by accreditation by a nationally recognised accreditation authority according to EN-ISO-17025 for the test in question and/or by validation of their methods by participation in relevant ring tests.

The EU legislation on food safety for oils and fats has stimulated the development of more accurate and efficient analytical methods for the determination of, for example, PAHs in oils and fats (Stijn et al., 1996), of dioxins in oils and fats (www.fediol.be), and of mineral oil in oils and fats (www.mvo.nl).

The Central Science Laboratory (CSL) in the U.K. organises several ring tests every year for contaminants in oils and fats. Their ring tests focus on the following contaminants:

- OC-, OP, and pyretheroid pesticides in oils and fats.
- Dioxin in oils and fats, and in fresh bleaching earth.
- Mycotoxins-zearalenone (ZEN), deoxynivanol (DON), and aflatoxin in oilseed meals.
- Heavy metals — lead and cadmium in oilseed meal.
- *Salmonella* in oilseed meals.
- PAHs in oils and fats.

These ring tests are coordinated by FEDIOL. Participating laboratories that fulfill the requirements qualify for inclusion in the FEDIOL list of recommended laboratories (see www.fediol.be).

References

Biernoth, G. and Rost, H.E. (1968), *Arch. Hyg. Bakt.*, **152**, 238.

Brink, H.B. ten et al. (2002), Removal of pesticides during oil processing, *Poster presented during the 2nd EuroFedLipid Congress, Strasbourg.*

Codex (1969), Codex Alimentarius Commison Report CAC/RCP-1: Recommended International Code of Practice, General Principles of Food Hygiene, Rev. 3 (1997).

EU (1979), Council Directive (EEC) No 117/1979 of 21 December 1978 prohibiting the placing on the market and use of plant protection products containing certain active substances, *Off. J.*, L 33, 08/02/1979.

EU (1990), Council Directive (EEC) No 642/1990 of 27 November 1990 on the fixing of maximum levels for pesticide residues in and on certain products of plant origin, including fruits and vegetables, *Off. J.*, L 350, 14/12/1990.

EU (1991), Council Directive (EEC) No 414/1991 of 15 July 1991 concerning the placing of plant protection products on the market, *Off. J.*, L 230, 19/08/1991.

EU (1993), Council Regulation (EEC) No 315/1993 of 8 February 1993 laying down Community procedures for contaminants in food, *Off. J.*, L 37, 13/02/1993.

EU (1998), Commission Directive 98/53/EC of 16 July 1998 laying down sampling methods and the methods of analysis for the official control of the levels of certain contaminants in foodstuffs, *Off. J.*, L 201, 17/07/1998.

EU (2001a), Commission Regulation (EC) No 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs, *Off. J.*, L 77, 16/03/2001.

EU (2001b), Council Regulation (EC) No 2375/2001 of 29 November 2001 amending Commission Regulation (EC) No 466/2001 setting maximum levels for certain contaminants in foodstuffs, *Off. J.*, L 321/1, 06/12/2001.

EU (2002a), Commission Directive 2002/27/EC of 13 March 2002 amending Directive 98/53/EC laying down the sampling methods and the methods of analysis for the official control of the levels of certain contaminants in foodstuffs, *Off. J.*, L 75/44, 16/03/2002.

EU (2002b), Commission Regulation (EC) No 221/202 of 6 February 2002 amending Regulation (EC) 466/2001 setting maximum levels for certain contaminants in foodstuffs, *Off. J.*, L 37/4, 07/02/2002.

EU (2004), Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs, *Off. J.*, L 139/1, 30/04/2004.

EU (2005a), Commission Regulation (EC) No 208/2005 of 4 February 2005 amending Regulation (EC) No 466/2001 as regards polycyclic aromatic hydrocarbons, *Off. J.*, L 34/3, 08/02/2005.

EU (2005b), Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene, *Off. J.*, L 35/1, 08/02/2005.

EU (2006a), Commission Directive (EC) No 32/2006 of 3 February 2006 amending Annexes I and II to Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed as regards dioxins and dioxin-like PCBs, *Off. J.*, L 32/44, 04/02/2006.

EU (2006b), Commission Recommendation (EC) No 88/2006 of 6 February 2006 on the reduction of the presence of dioxins, furans and PCB's in feedingstuffs and foodstuffs, *Off. J.*, L 42/36, 14/02/2006.

EU (2006c), Commission Regulation (EC) No 199/2006 of 3 February 2006 amending Regulation (EC) no 466/2001 setting maximum levels for certain contaminants in foodstuffs as regards dioxins and dioxin-like PCBs, *Off. J.*, L 32/34, 04/02/06.

FEDIOL (2006), Risk analysis of coconuts, *www.fediol.be.*

Fondo de Investigaciones Sanitarias (1990), *Toxic Oil Syndrome References Archive*, Ministerio de Sanidad y Consumo, Madrid.

Gooding, C.M.B. (1966), *Chem. Ind.*, 344.

Parker, W.A. and Melnick, D. (1966), *J. Am. Oil Chem. Soc.*, **43**, 635.

Ranasinghe, T.K.G. (1999), Coconut processing in modern coconut management, in *Intermediate Technology Publications FAO*, Ohler, J.G., Ed., University of Leiden, The Netherlands, 343–354.

Rohrbeck, M. (2005), Dioxins: a hot topic for the food industry, *inform*, **16**, 341–343.

Stijn, F. van et al. (1996), Determination of polycyclic aromatic hydrocarbons in edible oils and fats by on-line donor-acceptor complex chromatography and high performance liquid chromatography with fluorescence detection, *J. Chrom. A*, **750**, 263–273.

Thomas, A. (1976), *Die Einfluss der Raffination auf die Konzentration von Spurenelemente in Ölen und Fetten, Fette Seifen Anstrichm.*, **78**, 141–144.

Woodgate, S. and Veen, J. van der (2004), The role of fat processing and rendering in the European Union animal production industry, *Biotechnol. Agron. Soc. Environ.*, **8**, 283–294.

4

MODIFICATION PROCESSES AND FOOD USES

A. J. Dijkstra

4.1 Introduction

This chapter describes a number of applications of oils and fats in foods. In general, these applications do not use a straight, nonmodified oil, but use a blend of two or more fatty intermediates that has been designed and produced with the particular application in mind. There are exceptions in that, for instance, corn germ oil or sunflower seed oil may be sold as such, but even in the field of liquid oils, producers try to increase their income by selling special blends of liquid oils as "salad oil," "deep frying oil" or even "fondue oil."

Arriving at these "special blends" makes more demands on marketing ingenuity than on technological expertise since blending oils and/or fats is the most simple and by far the cheapest of all oil modification processes (Dijkstra, 2002). However, the costs of the modification process or processes needed to arrive at the right blend are often not its most decisive cost element. The raw material costs are certainly more important in absolute terms and the variations in raw material costs can be more important in relative terms (Young, 1994).

At a certain point in time, using palm oil in a blend may be the cheapest way to arrive at the specified blend properties, but what if palm oil gets more expensive? In general, the price of other oils will then also go up, but perhaps not to the same extent. Then, a situation may arise where exchanging some or all of the palm oil for a hydrogenated liquid oil like soya bean oil, leads to a cheaper blend even though some of the other blend components may have to be exchanged for more expensive components. In this example, the hydrogenation process has enabled a more efficient use of the raw materials on offer. In other instances, interesterification and/or fractionation may play a similar role by converting a low-cost raw material into a blend component that causes that blend to be cheaper (Young, 1985).

Linear programming can optimise the fat blends used in the applications discussed in the second half of this chapter by calculating the cheapest blend that meets the blend specifications. The inputs required are the properties and the cost prices of the components that can be used in the blend on the one hand, and the property requirements of the blend on the other. The output of the linear programming calculation is a blend composition — so much of this hard stock and so much of this liquid oil, etc.

No linear programming software is needed for the optimisation of a blend to be sold as straight salad oil. This is simply the cheapest oil on offer, be it soya bean oil or canola. However, if this salad oil has to contain a certain minimum of linoleic acid, this might determine the soya bean oil content if the latter happens to be more expensive than canola. In order to save money, the linear programming calculation would minimise the soya bean oil content of the blend and prescribe as much rapeseed oil as possible while respecting the minimum linoleic acid content.

Some bright marketing person might then suggest limiting the linolenic acid content of this oil blend so as to improve its shelf life, but since both soya bean oil and canola have a linolenic acid content close to 10%, another oil with a lower linolenic acid content, such as sunflower seed oil or corn germ oil, has to be included in this blend. Because the original blend did not contain any sunflower seed oil, it follows that this oil is more expensive than soya bean oil and rapeseed oil. Including it in the blend to reduce its linolenic acid content, thus, will increase the price of the blend.

A general conclusion can be drawn from the above example. Constraints on the blend (conditions it has to meet) may increase the number of blend components and, thereby, can also increase the blend cost. Limiting the *trans* isomer content of a fat blend is also likely to make the blend more expensive and increase its number of components.

The three oil modification processes to be discussed (hydrogenation, interesterification, and fractionation) each have their own cost (Kellens, 2000). In general, hydrogenation is the most expensive of these three processes, with the cost of investment and the hydrogen each accounting for about one-third of the total cost. Interesterification is cheaper and in this case, investment and yield loss each account for just under a third of the total cost. Dry fractionation is somewhat cheaper again, with the investment accounting for over half the total cost on the basis of assuming it is written off over 5-year period.

With hydrogenation and interesterification, the cost of processing is just added to the raw material costs to arrive at the cost of the final product. With fractionation, two or more products arise, and it is a matter of choice how the processing costs are allocated to these products. In fact, some co-products may have a lower value than the starting material so that the other product or products will have to bear the total processing cost as well as the markdown on the co-product. A plant producing palm midfraction for a particular downstream, internal application, and, thus, selling some of the co-products (palm stearin and high-IV olein), once discovered that the selling price of this olein was so attractive that producing more midfraction than needed and using it internally in blends for other applications was a profitable exercise because of the additional olein sales. Accommodating this additional midfraction in these blends was no problem because its low cost price led the linear programming of blend compositions to incorporate it into the blends wherever technically possible. An overall view, therefore, is essential when considering cost aspects of the fractionation process.

References

Dijkstra, A.J. (2002), Hydrogenation and fractionation, in *Fats in Food Technology*, Rajah, K.K., Ed., Sheffield Academic Press, Sheffield, U.K., 123–158.

Kellens, M.J. (2000), Oil modification processes, in *Edible Oil Processing*, Hamm, W. and Hamilton, R.J., Eds., Sheffield Academic Press, Sheffield, U.K., 127–173.

Young, F.V.K. (1985), Interchangeability of fats and oils, *J. Am. Oil Chem. Soc.*, **62**, 372–376.

Young, F.V.K. (1994), Modification processes, in *The Lipid Handbook*, 2nd ed., Gunstone, F.D., Harwood, J.L., and Padley, F.B., Eds., Chapman & Hall, London, 277–285.

4.2 Hydrogenation

Following the discovery by Sabatier and Senderens in 1897 that hydrogen can be added to double bonds in organic compounds when their vapours are passed over certain metal catalysts, such as nickel, in the presence of hydrogen, the hydrogenation of triglyceride oils was invented by Normann in 1901 (Normann, 1903). In his obituary, Kaufmann (1939) described the early industrialisation of the newly invented process and stressed the importance of the invention: It allowed plastic fats to be made from liquid oils and, thus, made the margarine and shortening industries less dependent on the limited availability of fats, such as tallow. In addition, hydrogenation opened up markets for whale and fish oils, which are too unstable for food use without hydrogenation.

The instability of these marine oils is not only due to their high degree of unsaturation, but also to the fact that animal oils and fats do not contain stabilising compounds that occur naturally in vegetable oils, of which tocopherols may be only one example. The role of these stabilising compounds is clearly demonstrated by the fact that the addition of a vegetable oil to fish oil greatly retards the deterioration of the latter. In fact, their role could well be more important than the degree of unsaturation, since a study of the influence of various fatty acids on the rate of oxidation of oils and fats (Dijkstra et al., 1995; 1996) showed the weighting factor at 120°C (a measure of instability) of linolenic acid (0.415) to be close to the weighting factor of linoleic acid (0.389), whereas both monounsaturated fatty acids and saturated fatty acids showed much lower weighting factors of 0.003 and 0.002, respectively.

Possibly the presence of doubly activated allylic hydrogen atoms in the methylene-interrupted polyenes is the cause of this stability difference between linolenic and linoleic acid on the one side and monoenes and saturated fatty acids on the other. The fact that linolenic acid is considered to be not much less stable than linoleic acid is also illustrated in Table 4.1, first shown by Haraldsson (1980) and subsequently quoted regularly (for instance, by Beckmann, 1983). In this table, the data from (Dijkstra et al., 1995; 1996) have been converted so that the rate of the saturated fatty acids has been set equal to unity.

Accordingly, the notion that linolenic acid is the primary cause of the instability of soya bean oil and that this

TABLE 4.1 Relative rates of oxidation of fatty acids

References	(Haraldsson, 1980)	(Dijkstra et al., 1996)
Temperature (°C)		120
Fatty acid	Relative rate	
stearic acid	1	1
oleic acid	10	1.3
linoleic acid	100	169
linolenic acid	150	180
α-eleostearic acid	800	

acid, therefore, should be eliminated by hydrogenation, plant breeding, or genetic modification may well be unfounded. Besides, soya bean oil is considered to be sufficiently stable to be used as salad oil in Europe, just like canola, which has a somewhat higher linolenic acid content than soya bean oil. It would appear that the poor stability of soya bean oil in the U.S. must have a cause other than its linolenic acid content, but it is not clear as of yet whether this poor stability originates from the occasionally incomplete removal of phosphatides by the Long Mix process or by too extreme deodorisation conditions (Dijkstra, 2005). The recently developed soya bean oil process (Tysinger et al., 2003; 2004; and various subsequent patents) describing the physical refining of expelled soya bean oil at a temperature range of 238 to 249ºC exemplifies a move towards less extreme conditions.

The hydrogenation process has had a profound influence on the edible oils and fats industry. It has not only allowed the use of whale and fish oils as raw materials for margarine, but has also allowed the U.S. to use soya bean oil as almost the sole raw material for the entire edible oils and fats industry, and opened up the way for the development of novel products, such as cocoa butter replacers (CBRs) based on *trans* isomer-rich, partially hydrogenated vegetable oils.

From the beginning, hydrogenation has been heterogeneous, so the industrial hydrogenation process is nearly always a batch process followed by the removal of the catalyst by filtration. The catalyst used for the hydrogenation of triglyceride oils has invariably been metallic nickel. Of course, the literature also reports on homogeneous (Augustine and Van Peppen, 1970; James, 1973) and precious metal catalysts (Rylander, 1970), but so far they have hardly been used industrially for edible oils.

Possible reasons have been listed by Schöön (1995): Homogeneous catalysts are not very active, they are expensive to synthesise, can be poisonous, and are difficult to recover, which means that the hydrogenated oil must be carefully purified. On the other hand, the oleochemical industry employs copper-based catalysts to produce fatty alcohols from methyl esters, wax esters, or fatty acids. If the unsaturation present in the fatty acid chain is to be maintained, zinc chromites are the preferred catalyst.

The hydrogenation reaction and process have been the subject of extensive research, but this was far from easy, especially before the advent of gas-liquid chromatography (GLC), when, for instance, fatty acid compositions had to be determined (or rather estimated), for example, by preparing lead salts. Besides, the complexity of the system was often unnecessarily aggravated by the use of soya bean oil as the hydrogenation substrate rather than simpler model compounds, such as fatty acid methyl esters (Dijkstra, 1997), diacyl ethylene glycol esters (Schilling, 1981), etc. Moreover, studying the hydrogenation process is also complicated by the simultaneous presence of three phases: liquid oil, gaseous hydrogen, and solid catalyst and, therefore, by mass transfer limitations. Proposing a kinetic model of a reaction is seriously hampered when the concentration of one of the reagents is not known.

Nevertheless, a certain degree of understanding has grown over the years, which has permitted the industrial processes of catalyst manufacture and oil hardening to be controlled to such an extent that reasonably reproducible results are obtained. Catalyst development has led to products permitting a reduced catalyst dosage and the use of pulse-NMR in product specification and quality control has also improved reliability and reduced the number of batches that have to be downgraded.

However, because of the possible nutritional effect of *trans* isomers, the future of the partial hydrogenation process is somewhat in doubt. *Trans* isomers have been linked to an increase in blood serum cholesterol content (Katan, 1995) and this increase is generally believed to cause heart disease, despite the fact that this causal effect has not been demonstrated (Ravnskov, 2000). Nevertheless and despite this lack of proof, margarine producers in Europe have agreed to limit the *trans* isomer content of consumer margarines and salad oils to a maximum of 1%. The *trans* isomer content of bakery margarines falls outside this agreement because these isomers are essential for the technical performance of these products. In the U.S. the amount of *trans* isomers per serving has to be mentioned on the label as from January 1, 2006 (Yurawecz, 2004)[1] and Denmark makes a distinction between the *trans* isomer content of dairy products and that of products that have been hydrogenated industrially (List, 2004); the legality of this distinction is doubtful.

Just as the labelling rules on saturated fatty acids make no distinction between nutritionally innocuous fatty acids like stearic acid, which is readily dehydrogenated to oleic acid, or behenic acid that is barely absorbed, and saturated fatty acids like lauric acid that are presently considered to be nutritionally less desirable, the labelling rules on *trans* isomers are equally indiscriminate (Dijkstra, 2006) and, therefore, scientifically unsound. Moreover, these rules force producers to introduce more expensive and nutritionally inferior products just because label-wise, they look like an improvement. These scares are nothing new. They have been raised in the past and subsequent fears have also been allayed (Applewhite, 1981). Now they have re-emerged and, in all likelihood, evidence will be reported in due course that will force the pendulum to swing back again. After all, mankind has been consuming *trans* isomers of fatty acids since ruminant-derived products started to appear on the menu, and that is quite some time ago.

Other factors influencing the hydrogenation of edible oils are that less fish oil is hydrogenated today because

[1] If a serving (14 g) contains less than 0.5 g *trans*-isomers, it can be labelled "zero-trans." This means that the fat blend used to prepare a 60% spread may contain up to 6% *trans*-isomers for the spread still to qualify as "zero-trans."

most fish oil is used for aquaculture feeds (Barlow, 2004). On the other hand, more and more vegetable oils are fully hydrogenated before being interesterified with one or more liquid oils to provide a hard stock that is low in *trans* isomers, which should help to allay fears about the nutritional effects of *trans* isomers (List, 2004).

4.2.1 Selectivities

After the hydrogenation of triglyceride oils had been invented, many authors have studied the mechanism of this reaction to improve process control and to develop novel hydrogenation products and improved hydrogenation catalysts. They wondered if a fatty acid on the middle 2-position of the glycerol backbone of a triglyceride reacted equally readily as the same fatty acid on one of the outer 1,3-positions (positional selectivity). They asked themselves whether the hydrogenation of polyunsaturated fatty acids was through consecutive steps or through a shunt reaction whereby hydrogen is added simultaneously to several double bonds in the same fatty acid (Bailey, 1949; Mounts and Dutton, 1967).

In 1937, Bushell and Hilditch studied the hydrogenation of mixtures of different synthetic triglycerides and concluded that "the tri-unsaturated glycerides are attacked more rapidly than the di-oleo-glycerides, and the latter somewhat more so than the mono-oleo-compounds." Accordingly, they concerned themselves with the reaction rates of triglycerides rather than those of unsaturated fatty acids. In this respect, their approach differs fundamentally from the approach taken by Bailey and Fisher (1946) who introduced the "common fatty acid pool" by suggesting, "In the case of a number of different fatty acids existing together in an oil and competing for hydrogen, it appears reasonable to assume that, of an infinitesimal amount of hydrogen absorbed, the fraction going to each acid will depend upon the concentration of the acid and a constant expressive of the affinity of the acid for hydrogen under the particular conditions of hydrogenation then obtaining" (see also Bailey and Fisher, 1946).

This "common fatty acid pool" assumption subsequently led to the concept of fatty acid selectivity as, for instance, described by Boelhouwer et al. (1956). This concept assumes that the rates of hydrogenation of the various unsaturated fatty acids depend in an identical manner on the hydrogen concentration. Consequently, the ratio of the rates of reaction of two different unsaturated fatty acids, for example, linoleic acid and oleic acid, is only a function of the concentration of these acids and their rate constants. Since the concentration of these acids can be determined experimentally, the ratio of the hydrogenation rate constants can be calculated (Albright, 1965; Schmidt, 1968); it is called the "fatty acid selectivity." Accordingly, the linolenic acid selectivity indicates how much faster linolenic acid reacts than linoleic acid, and the linoleic acid selectivity indicates how much faster linoleic acid reacts than oleic acid.

During the hydrogenation process, double bonds are not only saturated, they may also shift their position along the fatty acid chain (positional isomerisation) and/or undergo *cis-trans* isomerisation. Because *trans* isomers strongly affect the physical properties of the triglycerides, the extent of the formation of these isomers has to be controlled. It is generally expressed as the "*trans* selectivity" or as the "isomerisation index," which are defined as the increase in *trans* content (expressed as % elaidate) divided by the decrease in iodine value or by the decrease in double bond content (in % on triglycerides), respectively, as observed in the early stages of the hydrogenation process.

Finally, the literature also mentions "triglyceride selectivity," a quantitative definition of which has been given by Coenen (1981). He qualifies this triglyceride selectivity as "high when the attack on the fatty acid groups in the triglyceride is random, that is as if they reacted as independent molecules. It is low when the attack on the three fatty acids in a triglyceride is correlated." As will be discussed below, Coenen links his triglyceride selectivity to the catalyst pore structure.

4.2.1.1 Positional selectivity

Several authors (Bushell and Hilditch, 1937; Mattson and Volpenhein, 1962; Tümer et al., 1964; Schilling, 1968; Hashimoto et al., 1971b; Drozdowski, 1977; Paulose et al., 1978; Ilsemann et al., 1979; Kaimal and Lakshminarayana, 1979; Suzuki and Murase, 1981; Strocchi, 1982; Beyens and Dijkstra, 1983; Fan et al., 1986) have investigated the occurrence of positional selectivity and in doing so have reached contradictory conclusions. As a summary of a detailed examination of the evidence presented in the literature in favour of the existence of positional selectivity, it can be concluded that this evidence is weak. Most of the articles examined suffer from analytical inaccuracies and/or show internal inconsistencies. Besides, some authors have studied nonrandomised triglyceride oils and, thereby, have not eliminated the potential influence of triglyceride selectivity. Accordingly, for practical purposes, it can be assumed that unsaturated fatty acids at the 1,3-positions and the 2-position of a triglyceride react at equal rates.

4.2.1.2 Triglyceride selectivity

In the first of his papers dealing with triglyceride selectivity, Schilling (1968) describes the hydrogenation of tri-olein and then compares the rates of reaction of this tri-olein and resulting oleostearins. He concludes that the assumption that the rate of reaction is independent of the degree of unsaturation of his oleins provides a much better fit to the experimental data than the assumption that this rate is proportional to the number of oleic acid moieties in the olein. He also hydrogenated an

equimolecular mixture of tri-olein and mono-olein and again observed that both triglyceride species reacted at the same speed. In addition, he observed a strong tendency for the immediate consecutive hydrogenation of residual double bonds. These conclusions and observations are not in accordance with the concept of the "common fatty acid pool" (Bailey, 1946).

In subsequent papers (Schilling, 1977; 1978), he describes hydrogenation experiments of a mixture of trilinolenin and dipalmito-monolinolenin; he chose this particular mixture for analytical reasons. On the basis of the rate of reaction of his starting material, he concludes that the trilinolenin does not react three times as fast as the monolinolenin and, thus, provides firm evidence that the "common fatty acid pool" concept is not correct. Again he notices some simultaneous hydrogenation of all fatty acids in an adsorbed triglyceride and he also notices that this is catalyst-dependent.

This catalyst dependency has also been mentioned by Linsen (1971a; 1971b) who reports the largest amount of stearic acid when using a catalyst with pore widths between 20 and 25 Å (i.e., narrow pores). Mass transport limitations presumably cause any oleic acid moiety formed to be further hardened to stearic acid when trilinoleate is used as substrate. On the other hand, with methyl linoleate as a substrate, the reaction is more selective in that far less stearate is formed (Coenen, 1978). This is tentatively explained by the assumption that methyl esters are more mobile than triglycerides and leave the catalyst pores before they have been hydrogenated a second time.

Beyens and Dijkstra (1983) tackled the triglyceride selectivity concept with stereochemistry in mind; they were probably inspired by Drozdowsky (1977) in this respect. Studying triglyceride selectivity involves looking at how individual triglyceride classes react during hydrogenation. This implies that these classes must be hydrogenated as a mixture so as to eliminate variables, such as temperature, catalyst properties (amount, activity, possible activation, and/or poisoning), and hydrogen pressure/concentration. But hydrogenating a mixture of components and finding out what happens to these components requires a separation of the mixture into its original classes/components after hydrogenation.

They solved this separation problem by choosing components that could be separated by isocratic-reversed phase HPLC, since this technique also allows fractions to be collected for further analysis. Component A consisted of randomised sunflower seed oil, and component B consisted of an interesterified mixture of 20.0 wt% sunflower seed oil and 80.0 wt% Radianol®[2], a synthetic triglyceride containing only C8:0 and C10:0 fatty acids. Component A encompasses the partition numbers 42 to 50, whereas component B elutes much earlier and separately (partition numbers 24 to 40).

A mixture of 17.5 wt% of component A and 82.5 wt% of component B was hydrogenated and samples were taken every 20 minutes. The samples were separated into their original components by HPLC and their fatty acid composition was determined. Table 4.2 summarises the results. It shows that the linoleic acid in component A (randomised sunflower seed oil) is less "reactive" than the linoleic acid in component B. In fact, extrapolation to $t = 0$ leads to a reactivity ratio of 2/3.

This difference in reactivity was tentatively explained on stereochemical grounds because the linoleic acid moiety in component B triglycerides is surrounded by short chain fatty acids and, therefore, more accessible and could react faster.

However, it can also be argued that the linoleic acid in component B is almost the only unsaturated acid present, whereas the triglycerides of component A (randomised sunflower seed oil) will on average contain more than two linoleic acid moieties. These moieties are observed to react more slowly than the moieties in component B, which is also in accordance with the observations of Bushell and Hilditch (1937), Schilling (1968; 1977; and 1978), and even Coenen (1978).

Consequently, instead of explaining away observations that are inconsistent with the "common fatty acid pool" concept (Bailey and Fisher, 1946) on the basis of catalyst pore size or stereochemistry, this concept should be rejected on the basis of contradictory experimental evidence and lack of supporting evidence. However, rejecting the "common fatty acid pool" concept implies that all literature based upon this concept has to be re-evaluated.

TABLE 4.2 Fatty acid compositions of components A and B

Time (min)	C16:0 (%)	C18:0 (%)	C18:1 (%)	C18:2 (%)
		Component A		
0	6.94	4.60	18.14	69.79
20	7.84	4.73	24.77	63.20
40	7.90	4.88	31.46	55.76
60	6.98	4.42	41.63	46.96
80	6.94	4.34	50.72	37.99
100	6.95	4.43	61.66	26.96
120	6.71	4.89	73.73	14.67
		Component B		
0	6.07	3.44	16.4	73.59
20	6.24	3.66	26.57	63.53
40	6.27	3.80	38.59	51.35
60	6.06	3.81	50.28	39.15
80	6.25	4.05	60.53	29.17
100	6.08	4.15	70.38	19.38
120	6.36	4.58	80.48	8.57

Source: Beyens, Y. and Dijkstra, A.J. (1983), in *Fat Science 1983, Proceedings of 16th ISF congress, Budapest*, Holló, J., Ed., Akadémiai Kiadó, Budapest, 425–432.

[2] Registered trademark of Oleon N.V., Oelegem, Belgium.

4.2.1.3 Fatty acid selectivities

As mentioned above, the fatty acid selectivity is based on the "common fatty acid pool" concept. It is defined as the ratio of two rate constants, so that the fatty acid selectivity itself should also be a constant. The absolute value of this constant may be catalyst-dependent and may also depend upon the temperature, since the rate constants may differ in the way they depend upon temperature, but during an isothermal hydrogenation, the fatty acid selectivity should be constant. As shown in Table 4.3 (Dijkstra, 1997), this is not the case. During the early stages of an hydrogenation experiment involving sunflower seed oil, the linoleic acid selectivity is about 50 to 70, but once the linoleic acid concentration has fallen below 20%, the selectivity (being a ratio of two constants) drops to much lower values. This anomaly calls for an explanation.

Dijkstra (1990; 1997) has given a possible explanation by describing a quite simple experiment. It entails an isothermal hydrogenation of sunflower seed oil at atmospheric pressure in an autoclave provided with a variable speed agitator. The vent of the autoclave is connected to a wash bottle to detect when more hydrogen is sparged into the bottom of the autoclave than reacts. The hydrogenation is started with a fairly low rate of agitation and an excess of hydrogen, but, once underway at full catalytic activity, the rate of hydrogen supply is manually decreased until no more hydrogen bubbles through the wash bottle and the hydrogen supply matches the ability of the agitator to dissolve the hydrogen into the oil, and the ability of the reaction mixture to make it disappear by reacting.

At this point even a slight decrease in agitator speed causes hydrogen to bubble through the wash bottle, so the hydrogenation is clearly carried out under mass transfer limitation. These conditions are also called "selective" since hardly any stearic acid is formed during the early stages of the process. Moreover, it shows that the reaction rate of hydrogenation depends on the hydrogen concentration, since lowering this concentration by decreasing the speed of agitation causes the rate to fall so that not all hydrogen reacts and some reaches the wash bottle.

TABLE 4.3 Linoleic acid selectivity

Time (min)	C18:0 (%)	C18:1 (%)	C18:2 (%)	Linoleic Acid Selectivity
0	4.15	18.5	77.35	
80	4.40	45.0	50.6	59
100	4.60	52.2	43.2	40
120	4.80	60.5	34.7	55
140	5.10	69.5	25.4	68
160	5.50	77.5	17.0	72
180	7.00	83.8	9.2	33
200	16.6	80.5	2.9	10

Source: Dijkstra, A.J. (1997), *inform*, **8**, 1150–1158.

The hydrogenation is allowed to continue and for quite some time, the rate of agitation hardly needs any adjustment in order to balance hydrogen supply and demand. However, when the residual linoleic acid content of the reaction mixture has fallen to some 15%, the rate of agitation has to be steadily increased to balance the constant hydrogen supply with demand. This observation allows a number of conclusions to be drawn.

It shows that, at that point in time, a further decrease in the degree of unsaturation of the oil causes a decrease in reactivity that has to be compensated for by an increase in the hydrogen concentration by increasing the rate of agitation. Since a decrease in unsaturation causes a decrease in reaction rate, this rate, therefore, not only depends on the hydrogen concentration but also on the degree of unsaturation of the substrate. This raises the question of why this dependence did not manifest itself during the early stages of the hydrogenation experiment, when the degree of unsaturation also decreased.

This question can be answered by assuming that during the early stages of the hydrogenation experiment, when the oil was still highly unsaturated and, thus, highly reactive, this high reactivity kept the hydrogen concentration low. As and when the experiment proceeded and the reactivity of the oil decreased, the rate of reaction was maintained and this implies that the hydrogen concentration gradually increased. Since this increase did not require an increase in the rate of agitation, it can be concluded that there was no change in the driving force for hydrogen dissolution and that the difference between the hydrogen solubility and its concentration did not change. Since the concentration did change to compensate for the decrease in reactivity of the oil, it follows that the concentration must have been only a small fraction of the solubility. Only then could a large relative change in concentration correspond to a negligible absolute change in the driving force for dissolution.

During industrial hydrogenations, the hydrogen concentration in the oil is very low when the reaction mixture is quite reactive and the catalyst is sufficiently active to maintain this low hydrogen concentration. It increases gradually until the decrease in reactivity of the mixture forces it to increase more rapidly. This rapid increase coincides with the sudden decrease in linoleic acid selectivity (cf. Table 4.2) and this coincidence is the basis of the mechanism (Dijkstra, 1997; 2002a; 2006) to be discussed later, that suggests that the additions of hydrogen to linoleic acid and oleic acid are of a different order with respect to hydrogen.

There is some support in the literature for this difference:

- Hashimoto et al. (1971a) conclude that the rate of hydrogenation of diunsaturated groups to monounsaturated groups and the rate of geometrical isomerisation between monounsaturated groups are half order with respect to the hydrogen concentration,

whereas the rate of hydrogenation of monounsaturated groups is first order in hydrogen.
- Bern et al. (1975) derive a lower order in hydrogen for linoleic acid than for monoenes, but, on the other hand, report different values for different monoenes (C18:1, C20:1, and C22:1).
- Ahmad et al. (1979) stated that "kinetic measurements in these laboratories have indicated that, for nickel catalysts, the rate of hydrogenation is proportional to the hydrogen pressure, whereas the rate of isomerization is proportional to the square root of the hydrogen pressure."
- Lidefelt et al. (1983) arrived at a first order in hydrogen for dienes and an order of 3/2 for monoenes when hydrogenated in the gas phase.
- Krishnaiah et al. (1990) concluded that the rate of linoleate disappearance is proportional to the square root of the hydrogen concentration.
- Jonker (1999) arrived at a first order in hydrogen for the hydrogenation of methyl oleate.

There appears to be a degree of consensus that diene hydrogenation has a different and lower order with respect to hydrogen than monoene hydrogenation. This consensus, therefore, makes the current definition of linoleic acid selectivity obsolete because this definition is based on the assumption that both the rate of hydrogenation of linoleic acid and the rate of hydrogenation of oleic acid depend on the hydrogen concentration in an identical manner. However, this definition is also linked to established expressions, such as "a selective hydrogenation," which expressions are meaningful, but pertain not to the catalyst but to the hydrogenation process conditions.

4.2.1.4 *Trans* selectivity

Trans selectivity has been defined as the ratio of the percentage increase in *trans* isomer content and the decrease in iodine value (or the decrease in percentage of double bonds). This definition is based on the observation that, if during a partial hydrogenation run, the *trans* isomer content is plotted against the decrease in iodine value, a straight line results, the slope of which can be easily determined. Of course, when the hydrogenation is continued, the *trans* isomer content will decrease again to reach zero at complete hydrogenation.

It is very convenient for definition purposes that such a straight line is observed, but it is nevertheless quite remarkable. It means that:

- The rate of *trans* isomer formation is zero order in iodine value or double bonds.
- But it is nevertheless linked to the rate of hydrogenation of the double bonds.
- Whereby, a decreasing total number of double bonds goes on producing *trans* isomers at a constant rate.
- Although the number of *cis* double bonds decreases even faster.
- While *trans* isomers can re-isomerise to yield *cis* isomers.

This isomerisation is governed by the thermodynamic constant of the *cis-trans* equilibrium. This has been reported by Veldsink et al. (1997) on the basis of the heat of hydrogenation of both isomers as $\Delta H_{iso} = -4.1$ kJ/mole. By determining the position of this equilibrium, the same value is arrived at (Dijkstra, 2006). Some *cis-trans* equilibrium positions calculated on the basis of this value have been listed in Table 4.4. Statements such as "that the maximum obtainable *trans* level is 67%" (Van Duijn, 2000) and claims of *trans* levels above 90% (Baltes, 1970; 1972) should be disregarded.

Another publication dealing with the formation of *trans* isomers (Coenen and Boerma, 1968) describes an experiment that is, in principle, quite elegant in that it employs high erucic acid rapeseed oil as hydrogenation substrate and, thus, its erucic acid (C22:1) as an internal marker. It notes that the formation of behenic acid (C22:0) coincides with the formation of brassidic acid (*trans* C22:1), and then concludes that monoenes can only be isomerised when they are also hardened. This is an undue generalisation.

High erucic acid rapeseed oil only contains some 14% linoleic acid, so the substrate is not representative of what happens during the early stages of the hydrogenation of soya bean or sunflower seed oil; the hydro-genation was also carried out at 100°C and, therefore, is even less representative of industrial hydrogenation conditions. Moreover, a repeat of the experiment at a higher temperature showed that monoenes (C22:1 and C20:1) isomerised without being saturated (W. Meeussen, personal communication). Accordingly, any mechanism to be suggested must take into account that monoenes can isomerise while polyenes are being hardened and monoenes are not.

Moreover, the notion that *trans*-isomers can only originate from dienes when no saturates are formed is also contradicted by the observation that the *trans* selectivity can be larger than unity. In fact, this selectivity was found to depend upon the ratio of monoenes to dienes in the

TABLE 4.4 *Cis-trans* equilibrium compositions for various temperatures

Temperature (°C)	Trans (%)	Cis (%)	Trans/cis
20	84.33	15.67	5.38
60	81.46	18.54	4.40
100	78.95	21.05	3.75
140	76.75	23.25	3.30
180	74.81	25.19	2.97
220	72.53	27.47	2.72
260	71.61	28.39	2.52

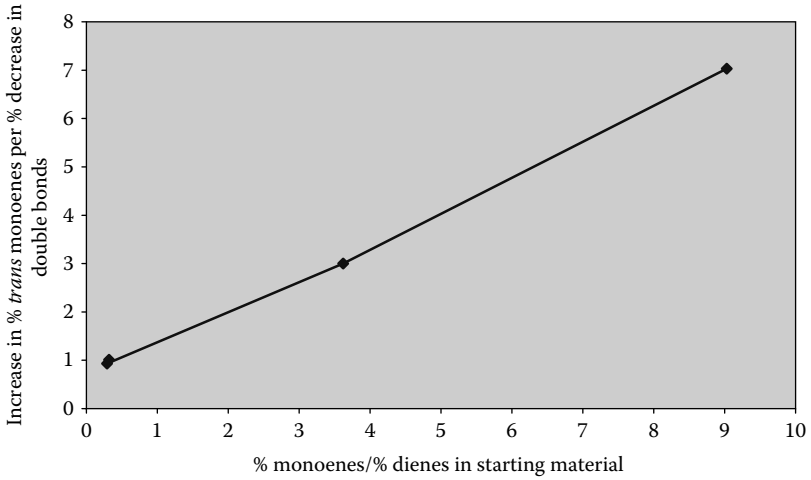


FIGURE 4.1 *Trans* isomer formation as a function of unsaturates composition.

starting material as illustrated in Figure 4.1 (Dijkstra, 1997; 2006).

4.2.2 Hydrogenation mechanism

Like any mechanism, the hydrogenation mechanism to be proposed should be in accordance with available experimental observations and preferably not contradict generally accepted theories. Accordingly:

- The hydrogenation mechanism should explain why a low hydrogen concentration, as during the early stages of a hydrogenation run, leads to a preferential saturation of polyunsaturated fatty acids.
- Similarly, it should explain why this "selectivity" decreases when the hydrogen concentration is deliberately increased, or increases of its own accord when the reactivity of the reaction mixture decreases.
- It should explain how this increased hydrogen concentration suppresses isomerisation.
- It should also provide an explanation of why a high monoene to polyene ratio leads to an increase in *trans* isomer formation.
- It should predict any difference between the rates of reaction of *cis* isomers and *trans* isomers.
- It should preferably also explain why some catalysts promote *trans* isomer formation and why others apparently suppress this formation.

The mechanism as proposed by Dijkstra (1997; 2002a; 2006) is based on the mechanism put forward by Horiuti and Polanyi in 1934. This mechanism assumes the two hydrogen atoms required for the saturation of a double bond to be added consecutively. Accordingly, their mechanism assumes a half-hydrogenated intermediate, which can also revert to its starting material. By assuming the stability of this half-hydrogenated intermediate to depend upon the number of double bonds in its original, nonhydrogenated state, a distinction can be introduced that may help to explain the experimental evidence.

The reactions depicted in Figure 4.2 commence with the hydrogen reactions involving (1) adsorption onto the catalyst surface, which has been indicated by an asterisk (*), and (2) subsequent dissociation. Both reactions (1) and (2) are considered to be reversible and fast. In step (3), the starting material *cis,cis*-linoleic acid (*c,c*-D, with D standing for diene) attaches itself reversibly to the catalyst surface. Because of the nonvalidity of the "common fatty acid pool" concept, the rate of attachment is not quite proportional to the linoleic acid concentration, but it can nevertheless be assumed that a higher concentration will cause the rate to increase.

In reaction (4), a hydrogen atom is added to one of the double bonds of the attached linoleic acid, as a result of which this double bond loses its configuration: *c,c*-D* becomes the intermediate *c*-DH*, which has only one *cis* bond left. This intermediate *c*-DH* can react in three ways. It can revert back to linoleic acid via step (-4), it can dissociate under formation of a *trans* isomer (*c,t*-D*), which has been indicated as reaction (5) in Figure 4.2, or it can react with a second hydrogen atom in accordance with the Horiuti-Polanyi mechanism in step (10) and form a monoene (*c*-M) that still has one of the original *cis* double bonds. This second addition of a hydrogen atom is assumed to be irreversible. The adsorbed *trans* isomer (*c,t*-D*) can desorb from the catalyst surface in step (-6) and, thereby, complete the sequence of adsorption (3), hydrogen addition (4), hydrogen release (5), and desorption (-6) leading to *cis-trans* isomerisation.

As shown in Figure 4.2, there are further sequences leading to *cis-trans* isomerisation reactions, such as (6), (7), (8,) and (-9) leading to the formation of *t,t*-D from *c,t*-D, and (12), (14), (15), and (-13) leading to the formation of *t*-M from *c*-M. Since all the steps listed are reversible, there will also be *trans-cis* isomerisation reactions, but given the free energy difference between the two

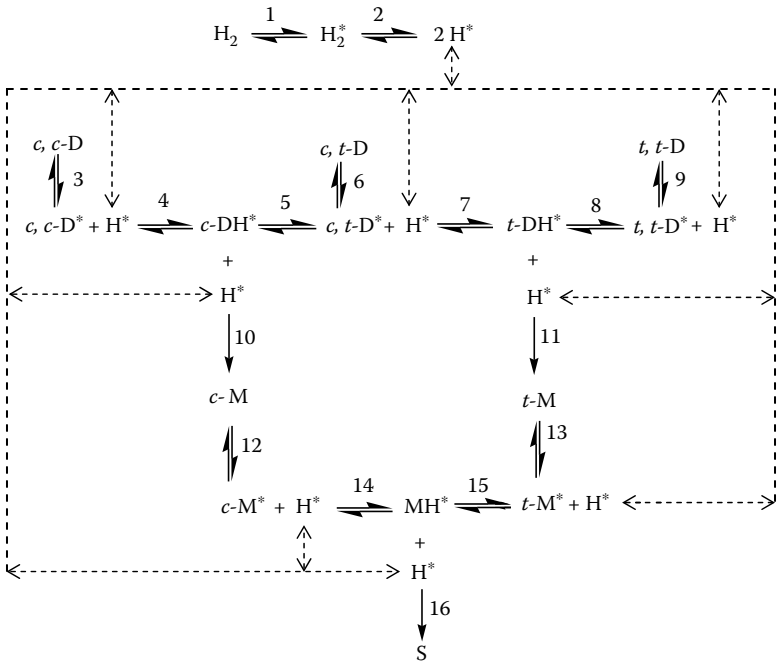


FIGURE 4.2 Reactions involved in the hydrogenation of dienes to saturates.

isomers, the latter isomerisation reactions will be less prevalent, at least in the beginning of the reaction.

Figure 4.1 shows the number of *trans* monoenes formed per double bond being saturated as function of the monoene-diene ratio. In the absence of monoenes, i.e., when this ratio equals zero, extrapolation shows that this ratio equals about 0.7 or in other words, the sequence comprising steps (3), (4), (5), (7), and (11) leading to elaidic acid (*t*-M) is favoured over the sequence (3), (4), and (10) leading to oleic acid (*c*-M). Thermodynamically, step (7) leading to a *trans* isomer is favoured over step (–5) leading to the *cis* isomer and that partially explains the aforementioned ratio of 0.7. Another aspect of the explanation of this ratio is the implication that the equilibrium reactions involved in the sequences leading to the formation of elaidic and oleic acid are fast in comparison with these formations. For a full and detailed discussion of this mechanism see (Dijkstra, 2006).

The catalyst industry has developed special, sulfur-treated catalysts to promote *trans* isomer formation (Beckmann, 1983). These catalysts (Rijnten and Eikema, 1975) are less active than the nontreated, normal nickel catalysts and the *trans* selectivity of these catalysts does not depend upon the temperature of the hydrogenation reaction. In the case of the nontreated catalyst, an increase in reaction temperature will cause the catalytic activity to increase so that the hydrogen concentration is decreased. Sulfur poisoned catalysts apparently behave in an unexpected manner and this raises the question of why their behaviour is so different. A speculative explanation has been put forward by Eckey (1954) who looked at the density (or concentration) of the hydrogen at the catalyst surface. If this is high, the likelihood of there being two hydrogen atoms sufficiently close together to add to the double bond is also high. If this density is low, and only one hydrogen can react at the time, isomerisation will be favoured. According to Eckey, poisons deactivate some active sites for hydrogen dissociation and, thereby, decrease the atomic hydrogen density. This lower density causes the rates of steps (4), (7), (10), (11), (14), (-15), and (16) to decrease, but does not affect the equilibrium constants involved. Accordingly, the equilibrium concentration of the half-hydrogenated intermediates c-DH*, t-DH*, and MH* will be decreased and since the hydrogen concentration was already low, isomerisation will be favoured over hydrogenation. The decrease in hydrogenation activity observed for sulfur-poisoned catalysts supports this tentative explanation.

It also explains the lower activity of sulfur-poisoned catalysts, but what about the lack of temperature dependence of the *trans* selectivity of these catalysts? Non-poisoned catalysts show an increased *trans* selectivity at increased temperatures because their increased activity lowers the hydrogen concentration in the bulk of the oil. This lowers the concentration of adsorbed hydrogen and, thus, of atomic hydrogen which, as explained above, favours isomerisation over saturation.

Poisoned catalysts will presumably also become more active when the temperature is raised, but this does not alter the geography of their active sites. If "the likelihood of there being two hydrogen atoms sufficiently close together to add to the double bond" was low at low temperature, it will also be low at high temperatures and, thus, the *trans* selectivity will not be affected.

Lowering the hydrogen concentration on the catalyst surface by poisoning the catalyst or raising the reaction temperature favours isomerisation. This implies that increasing the hydrogen concentration favours saturation over isomerisation. Accordingly, lowering the reaction temperature (Van Toor et al., 2005) not only necessitates the use of much more catalyst to prevent the rate of hydrogenation to fall to unacceptably low levels, it also favours saturation over isomerisation and leads to what is called a low linoleic acid selectivity (Dijkstra, 2006).

The above pertains to the normal nickel catalyst, but the catalyst industry has also developed a platinum catalyst (Beers and Mangnus, 2004; Beers et al., 2004; Beers et al., 2005) that has a high linoleic acid selectivity and a low isomerisation index; it just catalyses the partial saturation of polyunsaturated fatty acids. In theory, this can be achieved by modifying the catalyst properties in such a way that its surface has a reduced affinity for monoenes as expressed by the equilibrium constants K_{12} and K_{13} relative to the equilibrium constants K_3, K_6, and K_9 pertaining to the dienes. At the time of writing, no patent application had as yet been published, so it is not yet clear how the modification has been realised.

Similar modifications of the catalytic properties have been reported by Cahen (Cahen, 1979; 1980) who added a basic compound containing nitrogen, such as ammonia, uream aliphatic amines, etc., to nickel catalysts and noted an increased selectivity. A competition for active sites on a platinum catalyst between monoenes and an additive, such as tetra ethyl ammonium or liquid ammonia, is also reported to lead to improved linoleic acid selectivity and low *trans* formation (Kuiper, 1980; 1981a; 1981b).

Several authors (Feuge et al., 1953; Feuge and Cousins, 1960; Cousins, 1963) have published graphs showing the *trans* isomer content as a function of some parameter indicative of hydrogenation progress. When these graphs follow the hydrogenation to completion, they invariably follow the same curve near the end of the process, which curve is indicative of the *cis*-*trans* equilibrium. This shows that it is not possible to generate a higher *trans* isomer content than that which corresponds to equilibrium and also that both isomers have the same hydrogenation rate constants. This calls for an explanation of how the *cis*-*trans* equilibrium is reached.

In this context it has been suggested that *cis* isomers (such as *c*-M* in Figure 4.2) react with atomic hydrogen in step (14) to form an adduct (MH*) that is neither *cis* nor *trans* and that *trans* isomers (such as *t*-M*) form the same adduct (MH*) after a hydrogen atom has been added to their double bond in step (-15). This adduct is equally likely to dissociate into a *cis* isomer (-14) as into a *trans* isomer (15), but because of the energy difference between the two isomers, the activation energy for the hydrogen addition will be lower for the *cis* isomer. The rate constant of step (14), thus, will be larger than the rate constant of step (-15) and

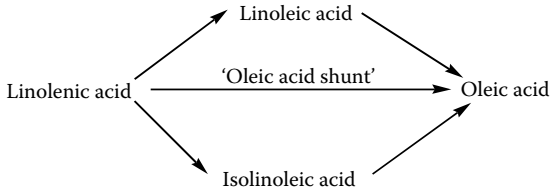


FIGURE 4.3 Shunt reactions.

at equilibrium, there will be more *trans* isomers than *cis* isomers because the latter are more reactive.

The literature regularly mentions "shunt reactions" taking place during the hydrogenation of polyunsaturated triglycerides. The "oleate shunt," being a "direct-through" reaction of linolenic acid to oleic/elaidic acid, was suggested by Bailey (1949) as illustrated in Figure 4.3 above:

Subsequent authors (Mounts and Dutton, 1967) even suggested stearate shunts in which linolenic acid and linoleic acid would react straight through to stearic acid. Their conclusion that these reaction paths existed was based on otherwise inexplicable deviations from kinetic models. In retrospect, the validity of these models is doubtful, since they do not take triglyceride selectivity or the order with respect to hydrogen of the various fatty acid moieties into account. Besides, the only difference in reaction rate between fatty acid isomers taken into account is between linoleic acid and isolinoleic acid (Δ^9, Δ^{15} -octadecadienoic acid). Accordingly the models used are oversimplifications that do not provide valid evidence of the existence of shunt reactions. On the other hand, the evidence of the existence of a shunt reaction when the model compound methyl sorbate (2,5-hexadienoic acid methyl ester) is hydrogenated in the presence of a palladium metal catalyst, is quite convincing (Heldal and Frankel, 1984). Nickel, on the other hand, does not lead to such a shunt reaction.

4.2.3 Hydrogenation reaction partners

4.2.3.1 Hydrogenation catalysts

From the very beginning, nickel has been the metal of choice for the hydrogenation of edible oils and fats. Although Raney nickel has been used as a hydrogenation catalyst in the laboratory (Schilling, 1968), industrial nickel catalysts have always been supported. Pumice stone was mentioned by Normann (1903), diatomaceous earth (kieselguhr) has been used extensively and more recently, synthetic supports based on silica and/or alumina started being used. Catalysts as supplied contain some 18 to 25 wt % nickel, up to 20 wt % support material, the remainder being fully hydrogenated vegetable fat. They are supplied in drums as pellets or flakes.

During storage, some oxygen may permeate into these flakes and this leads to the formation of a nickel oxide film. This film should not be regarded as a solid oxide coating, but rather as a slight haze formed by an incomplete

monolayer, since the temperature required to reduce nickel oxide is much higher than the temperature used during hydrogenation. The reduction of the oxide haze shows up as an induction period that is characterised by a low rate of reaction and, thus, a high concentration of hydrogen and slow temperature rise.

Such a low reaction rate may also result from catalyst inactivation by catalyst poisons present in the oil and/or the hydrogen. During industrial hydrogenation processes, this loss of activity is a costly nuisance, so there has been an economic incentive to study its causes. Accordingly, quite a few papers have been published on catalyst poisoning and the subject has also been reviewed (Irandoust and Edvardsson, 1993) as part of the ASHLI project (Advanced Selective Hydrogenation of Lipids).

With respect to the effect of phosphorus, the literature is confusing. Its effect was studied by adding lecithin to soya bean oil (Merker, 1959; Drozdowski and Zajac, 1977) and to highly purified fatty acids (Klimmek, 1984) and a decrease in reaction rate was noted in both cases. Chu et al. (1992) attempted to correlate the residual phosphorus content of soya bean oils that had undergone different laboratory refining steps with their rate of hydrogenation and noted lower rates of high phosphorus oils and a wide spread in their data (Szukalska, 2000). This spread was subsequently explained qualitatively by the observation that hydratable phosphatides do indeed inactivate the hydrogenation catalyst, but that nonhydratable phosphatides are virtually inert in this respect (Dijkstra and Maes, 1991). This observation must also be taken into account when interpreting the results reported by Drigalla et al. (1993) when hydrogenating TOP-oil. They note a decrease in rate of hydrogenation, but conclude that this is mainly due to the difference in sulfur content of the various samples.

The review (Irandoust and Edvardsson, 1993) also mentions the strong rate retarding effect of soaps and the lesser effect of free fatty acids. It quotes Drozdowski and Goraj-Moszora (1980) who report the observation that hydrogenating in the presence of bleaching earth can partially restore the catalytic activity presumably by converting soaps into free fatty acids. Another explanation of this observation might be that the oil contained another catalyst poison that was adsorbed by the bleaching earth. The experiment whereby soaps were added to poison-free oil was apparently not carried out.

The review (Irandoust and Edvardsson, 1993) does not mention that the hydrogenation of oil containing free fatty acids causes these acids to be preferentially hydrogenated (P.J. Maes, personal communication) and that, simultaneously, *trans* isomer formation in the triglycerides is somewhat suppressed (Dijkstra, 2006). It could be that the free fatty acids compete with the monoenes for active catalyst sites and, thus, reduce their concentration and as a consequence their isomerisation.

Among the nonnickel catalysts, copper received a great deal of attention when it was observed (De Jonge et al., 1965; Kuwata et al., 1965; De Jonge and Erkelens, 1966; Koritala and Dutton, 1966; Okkerse et al., 1967) that this metal hardly catalyses the saturation of monoenes at all. It, therefore, could be used to reduce the linolenic acid content of soya bean oil and, thus, hopefully increase its stability; that is, provided residual copper levels could be maintained at very low levels. With respect to the mechanism induced by this catalyst, it was proposed quite early on that the actual species being hydrogenated could well be a conjugated polyene and that conjugation should precede hydrogenation (Koritala et al., 1970). For this conjugation, a Horiuti-Polanyi mechanism was proposed (Koritala, 1970) involving temporary addition of a hydrogen atom followed by its return to the catalyst. As pointed out much later (Dijkstra, 2002b), this proposal could only partially explain experimental observations, so that a different conjugation mechanism involving hydrogen abstraction followed by hydrogen addition was suggested.

In edible oil modification, noble metals have also been studied as hydrogenation catalysts, but so far, the care required for their recovery has prevented them from being used industrially. However, in this context, two developments should be mentioned. The first development concerns the "supercritical" hydrogenation (Härröd and Møller, 1996) in which the substrate to be hydrogenated is dissolved into a solvent such as, for example, supercritical propane, with which hydrogen is fully miscible. This way, the hydrogen concentration can be increased by orders of magnitude and provided the catalyst is sufficiently active, the reactor volume can be decreased by orders of magnitude.

However, the high hydrogen concentration affects the selectivity of the reaction so that it looked as if the process is only suitable for full hydrogenation. This requires a very active hydrogenation catalyst and noble metals, such as palladium or platinum, have been suggested to this end. Their high price should be less of a deterrent in the case of "supercritical" hydrogenation, where the catalyst is in a fixed bed and can easily be recuperated for regeneration. However, more recent developments in this field (Härröd et al., 2004) claim that during the hydrogenation of rapeseed oil, when "all the polyunsaturated fatty acids are hydrogenated to monounsaturated fatty acids, no *trans* fatty acids and no saturated fatty acids are formed." The patent concerned (Härröd et al., 2005) does indeed describe examples supporting this claim, but the productivity in these examples is a few orders of magnitudes lower than for current industrial hydrogenations. The high pressure requirements, the increased size, and the amount of noble metal catalyst required, may well increase the investment to unacceptably high levels.

4.2.3.2 Hydrogen

If a hydrogenation plant is located reasonably close to a hydrogen grid, purchasing hydrogen from the consortium

operating this grid is by far the most convenient and often the cheapest way of providing this plant with one of its essential raw materials. An additional advantage is that the pipelines act as a buffer vessel that can take care of peak demands in the hydrogenation plant.

Another supply system that takes care of peak demands involves hydrogen under pressure and stored in cylinders and liquefied hydrogen. The former requires little infrastructure and, therefore, is suitable for low and/or intermittent demand. The latter is particularly suited for larger hydrogenation plants situated sufficiently near a hydrogen source with liquefaction facilities.

Other hydrogenation plants have to generate their own hydrogen and several processes have been developed to this end:

- As mentioned by Kaufmann (1939), Normann had difficulty in obtaining the hydrogen required by his newly invented process (1903), so on his initiative a retort was built in which glowing iron swarf was allowed to react with water vapour; not surprisingly this process was short lived.
- Another process is based upon the thermal dissociation of ammonia (Van de Erve et al., 1948). The process itself is reasonably cheap, but it requires a rather expensive gas separation and hydrogen purification step (Allmendinger, 1979).
- The steam reforming process converts natural gas, LPG, or naphtha and steam into synthesis gas in the presence of a nickel catalyst at a temperature in the range of 800 to 900°C. Then the carbon monoxide present in this synthesis gas is converted to carbon dioxide by reaction with steam in a shift reactor, and the gas is cooled to condense water still present. If the feedstock contains sulfur, purification is necessary. Small amounts of dihydrogen sulfide can be removed by chemisorption in a zinc oxide bed. Large amounts of dihydrogen sulfide can be removed by washing. Finally, the hydrogen is purified in a pressure swing adsorption (PSA) unit using aluminium silicate molecular sieves (Pedersen and Agertoft, 1999).
- Methanol can also be used as the raw material for hydrogen production (Voeste, 1973). Cracking in the presence of water and a copper catalyst is carried out at 250 to 300°C, which is much lower than needed for the steam reforming of hydrocarbons and, thus, leads to a much lower carbon monoxide content. Therefore, a separate shift reactor is not needed and the carbon monoxide present in the tail gas of the PSA unit can be used as fuel to heat the reagents.
- Electrolysis of water is also widely used for the production of hydrogen (Vanden Borre, 1983). The process has the advantage that its output can be varied between 0 to 100% but the investment needed for the electrolyser does not profit from an economy of scale.

As shown in Table 4.5 the consumables of the various processes differ widely. Accordingly, choosing the optimal process depends very much on local conditions, such as the cost of electricity and an outlet for oxygen. Generally speaking though, electrolysis is the preferred process for smallish (say <200 Nm^3/h) and especially intermittent demand. The steam reforming process is usually the cheapest process for large and continuous demand; the methanol cracking process, requiring a lower investment than the steam reforming process is often competitive (Daum, 1993).

Modern hydrogen plants produce 99.99% pure hydrogen. This is better than actually required by the hydrogenation process. For this process, the absence of sulfur as hydrogen sulfide and carbon monoxide is essential since these gases react with nickel. The steam reforming process therefore includes a desulfurisation step by hydrogenation on cobalt and molybdenum trioxide (Allmendinger, 1979) and conversion of residual carbon monoxide to methane is also mentioned (Voeste, 1973). Water should also be absent since it causes hydrolysis of the oil. The presence of some nitrogen and/or methane does not affect the hydrogenation reaction, but these gases do accumulate in the headspace of the autoclave and can cause erroneous hydrogen pressure readings.

4.2.3.3 Triglyceride oil

Impurities in the oil to be hydrogenated can also affect the nickel catalyst and, therefore, the hydrogenation process and product. They can totally inactivate the catalyst added so that more catalyst must be added to initiate the hydrogenation reaction. They can partially inactivate the catalyst so that the hydrogenation proceeds as if there were

TABLE 4.5 Consumables of hydrogen production processes for 100 Nm^3 high purity hydrogen

	Ammonia	Steam Reforming	Methanol	Electrolysis
Raw material	51 kg ammonia	49 Nm^3 natural gas	65 kg methanol	80 kg water
Fuel	420 MJ			
Electricity	1 kWh	2.8 kWh	5.5 kWh	420–460 kWh
Cooling water	1.4 m^3		1.3 m^3	
Boiler feed or demi-water		180 kg	36 kg	
Export steam		135 kg		

less catalyst. Less catalyst will slow down the hydrogenation reaction so that the hydrogen concentration increases so that dissociation of the half-hydrogenated intermediate is suppressed in favour of full hydrogenation. A less selectively hydrogenated product with eventually a higher stearic acid content and a lower *trans* content is the result. On the other hand, the impurities can also poison the catalyst, which may promote *trans* isomer formation. Accordingly, impurities in the oil increase the cost, reduce plant capacity, and/or change the product properties. They should be eliminated or standardised by the standard purification processes, such as degumming and bleaching. Standardisation is also possible by adding impurities, such as lecithin (Merker, 1959), to control the hardness and plastic range of partially hydrogenated soya bean oil. Of course, the effect of these impurities is larger when a small amount of fresh catalyst is used than when a larger amount of recycled catalyst is used.

4.2.4 Industrial hydrogenation processes

Although continuous hydrogenation processes employing a fixed-bed catalyst have been described (Rosen, 1985) and have been evaluated even earlier (Osteroth, 1968), gradual changes in catalyst properties during operation leading to changes in product properties have prevented such processes from being used for the partial hydrogenation of triglyceride oils to any appreciable extent. For the hydrogenation of fatty acids, several plants operate continuous slurry reactors and claim reduced catalyst usage.

For triglyceride oils, industrial converters tend to be batch reactors, and since these autoclaves with their hydrogen dissolution systems are relatively expensive, savings in investment can result from using simpler and cheaper vessels for operations such as heat exchange that do not require an autoclave. The aim of the design of a hydrogenation processing plant as illustrated in Figure 4.4 is to ensure that the autoclave is used for no other purpose than the actual hydrogenation reaction.

Because hydrogen can form highly explosive mixtures with air, hydrogenation autoclaves are preferably located outside, so that wind can disperse any hydrogen leaking from the vessel and its piping; nevertheless, most hydrogenation plants are inside a building. Because the melting point of oils and fats increases on hydrogenation and can reach values of some 70°C on full hydrogenation, the piping connecting the autoclave to other vessels is preferably steam-jacketed or steam-traced. The filtration operation should be carried out inside a building, especially if a frame and plate type filter is used. If the autoclave is also inside a building, extensive safety measures are necessary.

As shown in Table 4.6, the ignition energy is very low which means that even small sparks resulting from static electricity suffice to cause an explosion. The table also shows that a concentration of only 4% hydrogen by volume in air will already support an explosion. Consequently, the risk of explosions inside the building, but outside the autoclave, is much larger than the risk of an explosion inside a reactor in operation.

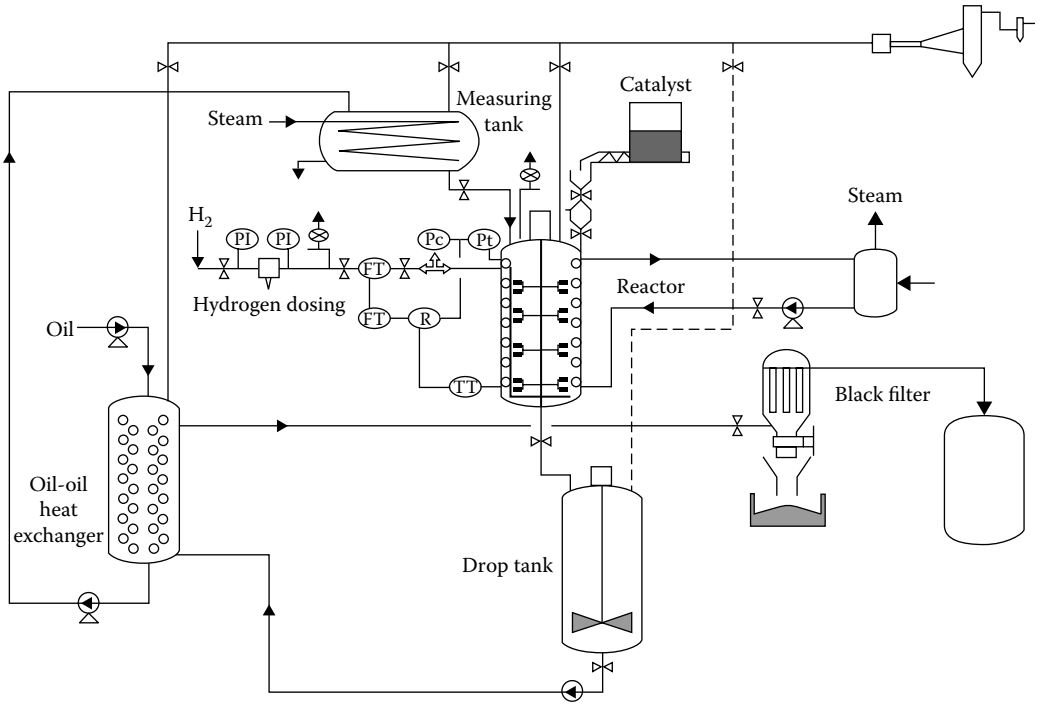


FIGURE 4.4 Simplified flow sheet of a semicontinuous hydrogenation plant.

TABLE 4.6 Safety data for hydrogen gas

Property	Units	Value
Density	kg/m^3	0.08
Self-ignition	°C	858
Minimum ignition energy	mJ	20
Ignition limits	%	4-75
Flame temperature	°	2318
Detonation limits in air	%	18–59

As explained by Roquette (1994), the design of the building should incorporate a central exit for exhaust air with a hydrogen detector. This exit must be at the highest point of the building to prevent pockets of an explosive hydrogen/air mixture being formed. For the evacuation of the reactor, a water ring pump is recommended, provided it is fitted with an automated water level control system.

The flow diagram of Figure 4.4 shows that the oil to be hydrogenated is first of all heated by oil-to-oil heat exchange by flowing over spiral tubes. The heat exchange vessel can be evacuated so that the oil is dried at the same time. This water removal is recommended since any water present causes some triglycerides to hydrolyse at the high temperature commonly used in the hydrogenation process. The resulting FFA will react with the nickel catalyst under formation of nickel soaps. This is a reversible reaction so metallic nickel will be regenerated, but its catalytic activity will not be restored at the same time.

From the heat exchange vessel, the oil is pumped to a measuring vessel from where the measured charge can be dropped into the actual autoclave. The measuring tank has steam coils allowing the oil to be heated if necessary. Measured amounts of catalyst can also be fed into the autoclave and the catalyst is held in suspension by the autoclave agitator that also ensures that the hydrogen dissolves into the oil. For temperature control, the autoclave has also been provided with cooling coils. From the autoclave, the hydrogenated batch is sent to a drop tank, which is also agitated to prevent the catalyst from settling. The hot hydrogenated oil is then pumped through the coils in the heat exchange vessel to the main catalyst filter and possibly to a downstream polishing filter as well (not shown in Figure 4.4).

Means should be provided to evacuate the autoclave not only to avoid the formation of an explosive air/hydrogen mixture but also to facilitate the oil transfer from the measuring tank into the autoclave, thus saving time, and to permit the catalyst to be sucked into the autoclave. Similarly, emptying the autoclave into the drop tank is faster if the latter has been evacuated.

4.2.4.1 Hydrogen dissolution systems

The most widely used hydrogen dissolution system is the Rushton or Flat Blade Turbine. A converter fitted with such an agitator is shown in Figure 4.5. Hydrogen gas is fed into the autoclave underneath this agitator and divided into small bubbles, which then dissolve when moving upwards through the oil. Hydrogen that is not dissolved collects in the headspace, and a special impeller may be fitted just below the oil surface to encourage hydrogen in the headspace to dissolve.

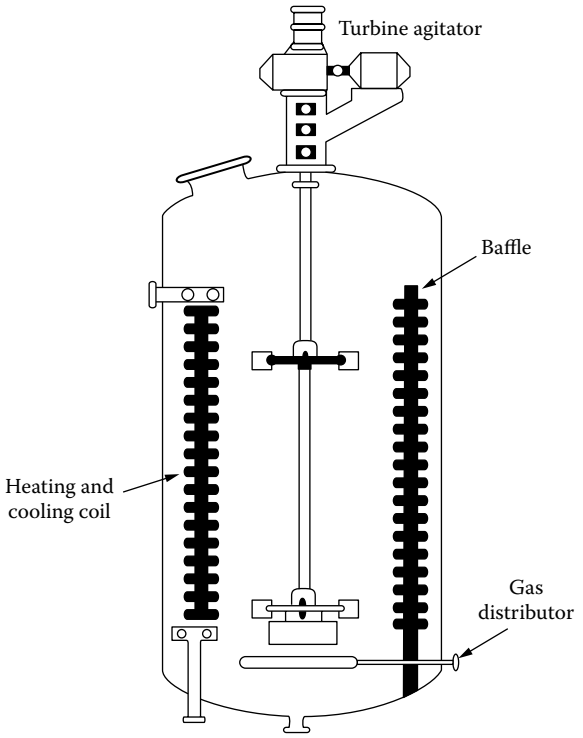


FIGURE 4.5 Hydrogenation converter.

Another way to prevent a pressure build-up that would eventually cause the hydrogen supply to be entirely closed, comprises an external recycling loop. Hydrogen is collected from the headspace by a pump that passes the hydrogen through a cooler and a condenser before returning it underneath the agitator. This recycling is especially important in the beginning of the hydrogenation process when the reaction may be sluggish (induction period) and/or at the end of full hydrogenations when the reactivity of the reaction mixture has substantially decreased.

An agitator that does not require such a recycling system because it has been designed to pull hydrogen from the headspace and dissolve it into the oil has been patented by Praxair, the former Linde Division of Union Carbide (Litz, 1984); the device is depicted in Figure 4.6. When the impeller rotates, it forces liquid down and, in doing so, it creates a vortex. Gas is pulled into the vortex and with baffles causing turbulence and high shear, the gas is dissolved into the oil.

A problem with this kind of agitator is that it only functions properly at a certain fixed liquid level. Given the quite sizeable thermal expansion coefficient of triglyceride oils (with a density decrease of some 0.00064 g/cm^{3}K (Rodenbush et al., 1999) or even somewhat higher (Noureddini et al., 1992), this means that the oil level in

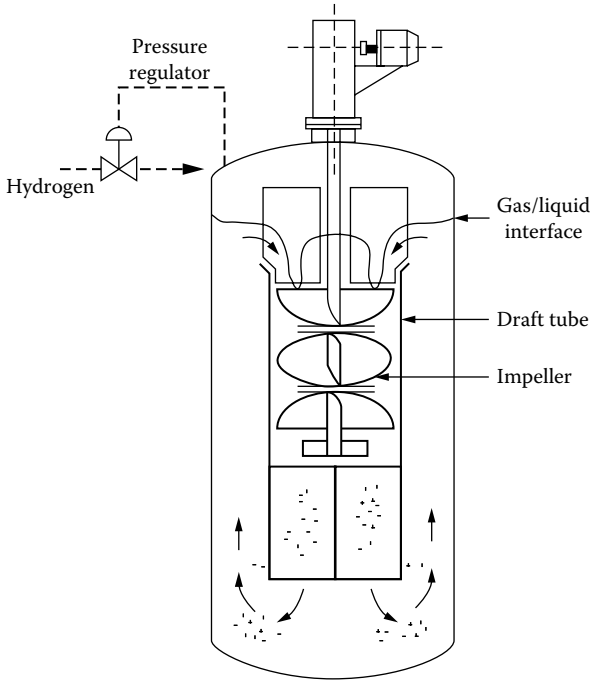


FIGURE 4.6 Helical impeller.

the autoclave will rise considerably during a hydrogenation run that may start at 150°C and finish at 220°C. To this end, a variable level agitator has been developed that maintains the same flow patterns and mixing performance as its fixed level counterpart, but ingests gas via a hollow shaft and eductor tubes rather than directly by vortex ingestion.

A third type of dissolution system is provided by the BUSS loop reactor (Duveen and Leuteritz, 1982). As shown in Kellens (2000, Figure 5.5), an external pump circulates the oil over the autoclave through a Venturi tube that sucks in hydrogen from the headspace and dissolves it into the oil. The loop also contains a heat exchanger for oil temperature control. Urosevic described some improvements to the BUSS loop reactor in 1986.

4.2.4.2 Hydrogen transfer and diffusion of reagents

The solubility of hydrogen gas in triglyceride oil is quite low. As shown in Table 4.7, 1 kg of oil or about 1.13 mol will dissolve only some 80 mg hydrogen or 4 mmol at hydrogenation temperature and atmospheric pressure. Consequently, hydrogen gas must be dissolved into the oil during the hydrogenation process.

All dissolution systems listed above have in common that their effectiveness can be described by an equipment parameter k_La, the volumetric liquid-side mass transfer coefficient, which is the product of the liquid phase mass transfer coefficient k_L (expressed in m/s) and the specific interfacial area a (expressed in m^2/m^3). Accordingly, k_La has the dimensions of sec^{-1} (Koetsier, 1997). It can be measured experimentally by measuring the rate (R) of hydrogenation at high catalyst concentration when the concentration of the dissolved hydrogen ($[H_2]_{bulk}$) can be assumed to be very low according to:

$$R = k_La\,([H_2]_{max} - [H_2]_{bulk}) \qquad (4.1)$$

where $[H_2]_{max}$ stands for the solubility of the hydrogen at that temperature, which has been determined independently (Wisniak and Stein, 1974; Andersson et al., 1974).

In practice, the rate of hydrogenation (R) is plotted against the catalyst loading (Ahmad et al., 1979). At low catalyst loading, this rate is first proportional to the catalyst loading, but at increased loading, the rate levels off to reach a plateau where the reaction is said to be "mass transfer controlled." The volumetric liquid-side mass transfer coefficient k_La is then estimated by dividing the rate at this plateau by the hydrogen solubility.

Because k_La is an equipment parameter, its value can vary over a wide range. For small laboratory reactors with highly efficient agitation, Koetsier (1997) estimates that "a maximum value for k_La of 1.2 s^{-1} would seem to be achievable." And indeed, a value of 1.1 s^{-1} has been reported for a laboratory reactor with an agitator speed of 750 rpm (Coenen, 1978), but a value as high as 1.8 s^{-1} has also been reported (Jonker, 1999).

Industrial reactors show much lower values. Bern et al. (1976) determined k_La values for 30 l, 500 l, and 24 m^3 reactors and found values as low as 0.05 s^{-1} for the industrial autoclave. If a stirring power of 2 kW/m^3 of oil is applied, Koetsier (1997) estimates k_La values of 0.10 to 0.15 s^{-1}. This is about one-tenth of what is possible in small-scale laboratory reactors. This has obvious implications for industrial hydrogenation processes.

Dissolving hydrogen into the oil is one aspect of hydrogen transfer. Another aspect is its diffusion towards the catalyst surface. If this were to be slow in comparison with

TABLE 4.7 Solubility of hydrogen in oil at atmospheric pressure

Temperature	(Schaffer and Haller, 1943)	(Tyutyunnikov and Novitskaia, 1958)	(Andersson et al., 1974)
40	4.7-5.4mL/100mL		
50		4.3-5.3 mg/100g	
60	6.8. mL/100mL		
120			2.9 mol/m^3
180			3.3 mol/m^3
220		7.3-8.6 mg/100g	

the volumetric liquid–side mass transfer coefficient k_La, this would have to be taken into account when studying reaction kinetics. However, in his exhaustive treatment of the subject, Koetsier (1997) clearly demonstrates that the rate constant pertaining to the transfer of hydrogen to the catalyst particles is an order of magnitude larger than k_La. Accordingly, in industrial hydrogenations, the effect of the mass transfer rate from the bulk of the oil to the catalyst particles, therefore, can be disregarded.

But what about the diffusion of the fatty reaction partners? In this context, Jonker (1999) reports original and highly relevant results. The experimental side of his work involves both hydrogenation experiments and an independent determination of the effective intraparticle diffusion coefficients via tracer pulse experiments using an HPLC method with a column filled with hydrogenation catalyst. His experimental findings allow him to conclude that in the hydrogenation of triglycerides, the reaction is controlled by their diffusion and that hydrogen diffusion is not a limiting factor. For fatty acid methyl esters, the situation is different in that intraparticle hydrogen diffusion may also be a limiting factor.

These findings explain why the rate of hydrogenation at high hydrogen concentration is lower than would be expected on the basis of this high concentration. At low hydrogen concentration, as inevitable in industrial reactions, this low concentration is the main factor limiting the rate of reaction, but as and when this limitation is reduced by an increasing hydrogen concentration, the intraparticle diffusion of the triglycerides becomes more and more important until it becomes the main limiting factor.

4.2.4.3 Hydrogenation process conditions

Industrial hydrogenations catalysed by a supported nickel catalyst require a starting temperature of at least 150°C. At lower temperatures, the reaction may be very slow to get underway, making the process and its duration less predictable. If the oil being hydrogenated has been bleached, as is standard in the U.S., a lower starting temperature of 110 to 150°C may be used. If the previous batch, but one which is used to preheat the batch to be hydrogenated, reached a temperature above 200°C, the starting temperature of >150°C can be reached by oil-to-oil heat exchange. If this does not lead to a sufficiently high oil temperature, preheating the batch in the measuring vessel with the steam coils is advocated.

During the hydrogenation process, the temperature is generally allowed to rise to 200 to 220°C and controlled at that level, but a lower temperature of 180 to 190°C has also been mentioned (Young, 1994). A considerably lower temperature (<70°C) is advocated by Van Toor et al. (2005) to arrive at a lower *trans* isomer content in the hydrogenation product. As pointed out in a recent review on *trans* isomer formation (Dijkstra, 2006), the effect is most noticeable for low degrees of hydrogenation and the low temperature also causes an increase in the formation of saturated fatty acids; the catalyst loading mentioned in the patent makes this process quite expensive.

When fish oil is hydrogenated, cooling may be instigated at an earlier stage to prevent the formation of cyclic isomers by maintaining the temperature at around 150°C (Coenen and Wieske, 1967), but once the highly unsaturated fatty acid content has decreased to a value equal to "0.2% of the square of the initial iodine value," the temperature can safely be allowed to increase to levels common to vegetable oil hydrogenations (see also Patterson, 1978).

The hydrogenation reaction is highly exothermic: A drop in iodine value of 1 unit causes the oil temperature in the autoclave to rise by 1.6 to 1.7°C. Since the temperature at the beginning of the reaction is usually at least 150°C, since the temperature during the reaction should not exceed 220°C, and since a decrease in iodine value in excess of 40 units is not uncommon, the autoclave has to be cooled. Besides, cooling also allows the temperature to be controlled, which may be advantageous for product property control. As explained by Kokken (1993) and illustrated by Figure 4.3, temperature control can be achieved by allowing demineralised water to be evaporated inside the cooling coils when heat has to be removed from the autoclave and by closing the steam valve if heat removal has to be interrupted. This may cause the pressure inside the coils to increase to some 25 bars.

4.2.4.4 Catalyst usage

In the U.S., edible oil processors tend to use a small amount (0.03 to 0.05% by weight of fresh catalyst with 20 to 25% nickel) for each batch and discard this catalyst after it has been used once. This approach is now also being adopted in Europe, but formerly, processors preferred to use the hydrogenation catalyst more than once and for the less active, partially poisoned catalyst promoting *trans* isomerisation, re-use is imperative for cost reasons. Both systems have their advantages and disadvantages:

- Using a small amount of catalyst makes it more susceptible to catalyst poisons present in the oil. Since the amounts of poison present in the oil may vary from batch to batch, the degree of poisoning may vary from batch to batch. Since catalyst poisoning affects the catalyst properties (activity and selectivity) in different ways, the catalytic properties will vary from batch to batch. Consequently, the poison level has to be kept as low as possible by bleaching the oil before hydrogenation.
- Catalyst that has been used only once may still be catalytically active and discarding this activity is a cost element.
- Partially poisoned catalyst will be less active than fresh catalyst, so more is needed. Moreover, the catalytic activities of partially poisoned catalyst will

differ from those of fresh catalyst. Accordingly, a kind of steady state with a hopefully constant degree of poisoning should be aimed for.
- Using a catalyst leads to catalyst attrition and this may cause the filterability to decrease; this may become so poor that plant productivity can only be maintained by discarding the entire amount of catalyst in circulation; this is also a cost element.
- Collecting used catalyst for re-use and providing a used catalyst dispensing system next to the system for the fresh catalyst is yet another cost element.
- Re-use also requires accurate administration and the execution of a set of procedures.

Two re-use systems are employed in industry. The most common system involves discarding a fraction of spent catalyst and replenishing this with fresh catalyst, and aiming at a steady state of catalytic properties. Another system involves using a batch of catalyst corresponding to a full filter over and over again, but using a bit more catalyst each time the batch is re-used to compensate for the loss in catalytic activity, and discarding the entire batch when its catalytic activity has been exhausted or its filtration performance has become unacceptably low. This latter system has the advantage of really exhausting the catalytic potential of the catalyst, but suffers from the disadvantage that increasing the catalyst loading on re-use does not take into account the fact that poisoning affects the various catalysed reactions in a nonuniform manner.

Consequently, the industry is moving towards a situation whereby highly active catalysts are used only once, and this implies bleaching the substrate. On the other hand, *trans* isomer promoting, prepoisoned catalysts that are less active so that a higher catalyst dosage is needed, are preferably re-used for cost reasons. Since the catalyst is already partially poisoned, further poisoning during usage, if any, will be marginal and, therefore, will only affect the catalytic activity but not the selectivities.

4.2.4.5 Posttreatment

The drop tank receiving the batch after the hydrogenation is deemed to be complete serves several purposes. It frees up the expensive autoclave so that this can be used for the hydrogenation of the next batch, and it also serves as a buffer tank in front of the filter. Since the oil in the drop tank still contains the hydrogenation catalyst, the tank is provided with an agitator to keep the catalyst in suspension.

As shown in Figure 4.3, a pump transfers the hydrogenated oil from the drop tank through the coils in the heat exchange vessel to the so-called "black filter." Because of the relatively high flow rate inside these coils, there is little risk of the catalyst settling inside these coils. The type of filter shown in Figure 4.3. is a pressure leaf filter requiring a precoat. This type of filter has the advantage of minimising the exposure of plant operators to nickel and requiring less labour than frame and plate filters. On the other hand, the large heel of this type of filter makes it unsuitable if frequent oil type changes are planned and the presence of a filter aid, such as, for instance, diatomaceous earth, complicates the re-use of the catalyst in the filter. Since the use of a precoat and filter aids are a cost element, catalyst manufacturers aim at ensuring a narrow particle size distribution between about 5 to 10 micron and advocate using the catalyst itself as a pre-coat (Ariaansz, 1993). Instead of a "black filter," a centrifugal separator can also be used to remove most of the catalyst (Logan, 1996). This method also avoids the need to use a filter aid and is highly suitable when the catalyst is to be re-used.

If the catalyst is to be re-used, it has to be collected, reslurried and stored as a slurry in a heated, agitated catalyst dispensing vessel that can hold a full filter load after it has been reslurried. If several different catalysts are in use, each catalyst requires its own catalyst dispensing vessel and each vessel requires its own dispensing system. Fitting these vessels with load cells and dispensing by weight has been found to be a convenient way of charging the desired amount of catalyst slurry to the autoclave.

If no re-use of the catalyst is contemplated, the spent catalyst still has to be disposed of. To this end, specialised firms purchase and process the spent catalyst to recuperate the nickel metal (Hennion, 1986).

The filtered oil still contains some 5 to 20 ppm nickel, both as nickel soap and as metallic nickel. This can be removed effectively (Kopp et al., 1989; 1991) by mixing a small amount of water into the oil, adding bleaching earth, drying, and removing the earth by filtration. (*Nota bene:* The resulting spent earth contains nickel and is not suitable to be mixed with meal. Depending upon local regulations, it may even be regarded as "hazardous waste.'")

Using a citric acid solution instead of water has the advantage that the acid converts the soaps into free fatty acids that will be removed during deodorisation. The use of a "white filter" as a polishing filter will also remove nickel present as particulate matter (Kokken, 1993).

4.2.5 Hydrogenation process and product control

4.2.5.1 Process control

Process control in hydrogenation should aim for reproducibility and, thus, ensure that subsequent batches of the same grade have almost identical compositions and, thus, properties. This means that both the extent of hydrogenation (drop in IV) and the various selectivities that characterise a hydrogenation run have to be controlled. In addition, this control must be quite accurate since small changes in IV and *trans* isomer content can have a large

effect upon the solid fat content (SFC), as illustrated by the equation below (P.J.A. Maes, personal communication):

$$\Delta N_{20} = -1.2\ \Delta(IV) + 0.5\ \Delta(\%trans) \qquad (4.2)$$

This equation shows that for a partially hydrogenated soya bean oil (melting point 35 ºC), a drop in IV of 1 unit will increase the SFC at 20 ºC (N_{20}) by more than 1.2% since this IV drop will also be accompanied by an increase in *trans* isomer content.

Process control should also deal with variations in catalyst activity and catalyst activation and with a variable and often unknown content of catalyst poisons in the oil. It is clear that product reproducibility can only result if the batch temperature and the hydrogen concentration in the oil follow standard profiles as a function of the extent of hydrogenation, which can be determined by calculating the accumulated hydrogen consumption from the hydrogen flow measurement.

According to a method described by Colen et al. (1990), good product reproducibility can be achieved if pertinent batch parameters (temperature and hydrogen concentration) are controlled as a function of the IV achieved; they replace the "time" variable with the "degree of hydrogenation" variable. To this end they construct a database comprising:

- The extent of hydrogenation as calculated from the measured hydrogen flow. This requires accurate flow measurement, for which the diaphragm gas meter is the most suitable type of meter (Kellens, 2000). It measures gas volumes over a wide range (1/100) with an accuracy greater than 99% and is a worthy successor to the turbine gas meter used previously (Kokken, 1993).
- Temperature.
- Rate of hydrogenation by calculating the rate of drop in IV.
- Hydrogen concentration in the oil.

The dissolved hydrogen concentration is calculated from the mass transfer Equation 4.1 given above (Koetsier, 1997), whereby:

$$[H_2]_{max} = p.m(T) \qquad (4.3)$$

where:

$m(T)$ = Henry's law constant
p = pressure

Having obtained a dataset that is characteristic of a certain hydrogenation grade, Colen et al. (1990) then ensured that subsequent runs aiming to produce that grade followed the same temperature and hydrogen concentration profiles. They controlled the temperature by adjusting the extent of cooling and achieved the same hydrogen concentration by varying the pressure in the autoclave. They also mention that control is more critical in the final stages of the hydrogenation than in the beginning.

Thus, if for one reason or other a hydrogenation run turns out to be slow, this will be signalled by a higher than normal hydrogen concentration, as shown by rewriting the mass transfer Equation 4.1 as:

$$[H_2]_{bulk} = [H_2]_{max} - \frac{R}{k_1 a} \qquad (4.4)$$

Accordingly, Colen et al. (1990) then decrease the autoclave pressure to decrease the driving force for hydrogen dissolution, as a result of which the dissolved hydrogen concentration will fall. Of course, this will slow down the rate of reaction even further, so that the operator will instinctively increase the pressure to speed up the reaction and, thus, gain time lost. However, he should have decreased the pressure; this action will ensure that the various selectivities are controlled at around the values shown by the dataset and, thereby, lead to a similar final product.

4.2.5.2 Product control

To ensure that final product properties are even closer to specifications, it is advisable to interrupt the hydrogenation before the expected end point and take a sample for quick analysis. One method of quick analysis could be the determination of IV and possibly *trans* isomer content by Fourier transform near infrared analysis (Cox et al., 2000). Another method could be the fast solid fat content measurement by pulse-NMR (Rutledge et al., 1988) involving the crystallisation of the sample in liquid nitrogen. In both cases, tables based on past performance should be used to calculate how much more hydrogen is to be added to the autoclave to obtain the target product.

After the batch has been filtered, a sample is usually fully analysed for fatty acid composition, *trans* isomer content, and SFC to decide whether or not the batch meets the batch specification. If so, it is sent to the storage tank for that particular hydrogenation grade. If not, various actions can be taken:

- If it is clear that the batch has been insufficiently hydrogenated, it can be rehydrogenated to reach the specification of the original grade.
- If it has been hydrogenated too far and, if a grade exists that is further hydrogenated, it may be rehydrogenated to meet the specification of that particular grade; if this particular grade is, for instance, fully hydrogenated oil, this is a safe and elegant way of disposing of out-of-specification material.
- If the batch is only just outside specification, a so-called "compensation batch" can be attempted in the hope that the mixture of these two batches will be more or less within specification. It often isn't

and, even if it is, the mixture will still have a different triglyceride composition, so this action is not recommended.
- If there is a general grade of nondescript fatty matter originating from recuperating fatty matter from margarine, the batch may be downgraded to this grade. This is the safest, but often also most expensive solution.

4.2.6 Miscellaneous hydrogenation processes

4.2.6.1 Hydrazine as a hydrogen source

When an unsaturated fatty acid or glyceride is dissolved in, for instance, ethanol, and hydrazine hydrate is added, and air is blown through the reaction mixture, some of the double bonds will be saturated (Schilling, 1961). Moreover, the double bonds that are not saturated do not isomerise positionally or geometrically. The hydrogenation mechanism has not been fully elucidated, but the formation of a diimide (HN = NH) resulting from the reaction of hydrazine with oxygen has been proposed. The hydrogen that is also observed among the reaction products could originate from a side reaction (Bitner and Dutton, 1968):

$$N_2H_4 + \tfrac{1}{2}O_2 \rightarrow H_2O + N_2 + H_2$$

The rates at which the various double bonds react have been studied by Schilling (1963) who established that terminal double bonds react much faster than midchain ones, but that otherwise all isolated double bonds were about equally reactive; two conjugated double bonds were found to react only slightly faster than a single isolated double bond (Ratnayake et al., 1990).

Despite the anticipated reduction in cost of hydrazine (Dutton et al., 1963), hydrazine is not used as a hydrogen source in industrial hydrogenations. For speciality products, its use could be considered, since it does not generate *trans* isomers. It could, for instance, be used for the partial hydrogenation of soy lecithin and thus improve its oxidative stability.

4.2.6.2 Catalytic transfer hydrogenation

In 1987, Arkad et al. reported the use of formic acid as the hydrogen donor for the hydrogenation of soya bean oil methyl esters. They tried several catalysts, but a palladium-on-carbon catalyst was the only one found to be effective. They observed a linolenic acid selectivity of around 5 and linoleic acid selectivity in excess of 100 and tentatively explained this observation by assuming a strongly hydrogen-deficient catalyst surface caused by the poor miscibility of the organic fatty acid methyl ester (FAME) and the aqueous potassium formate solution; the need for vigorous agitation and the relatively long reaction time support this assumption.

Basu and Chakrabarty showed (1966) that nickel can also catalyse the transfer hydrogenation by using an alcohol as a hydrogen donor, but despite the high temperatures used (200 to 250°C), they did not achieve full hydrogenation. Subsequently, Vijayalakshmi et al. (1988) used decalin (decahydronaphthalene) as a solvent *cum* hydrogen donor and again Pd/C as a catalyst. Since their reaction mixture was homogeneous, they observed both a faster reaction and full conversion. At partial conversion (0.5 h), the reaction product of the hydrogenation of methyl oleate showed methyl stearate (47.8%), *trans* monoenoate (35.0%), and *cis*-monoenoate (17.2%). Since the reaction was carried out under reflux conditions (b.p. decalin is 155.5°C) the *cis*-*trans* equilibrium was not quite reached.

It has been mentioned that the catalytic transfer hydrogenation process has the industrial advantage of not using gaseous hydrogen and operating at low temperature and atmospheric pressure (Šmidovnik et al., 1992), but, nevertheless, the process is not in use industrially.

4.2.6.3 Electrocatalytic hydrogenation

A hydrogenation process employing hydrogen produced *in situ* has also been reported (Yusem and Pintauro, 1992). It employs a Raney nickel powder cathode (Yusem et al., 1996) and a dispersion of oil in a water/*t*-butanol/tetraethylammonium/*p*-toluenesulphonate electrolyte at atmospheric pressure and moderate temperatures. With soya bean oil as the substrate, a brush hydrogenation was achieved with low linoleic acid and *trans* selectivities. Employing a Pd-black powder cathode and electrodepositing a second metal onto the Pd-black increased the selectivities (An et al., 1999) and produced a partially hydrogenated, low-*trans* soya bean oil with a lower hydrogenation odour than commercial oil (Warner et al., 2000). It was recommended for use in deep frying, etc., but so far, this recommendation has not been adopted by industry.

4.2.6.4 Hydrogenation of solutions of oil

In 1914, Maryott already suggested dissolving fatty acids or oil in a solvent, such as an alcohol, acetone, ether, and petroleum ether, or in solvents that are nowadays regarded as being potentially unhealthy, such as benzene, chloroform, carbon disulfide, or carbon tetrachloride, and hydrogenating their solution. The catalyzer of the palladium group he recommends is "preferably deposited out on some finely divided material as asbestos." The advantages claimed include an increase in the speed of the reaction "due to free diffusion of the reacting substances."

Quite some time later, Kaufmann (1958) claimed oils hydrogenated in a solvent containing less than 2% isoacids, but subsequently (Kaufmann and Mukherjee, 1965) he put this claim into perspective by explaining that this claim had been based on a lead salt separation of isomers, whereas determining the *trans* isomer content by IR also takes account of liquid *trans* isomers.

Disregarding some intermediate publications, interest in the hydrogenation of oil solutions revived when Härröd (Härröd and Møller, 1996) showed that the rate of hydrogenation can be vastly increased by dissolving the hydrogenation substrate in solvents, such as lower hydrocarbons, in the supercritical or near-critical state because then hydrogen is fully miscible with these solvents. Accordingly, the reaction is no longer limited by the hydrogen transport and, thus, very fast. Similarly, selectivities resulting from hydrogen transport limitations no longer pertain. Consequently, the supercritical process using standard catalysts will not supersede current selective hydrogenation processes, but could eventually be profitably employed to meet the demand for fully hydrogenated products. As mentioned above, the process claiming high selectivity and low *trans* isomer formation (Härröd et al., 2004) turns out to have a very low productivity (Härröd et al., 2005).

References

Ahmad, M.M. et al. (1979), Palladium-catalyzed hydrogenation of soybean oil, *J. Am. Oil Chem. Soc.*, **56**, 571–577.

Albright, L.F. (1965), Quantitative measure of selectivity of hydrogenation of triglycerides, *J. Am. Oil Chem. Soc.*, **42**, 250–253.

Allmendinger, R. (1979), Rationelle Wasserstofherstellung für fettchemische Prozesse, *Fette Seifen Anstrichm.*, **81**, 112–116.

An, W. et al. (1999), The electrochemical hydrogenation of edible oils in a solid polymer electrolyte reactor. II Hydrogenation selectivity studies, *J. Am. Oil Chem. Soc.*, **76**, 215–222.

Andersson, K. et al. (1974), Diffusivities of hydrogen and glyceryl trioleate in cottonseed oil, *J. Am. Oil Chem. Soc.*, **51**, 171–177.

Applewhite, T.H. (1981), Nutritional effects of hydrogenated soya oil, *J. Am. Oil Chem. Soc.*, **58**, 260–269.

Ariaansz, R.F. (1993), Hydrogenation theory, in *Proceedings of the Word Conference on Oilseed Technology and Utilization*, Applewhite, T.H., Ed., AOCS Press, Champaign, IL, 166–172.

Arkad, O. et al. (1987a), Catalytic transfer hydrogenation of soybean oil methyl esters using inorganic formic acid salts as donors, *J. Am. Oil Chem. Soc.*, **64**, 1529–1533.

Augustine, R.L. and Van Peppen, J.F. (1970), Some recent developments in the homogeneous catalyzed hydrogenation of olefins, *J. Am. Oil Chem. Soc.*, **47**, 478–481.

Bailey, A.E. (1946), Flavor reversion in edible fats, *Oil & Soap*, **23**, 55–58.

Bailey, A.E. (1949), Some additional notes on the kinetics and theory of fatty oil hydrogenation, *J. Am. Oil Chem. Soc.*, **26**, 644–648.

Bailey, A.E. and Fisher, G.S. (1946), Modifications of vegetable oils. V. Relative reactivities toward hydrogenation of the mono- di- and triethenoid acids in certain oils, *Oil & Soap*, **23**, 14–18.

Baltes, J. (1970), Selektive Härtung von Fetten und Fettsäuren mit Sulfiden der Übergangsmetalle als Katalysatoren, *Fette Seifen Anstrichm.*, **72**, 425–432.

Baltes, J. (1972), Process for the selective hydrogenation of fats and fatty acids, US Patent 3,687,989.

Barlow, S. (2004), Raw material supply issues within the fish oil industry, *Paper presented at the 3rd EuroFedLipid Congress, Edinburgh*, Abstracts page 5.

Basu, A.K. and Chakrabarty, M.M. (1966), Studies on conjugated hydrogenation: Nickel catalyst with alcohols, *J. Am. Oil Chem. Soc.*, **43**, 119–121.

Beckmann, H.J. (1983), Hydrogenation practice, *J. Am. Oil Chem. Soc.*, **60**, 282–290.

Beers, A.E.W. et al. (2004), Lowering the *trans*-content in edible oils, *Paper presented at the 3rd EuroFedLipid Congress, Edinburgh*, page 62 of the abstracts.

Beers, A.E.W. et al. (2005), Low *trans* hydrogenation of edible oils — New developments by Engelhard, *Paper presented at the 26th ISF World Congress and Exhibition, Prague*, page 78 in the abstracts.

Beers, A.E.W. and Mangnus, G. (2004), Hydrogenation of edible oils for reduced *trans*-fatty acid content, *inform*, **15**, 404–405.

Bern, L. et al. (1975), Kinetics of the hydrogenation of rapeseed oil: II. Rate equations of chemical reactions, *J. Am. Oil Chem. Soc.*, **52**, 391–394.

Bern, L. et al. (1976), Mass transfer and scale-up in fat hydrogenation, *J. Am. Oil Chem. Soc.*, **53**, 463–466.

Beyens, Y. and Dijkstra, A.J. (1983), Positional and triglyceride selectivity of hydrogenation of triglyceride oils, in *Fat Science 1983, Proceedings of 16th ISF Congress, Budapest*, Holló, J., Ed., Akadémiai Kiadó, Budapest, 425–432.

Bitner, E.D. and Dutton, H.J. (1968), Hydrogen formation during hydrazine reduction of oleic acid, *J. Am. Oil Chem. Soc.*, **45**, 603–606.

Boelhouwer, C. et al. (1956), Problems of selectivity in the hydrogenation of linoleic acid esters, *J. Am. Oil Chem. Soc.*, **33**, 143–146.

Bushell, W.J. and Hilditch, T.P. (1937b), The course of hydrogenation in mixtures of mixed glycerides, *J. Chem. Soc.*, 1767–1774.

Cahen, R.M. (1979), Hydrogenation process, US Patent 4,161,483, assigned to Labofina S.A.

Cahen, R.M. (1980), Hydrogenation catalyst and hydrogenation process, US Patent 4,229,361, assigned to Labofina S.A.

Chu, Y.-H. and Lin, L.-H. (1992), Effects of minor compounds on hydrogenation rate of soybean oil, *J. Am. Oil Chem. Soc.*, **69**, 880–883.

Coenen, J.W.E. (1978), The rate of change in the perspective of time, *Chem. Ind.*, 709–722.

Coenen, J.W.E. (1981), Selectivity in fat hydrogenation, *Riv. Ital. Sostanze Grasse*, **58**, 445–452.

Coenen, J.W.E. and Boerma, H. (1968), Absorption der Reaktionspartner am Katalysator bei der Fetthydrierung, *Fette Seifen Anstrichm.*, **70**, 8–14.

Coenen, J.W.E. and Wieske, T. (1967), Procédé d'hydrogénation catalytique de composés d'acides gras non saturés, notamment d'huiles alimentaires, French Patent 1.478.239, assigned to Unilever.

Colen, G.C.M. et al. (1990), Hydrogenation method, British Patent Application 2 230 020 A, assigned to Unilever.

Cousins, E.R. (1963), Hydrogenation of fats and oils. Isomerization during hydrogenation, *J. Am. Oil Chem. Soc.*, **40**, 206–210.

Cox, R. et al. (2000), Determination of iodine value with a Fourier transform–Near Infra Red based global calibration using disposable vials: an international collaborative study, *J. Am. Oil Chem. Soc.*, **77**, 1229–1234.

Daum, J.P. (1993), Determining the cost of hydrogen, *inform*, **4**, 1394–1399.

Dijkstra, A.J. (1990), On the mechanism of the hydrogenation of edible oils, in *Beauty is our Business. A Birthday Salute to Edsger W. Dijkstra*, Feijen, W.H.J., Gasteren, A.J.M. van, Gries, D., and Misra, J., Eds., Springer Verlag, New York, 102–111.

Dijkstra, A.J. (1997), Hydrogenation revisited, *inform*, **8**, 1150–1158.

Dijkstra, A.J. (2002a), Hydrogenation and fractionation, in *Fats in Food Technology*, Rajah, K.K., Ed., Sheffield Academic Press, Sheffield, U.K., 123–158.

Dijkstra, A.J. (2002b), On the mechanism of the copper-catalysed hydrogenation; a reinterpretation of published data, *Eur. J. Lipid Sci. Technol.*, **104**, 29–35.

Dijkstra, A.J. (2005), No solution yet to the linolenic acid mystery, *inform*, **16**, 283.

Dijkstra, A.J. (2006), Revisiting the formation of *trans* isomers during the partial hydrogenation of triglyceride oils, *Eur. J. Lipid Sci. Technol.*, **108**, 249–264.

Dijkstra, A.J. and Maes, P.J. (1991), Die Härtung entschleimter Öle, *Fat Sci. Technol.*, **93**, 468–468.

Dijkstra, A.J. et al. (1995), Interpreting oil stability data, in *Oils-Fats-Lipids 1995, Proceedings of the 21st ISF Congress*, Castenmiller, W.A.M., Ed., P.J. Barnes & Associates, Bridgewater, England, 629–637.

Dijkstra, A.J. et al. (1996), Interpreting the oil stability index, *Oléagineux Corps Gras Lipides/OCL*, **3**, 378–386.

Drigalla, P. and Pudel, F. (1993), Untersuchungen zur direkten Hydrierung von TOP-Rapsöl, *Fat Sci. Technol.*, **95**, 241–248.

Drozdowski, B. (1977), Effect of the unsaturated acyl position in triglycerides on the hydrogenation rate, *J. Am. Oil Chem. Soc.*, **54**, 600–603.

Drozdowski, B. and Goraj-Moszora, I. (1980), Reactivation of partially poisoned nickel catalyst by bleaching earth, *J. Am. Oil Chem. Soc.*, **57**, 171–174.

Drozdowski, B. and Zajac, M. (1977), Effect of concentration of some nickel catalyst poisons in oils on the course of hydrogenation, *J. Am. Oil Chem. Soc.*, **54**, 595–599.

Duijn, G. van (2000), Technical aspects of *trans* reduction in margarines, *Oléagineux Corps Gras Lipides/OCL*, **7**, 95–98.

Dutton, H.J. et al. (1963), Hydrazine-reduced linolenic acids as a source of C_9, C_{12} and C_{15} dibasic acids, *J. Am. Oil Chem. Soc.*, **40**, 175–179.

Duveen, R.F. and Leuteritz, G. (1982), Der BUSS-Schleifenreaktor in der Öl–und Fetthärtungsindustrie, *Fette Seifen Anstrichm.*, **84**, 511–515.

Eckey, E.W. (1954), *Vegetable Fats and Oils*, Reinhold Publishing Co., New York.

Fan, T. et al. (1986), Reactivity of fatty acids in the different positions of the triglycerides during hydrogenation of canola oil, *J. Am. Oil Chem. Soc.*, **63**, 898–901.

Feuge, R.O. and Cousins, E.R. (1960), Positional isomers formed during the hydrogenation of methyl oleate, *J. Am. Oil Chem. Soc.*, **37**, 267–271.

Feuge, R.O. et al. (1953), Modification of vegetable oils. XV. Formation of isomers during hydrogenation of methyl linoleate, *J. Am. Oil Chem. Soc.*, **30**, 454–460.

Haraldsson, G. (1980), Selective hydrogenation and subsequent fractionation of oil, in *Proceedings Symposium on Hydrogenation of Oils, Rimini, Italy,* American Soybean Association, 75–85.

Hashimoto, K. et al. (1971a), Kinetics of the hydrogenation of fatty oils, *J. Am. Oil Chem. Soc.*, **48**, 291–295.

Hashimoto, T. et al. (1971b), Hydrogenation of triolein with various catalysts on the selectivity toward the position of the acyl group, *J. Japn. Oil Chem. Soc.*, **20**, 10–15.

Hastert, R.C. (1998c), Past, present and future of the hydrogenation process, *Lipid Techn.*, **10**, 101–105.

Härröd, M., Hark, S. van den, Holmqvist, A., and Møller, P. (2004), Selective hydrogenation of triglycerides at supercritical single-phase conditions, *Paper presented at the 3rd EuroFedLipid Congress, Edinburgh,* page 85 in the abstracts.

Härröd, M. et al. (2005), Selective hydrogenation of functional groups in substrates and partially hydrogenated fatty acids and fatty acid derivatives, PCT Patent Application WO 2005/095306, assigned to Härröd Research AB.

Härröd, M. and Møller, P. (1996), Hydrogenation of substrate and products manufactured according to the process, WO Patent Application 96/01304, assigned to Poul Møller Ledelses- og Ingeniörrådgivning APS.

Heldal, J.A. and Frankel, E.N. (1984), Comparison of homogeneous and heterogeneous palladium hydrogenation catalysts, *J. Am. Oil Chem. Soc.*, **61**, 756–761.

Hennion, F.J. (1986), The reclamation of spent hydrogenation catalysts, in *World Conference on Emerging Technologies in the Fats and Oils Industry*, Baldwin, A.R., Ed., AOCS, Champaign, IL, 172–183.

Horiuti, I. and Polanyi, M. (1934d), Exchange reactions of hydrogen on metallic surfaces, *Trans. Faraday Soc.*, **30**, 1164.

Ilsemann, K. et al. (1979), Hydrogenation, isomerization and migration of unsaturated acyl moieties in natural triacylglycerols, *Chem. Phys. Lipids*, **23**, 1–5.

Irandoust, S. and Edvardsson, J. (1993), Poisoning of nickel-based catalysts in fat hydrogenation, *J. Am. Oil Chem. Soc.*, **70**, 1149–1156.

James, B.R. (1973), *Homogeneous Hydrogenation*, John Wiley & Sons, New York.

Jonge, A. de et al. (1965), Selective hydrogenation of linolenate groups in soybean oil, *Nature*, **206**, 573574.

Jonge, A. de and Erkelens, J. (1966), Traitement d'huiles, notamment d'huiles comestibles, French Patent 1.459.878, assigned to Unilever.

Jonker, G.H. (1999), *Hydrogenation of edible oils and fats*, Ph.D. thesis, Rijksuniversiteit Groningen, The Netherlands.

Kaimal, T.N.B. and Lakshminarayana, G. (1979), Effect of the position of linoleic acid in triacylglycerols on its hydrogenation rate, *J. Am. Oil Chem. Soc.*, **56**, 578–580.

Katan, M.B. (1995), *Trans* fatty acids and their effect upon lipoproteins in humans, *Ann. Rev. Nutr.*, **15**, 473–493.

Kaufmann, H.P. (1939), Wilhelm Normann, zum Gedächtnis, *Fette u. Seifen*, **46**, 259–264.

Kaufmann, H.P. (1958), Hydrogenation process and product, US Patent 2,852,541.

Kaufmann, H.P. and Mukherjee, K.D. (1965), Über den Chemismus der katalytischen Fetthydrierung in der Miscella, *Fette Seifen Anstrichm.*, **67**, 606–613.

Kellens, M.J. (2000), Oil modification processes, in *Edible Oil Processing*, Hamm, W. and Hamilton, R.J., Eds., Sheffield Academic Press, Sheffield, UK, 127–173.

Klimmek, H. (1984), Influence of various catalyst poisons and other impurities on fatty acid hydrogenation, *J. Am. Oil Chem. Soc.*, **61**, 200–204.

Koetsier, W.T. (1997), Hydrogenation of edible oils, technology and applications, in *Lipid Technologies and Applications*, Gunstone, F.D. and Padley, F.B., Eds., Marcel Dekker, Inc., New York, Basel, Hong Kong, 265–303.

Kokken, M.J. (1993), Hydrogenation in practice, in *Proceedings of the World Conference on Oilseed Technology and Utilization*, Applewhite, T.H., Ed., AOCS Press, Champaign, IL, 173–179.

Kopp, H.P.J. et al. (1989), Process for preparing refined oil, US Patent 4,857,237, assigned to Unilever.

Kopp, H.P.J. et al. (1991), Process for preparing refined oil, European Patent 0 259 981, assigned to Unilever.

Koritala, S. (1970), Hydrogenation of unsaturated fatty esters with copper-chromite catalyst: kinetics, mechanism and isomerization, *J. Am. Oil Chem. Soc.*, **47**, 463–466.

Koritala, S. et al. (1970), Selective hydrogenation with copper catalysts: II. Kinetics, *J. Am. Oil Chem. Soc.*, **47**, 266–268.

Koritala, S. and Dutton, H.J. (1966), Selective hydrogenation of soybean oil with sodium borohydride-reduced catalysts, *J. Am. Oil Chem. Soc.*, **43**, 86–89.

Krishnaiah, D. and Sarkar, S. (1990), Kinetics of liquid phase hydrogenation of cottonseed oil with nickel catalysts, *J. Am. Oil Chem. Soc.*, **67**, 233–238.

Kuiper, J. (1980), Selective hydrogenation, US Patent 4,228,088, assigned to Unilever.

Kuiper, J. (1981a), Process for the selective hydrogenation of triglyceride oils with a metallic catalyst in the presence of a diamine, US Patent 4,307,026, assigned to Unilever.

Kuiper, J. (1981b), Process for the selective hydrogenation of triglyceride oils with a metallic catalyst in the presence of ammonia, US Patent 4,278,609, assigned to Unilever.

Kuwata, T. et al. (1965), Process for improving flavor and taste of soybean oil, US Patent 3,169,981, assigned to Nikki Kagaku Kabushiki Kaisha.

Lidefelt, J.O. et al. (1983), The role of hydrogen in the selectivity of vapor-phase hydrogenation of methyl linoleate, *J. Am. Oil Chem. Soc.*, **60**, 608–613.

Linsen, B.G. (1971a), Selektivität bei der Fetthydrierung, früher und heute I, *Fette Seifen Anstrichm.*, **73**, 411–416.

Linsen, B.G. (1971b), Selektivität bei der Fetthydrierung, früher und heute II, *Fette Seifen Anstrichm.*, **73**, 753–758.

List, G.R. (2004), Processing and reformulation for nutrition labelling of *trans* fatty acids, *Lipid Techn.*, **16**, 173–177.

Litz, L.M. (1984), Process and apparatus for mixing a gas and a liquid, US Patent 4,454,077, assigned to Union Carbide Corporation.

Logan, A. (1996), Separation of nickel catalyst, *Oils & Fats Int.*, **12**, 29–31.

Maryott, C.H. (1914), Process for the reduction of unsaturated fatty acids and their esters, US Patent 1,097,456.

Mattson, F.H. and Volpenhein, R.A. (1962), Hydrogenation of a fatty acid is not influenced by the position it occupies on a triglyceride molecule, *J. Am. Oil Chem. Soc.*, **39**, 307–308.

Merker, D.R. (1959), Process of hydrogenating glycerides, US Patent 2,903,468, assigned to Swift & Company.

Mounts, T.L. and Dutton, H.J. (1967), Micro vapor-phase hydrogenation monitored with tandem chromatography-radioactivity. II Evaluation of catalyst selectivity for linolenate, *J. Am. Oil Chem. Soc.*, **44**, 67–70.

Normann, W. (1903), Process for converting unsaturated fatty acids or their glycerides into saturated compounds, British Patent 1 515, assigned to Herforder Maschinenfett-und Ölfabrik Leprince und Siveke.

Noureddini, H. et al. (1992), Densities of vegetable oils and fatty acids, *J. Am. Oil Chem. Soc.*, **69**, 1184–1188.

Okkerse, C. et al. (1967), Selective hydrogenation of soybean oil in the presence of copper catalysts, *J. Am. Oil Chem. Soc.*, **44**, 152–156.

Osteroth, D. (1968), Der gegenwärtige technische Stand der kontinuierlichen Fetthärtung, *Oléagineux*, **23**, 597–600.

Patterson, H.B.W. (1978), Hydrogenation of vegetable oils. Hydrogenation technology., *Oléagineux*, **28** , 355–359.

Patterson, H.B.W. (1983f), *Hydrogenation of Fats and Oils*, Applied Science Publishers, New York.

Paulose, M.M. et al. (1978), Isomerization and saturation of monounsaturated acyl moieties during catalytic hydrogenation influenced by their position in triacyl glycerols, *Chem. Phys. Lipids*, **21**, 187–194.

Pedersen, A.J. and Agertoft, M. (1999), Moneywise hydrogen production, *Paper presented at the 90th AOCS Annual Meeting & Expo, Orlando, FL.*

Ratnayake, W.M.N. et al. (1990), Studies on the mechanism of the hydrazine reduction reaction: applications to selected monoethylenic, diethylenic and triethylenic fatty acids of *cis* configurations, *J. Am. Oil Chem. Soc.*, **67**, 940–946.

Ravnskov, U. (2000), *The Cholesterol Myths Exposing the Fallacy that Saturated Fat and Cholesterol Cause Heart Disease*, New Trends Publishing, Inc., Washington, D.C.

Rijnten, H.T. and Eikema, E.T.J. (1975), Process for the preparation of partially sulfided metallic supported catalysts, US Patent 3,872,028, assigned to Unilever.

Rodenbush, C.M., Hsieh, F.H., and Viswanath, D.S. (1999), Density and viscosity of vegetable oils, *J. Am. Oil Chem. Soc.*, **76**, 1415–1419.

Roquette, E. (1994), Safety in hydrogenation plants, *Paper presented at the 85th AOCS Annual Meeting & Expo,* Atlanta.

Rosen, B.I. (1985), Continuous reduction of edible oils, US Patent 4,510,091, assigned to UOP Inc.

Rutledge, D.N., El-Khaloui, M., and Ducauze, C.J. (1988), Contribution à l'étude d'une méthode de contrôle rapide de la qualité des margarines par RMN-IBR, *Rev. Franç. Corps Gras*, **35**, 157–162.

Rylander, P.N. (1970), Hydrogenation of natural oils with platinum metal group catalysts, *J. Am. Oil Chem. Soc.*, **47**, 482–486.

Schaffer, P.S. and Haller, H.S. (1943), The solubility of gases in butter oil, cottonseed oil, and lard, *Oil & Soap*, **20**, 161–162.

Schilling, K. (1961), Der Verlauf der Hydrierung mehrfach ungesättigter Fettsäureester mit Hydrazin, *Fette Seifen Anstrichm.*, **63**, 421–425.

Schilling, K. (1963), Die relative Reaktions-Geschwindigkeit verschiedenartiger Doppelbindungen bei der Hydrierung mit Hydrazin, *Fette Seifen Anstrichm.*, **65**, 395–397.

Schilling, K. (1968), Über den Verlauf der Hydrierung von Triolein an Raney-Nickel, *Fette Seifen Anstrichm.*, **70**, 389–393.

Schilling, K. (1977), The mechanism of triglyceride hydrogenation, *Paper presented at the 9th Scandinavian Symposium on Lipids, Visly, Sweden* F-116–F-119.

Schilling, K. (1978), Der Reaktionsverlauf bei der Hydrierung von Triglyceriden. Simultanhydrierung von Tri- und Monolinolenin, *Fette Seifen Anstrichm.*, **80**, 312-314.

Schilling, K. (1981), Der Reaktionsverlauf bei der Hydrierung von Triglyceriden, Glykolester als Modellsubstrat, *Fette Seifen Anstrichm.*, **83**, 226–228.

Schmidt, H.-J. (1968), Hydrogenation of triglycerides containing linolenic acids. I. Calculation of selectivity, *J. Am. Oil Chem. Soc.*, **45**, 520–522.

Schöön, N.-H. (1995), Is a low *trans* content attainable by conventional hydrogenation of vegetable oils? in *Oils-Fats-Lipids 1995*, Castenmiller, W.A.M., Ed., P.J. Barnes & Associates, Bridgewater, England, 155–158.

Šmidovnik, A. et al. (1992), Catalytic transfer hydrogenation of soybean oil, *J. Am. Oil Chem. Soc.*, **69**, 405–409.

Strocchi, A. (1982), Fatty acid composition and triglyceride structure of corn oil, hydrogenated corn oil, and corn oil margarine, *J. Food Sci.*, **47**, 36–39.

Suzuki, K. and Murase, Y. (1981), Hydrogenation of edible oils and fats by nickel catalysts II. Triacylglycerol compositions of hydrogenated oils and fat by hydrogenation simulation, *J. Japn. Oil Chem. Soc.*, **30**, 841–848.

Szukalska, E. (2000), Effect of phospholipid structure on the kinetics and chemistry of soybean oil hydrogenation with nickel catalysts, *Eur. J. Lipid Sci. Technol.*, **102**, 739–745.

Toor, H. van et al. (2005), Low trans-fatty acid compositions; low-temperature hydrogenation, e.g., of edible oils, US Patent Application Publication 2005/0027136 A1, assigned to Cargill Incorporated.

Tümer, H.V. et al. (1964), Relative reactivity toward hydrogenation of the oleyl group in the 2- and 1,3-positions of triglycerides, *J. Am. Oil Chem. Soc.*, **41**, 413–415.

Tysinger, J.E. et al. (2003), Soybean oil process, US Patent 6,511,690 B1, assigned to Carolina Soy Products, Inc.

Tysinger, J.E. et al. (2004), Soybean oil process, US Patent 6,753,029 B1, assigned to Carolina Soy Products, Inc.

Tyutyunnikov, B.N. and Novitskaia, I.I. (1958), Au sujet de la dissolution de l'hydrogène dans les graisses, *Rev. Franç. Corps Gras*, **5**, 37–37.

Urosevic, D. (1986), Improvement of the Buss loop reactor in the oil and fat industry, in *World Conference on Emerging Technologies in the Fats and Oils Industry*, Baldwin, A.R., Ed., American Oil Chemists' Society, Champaign, IL, 128–132.

Van de Erve, A. et al. (1948), Use of dissociated ammonia for hydrogenation, *J. Am. Oil Chem. Soc.*, **25**, 60–63.

Vanden Borre, H. (1983), Hydrogen production by water electrolysis, in *Proceedings of the Third A.S.A. Symposium of Soybean Processing: Hydrogenation of Soy Oil*, 22–30.

Veldsink, J.W. et al. (1997), Heterogeneous hydrogenation of vegetable oils: a literature review, *Catal. Rev. Sci. Eng.*, **39**, 253–318.

Vijayalakshmi, P. et al. (1988), Hydrogenation of unsaturated fatty acid methyl esters using decalin for hydrogen transfer, *J. Am. Oil Chem. Soc.*, **65**, 939–941.

Voeste, Th. (1973), Über ein neues Verfahren zur Herstellung von Wasserstoff aus Methanol, *Fette Seifen Anstrichm.*, **75**, 360–362.

Warner, K. et al. (2000), Electrochemical hydrogenation of edible oils in a solid polymer elctrolyte reactor. Sensory and compositional characteristics of low *trans* soybean oils, *J. Am. Oil Chem. Soc.*, **77**, 1113–1117.

Wisniak, J. and Stein, S. (1974), Hydrogen solubility in jojoba oil, *J. Am. Oil Chem. Soc.*, **51**, 482–485.

Young, F.V.K. (1994), Modification processes, in *The Lipid Handbook*, 2nd ed., Gunstone, F.D., Harwood, J.L., and Padley, F.B., Eds., Chapman & Hall, London, 277–285.

Yurawecz, M.P. (2004), FDA requires mandatory labeling of *trans* fat, *inform*, **15**, 184–185.

Yusem, G.J. and Pintauro, P.N. (1992), The electrocatalytic hydrogenation of soybean oil, *J. Am. Oil Chem. Soc.*, **69**, 399–404.

Yusem, G.J. et al. (1996), Electrocatalytic hydrogenation of soybean oil in a radial flow-through Raney nickel powder reactor, *J. Appl. Electrochem.*, **26**, 989–997.

4.3 Interesterification

Whereas the hydrogenation process alters the fatty acid composition of oil, but retains its carbon number distribution, the interesterification process is the opposite in that it retains the fatty acid composition, but often leads to a different carbon number distribution. For the random interesterification process, this distribution is fully predictable and can be calculated on the basis of the fatty acid composition of the reaction mixture. Both the hydrogenation process and the interesterification process involve chemical reactions. Another chemical reaction, the esterification of glycerol with FFA, will also lead to a randomised triglyceride product, but for food applications, this product is often banned.

Both hydrogenation and interesterification are applications of known reactions to edible oil modification. The original interesterification reaction was described by Friedel and Crafts (1865) who heated a mixture of ethyl benzoate and amyl acetate to 300°C in a sealed tube and noted the formation of amyl benzoate and ethyl acetate. The earliest patent describing interesterification products (Grün, 1922) employs a two-step process. In the first step, triglycerides are allowed to react with glycerol to generate partial glycerides and, in the second step, these partial glycerides are esterified with, for instance, butyric acid and capronic acid to arrive at a synthetic butter fat.

Shortly afterwards, Normann (1924) described the reverse process, the first step of which involved a reaction with butyric or acetic acid and the second step an esterification

with glycerol of the free fatty acids liberated in the first step. Normann was also the first to produce acetoglycerides (1925) and he noted that whereas his starting material was hard, brittle, and could be reduced to a powder, the interesterification product was also hard but no longer brittle. It became malleable when manipulated, like wax. Accordingly, he remarked that this latter property constituted a particular technological value[3].

For this true interesterification between triglycerides, Normann (1925) mentioned various catalysts: aromatic or aliphatic sulphonic acids, tin and tin compounds, and alkali alcoholates, and he invariably employed high (140 to 250°C) reaction temperatures. The same catalyst types are listed by Van Loon (1926), but in more detail and in order to distantiate himself from the alkali refining process, he had to exclude the presence of water when using an alkali hydroxide as interesterification catalyst. Eckey (1945a), on the other hand, uses just water as the catalyst. This water leads to a partial hydrolysis of the triglycerides, but then, "by subsequently heating the fatty residue for about half an hour at 260°C, the free hydroxyl groups were practically eliminated by esterification with the free fatty acid."

Nowadays, industrial interesterification processes for food products employ much lower temperatures. When sodium methylate is used as the catalyst, the temperature is generally kept below 100°C and when an enzyme catalyses the reaction, the temperature is even lower to decrease the rate of denaturation of the enzyme. Only the reaction catalysed by the condensation product of glycerol and sodium hydroxide is carried out at temperatures around 150°C. Processes developed for nonfood products and involving the ester bond, such as hydrolysis, glycerolysis, or methanolysis, commonly employ high temperatures and sometimes superatmospheric pressures.

After WW II, the interesterification process was used in the U.S. to improve lard properties and in Europe, interesterification products are mostly used as hard stock components for margarine fat blends. Because interesterification permits such components to be *trans* isomer-free, their importance is expected to grow both in Europe and the U.S. Enzymatic interesterification leading to stereospecific acyl interchange is currently used in Japan and The Netherlands for cocoa butter equivalents and infant formula ingredients, respectively. Because of consumers' alleged phobia for all things chemical (Dijkstra, 2004a), enzymatic interesterification is also applied to hard stock components, in general.

4.3.1 Product composition

The most common interesterification process is the chemically catalysed randomisation process, also described as a "reshuffling" (Luddy et al., 1955). Unintentional and limited interesterification may take place when oil is heated to above say 200°C, as illustrated by the change in crystallisation characteristics of confectionery fats after their deodorisation (Willems and Padley, 1985). In addition to the chemically catalysed randomisation, there are also processes of directed interesterification in which the equilibrium corresponding to complete randomisation is disturbed by either distillative removal of the most volatile components, such as, for instance, FAME (fatty acid methyl ester) (Barsky, 1939; Eckey, 1945b) or the crystallisation of the highest melting components (Eckey, 1948a). This disturbance causes the components that have been removed to be replenished so that the remaining liquid fraction of the reaction mixture will be fully randomised. A directed interesterification process based on density differences of various interesterification reaction products and applying high pressure has been described by Barsky (1973).

Whether or not the latter process has ever been used is doubtful, but direct interesterification processes have been used. In the U.S., the process was used to improve the plastic range of lard and to eliminate its graininess on crystallisation (Hawley and Holman, 1956). In Europe, the process has been used by two separate companies in Belgium to produce a health margarine fat with a high (>66%) linoleic acid content and a low (<14%) saturated fatty acid content (Haighton and Kattenberg, 1974; De Lathauwer et al., 1980). At the time of writing, the processes are no longer in use.

The use of enzymes as an interesterification catalyst originally aimed at utilising their specificities, such as a 1,3-specificity. This allows nonrandomised products to be made which cannot be achieved by using a chemical catalyst. The product composition of enzymatically interesterified products can only be ascertained by determining their triglyceride composition. On the other hand, the product composition of randomised products can be calculated, as several authors have explained (Feuge et al., 1945; Norris and Mattil, 1946; Formo, 1954; Naudet, 1954; Kaufmann et al., 1958; Braun, 1960; Baltes, 1960; Coenen, 1974; Faur, 1977; Sreenivasan, 1978; Rozendaal and Macrae, 1997; Rousseau and Marangoni, 2002). Several of these authors not only show how to calculate the concentration or rather molar fraction of individual or groups of triglycerides, but they also provide tables with the number of isomers to be formed on randomisation in function of the number of different fatty acids (n) present in the reaction mixture. These tables can be somewhat confusing due to the way isomers have been dealt with.

In Table 4.8, isomers have been listed for monoacid (X_3), diacid (X_2Y), and triacid (XYZ) triglycerides. For each of these groups, the overall compositions of these triglycerides have been listed in the second column of Table 4.8 (A_3 … ABC) and the numbers per group correspond to the "Number without isomers" as listed in Table 4.9. Since the example is concerned with three different fatty acids A, B and C (n = 3), there are three monoacid

[3] Diese Beschaffenheit ist von besonderen technischen Wert.

TABLE 4.8 Isomers of X_3-, X_2Y-, and XYZ-triglycerides with three different fatty acids A, B, and C

Type of Triglyceride	Overall Composition	Left Stereo-Isomer	Symmetrical Isomer	Right Stereo-Isomer
X_3	A_3		AAA	
	B_3		BBB	
	C_3		CCC	
X_2Y	A_2B	BAA	ABA	AAB
	A_2C	CAA	ACA	AAC
	B_2A	ABB	BAB	BBA
	B_2C	CBB	BCB	BBC
	C_2A	ACC	CAC	CCA
	C_2B	BCC	CBC	CCB
XYZ	ABC	ABC		CBA
		ACB		BCA
		BCA		ACB

triglycerides, 3 × 2 diacid triglycerides and (3*2*1)/6 = 1 triacid triglyceride.

With respect to the diacid triglycerides X_2Y, the second column in Table 4.8 lists six triglycerides (A_2B … C_2B) having different fatty acid compositions and these correspond to what the literature (Coenen, 1974; Rozendaal and Macrae, 1997) refers to as "Number without positional isomers" in Table 4.9: $n*(n-1) = 3*2 = 6$. Each diacid triglyceride has three isomers, so the total number of isomers including positional and stereoisomers (last column Table 4.9) equals three times as many or 18 (=3*3*2). These 18 isomers have been listed in the third, fourth, and fifth column of Table 4.8. Omitting the third (or fifth) column and, thereby, disregarding stereoisomers and only taking positional isomers into account reduces the number to 12 (=2*n*(n - 1)=2*3*2). Similar arguments hold for the triacid triglycerides XYZ.

Probability calculus has been used to calculate these molar fractions. If the number of different fatty acids is set at n, the number of X_2Y compounds can be calculated by equaling the number of choices for the first fatty acid at n. Since the second fatty acid has to be a different one, there are (n – 1) different fatty acids left, so the number of X_2Y compounds equals $n*(n-1)$. If stereoisomers have not been taken into account, the number of positional isomers is double the number of compounds since now, a distinction is made between AAB and ABA. If stereoisomers are also taken into account and a distinction is made between AAB and BAA, the number of X_2Y compounds including all types of isomers equals $3*n*(n-1)$. When there are three different fatty acids (n = 3), this number equals 3*3*(3-1)=18; this is just the number given in Table 4.8.

TABLE 4.9 Number of triglyceride isomers

Triglyceride type	Number Without Isomers	Number With Only Positional Isomers	Number With Positional and Stereo-Isomers
X_3	n	n	n
X_2Y	$n(n-1)$	$2\ n(n-1)$	$3\ n(n-1)$
XYZ	$n(n-1)(n-2)/6$	$n(n-1)(n-2)/2$	$n(n-1)(n-2)$
Total number	$(n^3 + 3\ n^2 + 2\ n)/6$	$(n^3 + n^2)/2$	n^3
n = 2	4	6	8
n = 3	10	18	27
n = 4	20	40	64
n = 5	35	75	125
n = 6	56	126	216

TABLE 4.10 Calculation of triglyceride composition

Type of Triglyceride	Method of Calculation	Molar Percentage
S_3	0.80 × 0.80 x 0.05	3.2
SUS	0.80 × 0.80 × 0.95	60.8
SSU and USS	2 × 0.80 × 0.05 × 0.20	1.6
SU_2	2 × 0.80 × 0.95 × 0.20 +	30.4
	0.20 × 0.20 × 0.05	0.2
U_3	0.20 × 0.95 ×0.20	3.8
	Total	100.0

Stereoisomers should also be taken into account when calculating the mole fraction of certain triglycerides or groups of triglycerides in a randomised product. If the molar fraction of fatty acid A is denoted by *a,* the molar fraction of fatty acid B as *b*, etc., the molar fraction of the triglyceride A_2B in the randomised oil must be provided with a weighting factor of 3 and equals $3*a^2*b$ since A_2B denotes three stereoisomers: AAB, ABA, and BAA. Similarly, a weighting factor of 6 is required for triglycerides containing three different fatty acids A, B and C since there are six positional and stereoisomers involved: ABC, ACB, BAC, BCA, CAB, and CBA.

As an example, the triglyceride composition of a fat will be calculated that has not yet been randomised. Its overall content of saturated fatty acids (S) equals 55 mol% and the saturated fatty acid content of the 2-position was found to be 5 mol%. Consequently, the 1- and 3-positions contain (3*55 – 5)/2 = 80 mol%. Therefore, the outer positions contain 20 mol% unsaturated fatty acids (U) and the 2-position contains 95 mol% U. The calculation of the triglyceride composition has been given in Table 4.10 and this table also shows weighting factors where appropriate.

4.3.2 Interesterification mechanisms

4.3.2.1 Glycerolate mechanism

Until recently, the interesterification mechanism involving a glycerolate anion as the active intermediate was generally accepted. It had been introduced as a possibility by Eckey (1948b) who noted the formation of an equivalent amount of FAME when adding sodium methanolate catalyst to triglyceride oil and subsequently (1960), Baltes suggested that the sodium compound formed is a diacylglycerolate, and proposed interesterification reaction mechanisms involving this sodium

diacylglycerolate as the active intermediate. These proposals have been dutifully repeated by subsequent authors, amongst which: (Going, 1967; Arbeitskreis "Technologien der industriellen Gewinnung und Verarbeitung von Speisefetten", 1973; Coenen, 1974; Naudet, 1976; Rozendaal, 1990; Rozendaal, 1992; Marangoni and Rousseau, 1995; Dijkstra, 2001; Rousseau and Marangoni, 2002). Consequently, they were no longer questioned and became almost universally accepted.

However, in 2004 Liu observed that the fatty acid being exchanged must have an α-hydrogen for it to react and thereby demonstrated that the glycerolate mechanism as suggested by Baltes (1960) was inconsistent with his observation. Shortly afterwards, Dijkstra listed a number of additional arguments repudiating the glycerolate mechanism during an oral presentation (2004b):

- As pointed out before (Coenen, 1974), the glycerolate mechanism does not satisfactorily explain the observation (Weiss et al., 1961) that intramolecular rearrangement precedes the intermolecular rearrangement, which observation has subsequently been confirmed independently (Freeman, 1968).
- With respect to the soap formation on catalyst inactivation, Rozendaal (1997) assumes a saponification reaction, but given the small amount of caustic soda and the relatively large amount of water used to inactivate the catalyst, the caustic solution is too weak to cause saponification, let alone total saponification.
- Moreover, when acidified water is used to inactivate the catalyst, free fatty acids are found in an amount that is equivalent to the amount of catalyst (sodium methanolate) added (Burgers et al., 1965).
- Accordingly, the inactivation of the catalytically active intermediate leads to the formation of soap (or free fatty acid) and also to a diglyceride, such as dicapryline (coenen1974b). If the diacylglycerolate were to split off a fatty acid moiety, this would not lead to the formation of a diglyceride, but of a monoglyceride; such monoglycerides are not observed.
- The glycerolate mechanism fails to explain the thermal instability of the catalytically active intermediate. At ambient temperature, an activated reaction mixture can retain its catalytic activity for several hours but at 100°C, this activity disappears within an hour and at 150°C, the activity disappears so fast that no interesterification is observed after sodium methanolate addition (D. Meert, personal communication).
- Finally, the glycerolate mechanism does not offer an explanation for the rate acceleration caused by acetone (Muller and Kock, 1974), dimethyl sulphoxide (Artman et al., 1968), or aprotic substances selected from the group consisting of dimethyl formamide, dimethyl acetamide, etc. (Sreenivasan, 1973).

Accordingly, a novel, but speculative mechanism to be referred to as the "enolate mechanism" was proposed (Dijkstra, 2004b) and during the discussion following the oral presentation, Poppe suggested an experiment that would provide independent support for this speculative mechanism. The experiment was duly carried out and provided the anticipated support; the enolate mechanism *(vide infra)* and its experimental support have been reported in a joint paper (Dijkstra et al., 2005).

4.3.2.2 WEISS mechanism

Apart from the glycerolate mechanism, other mechanisms have been proposed in the literature. In 1961, Weiss et al. observed that the formation of the catalytically active intermediate has higher activation energy than the actual interesterification reaction. Accordingly, they proposed that this intermediate is an enolate anion formed according to Figure 4.7.

The above reaction does not explain the formation of FAME, but this can be explained by assuming a two-step enolate formation with the first step leading to the formation of a glycerolate anion and FAME as illustrated in Figure 4.7 and the second step to the formation of the enolate anion according to Figure 4.8.

This two-step enolate formation could well be supported by the alkoxide absorption at 1565 cm^{-1} as reported by Coenen (1974) and the presence of the β-keto ester maximum absorption peak in the infrared spectra as reported by Weiss et al. (1961). They propose that this β-keto ester is formed by the enolate anion and causes intramolecular interesterification according to Figure 4.9.

Intermolecular interesterification is analogous to the above reaction scheme and involves a second glyceride molecule. However, as pointed out by Heldal and Mørk (1981), "the observation that the concentration of the β-keto ester salt increases steadily with time does not seem to be in accordance with the WEISS mechanism. In case the interesterification proceeds via a β-keto ester intermediate, the concentration of this would be expected to reach a steady state after a comparatively short period of time

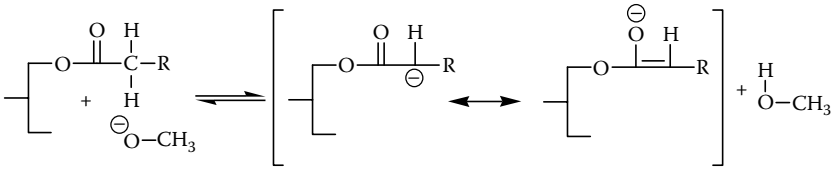


FIGURE 4.7 Formation of the enolate anion and its mesomeric structures.

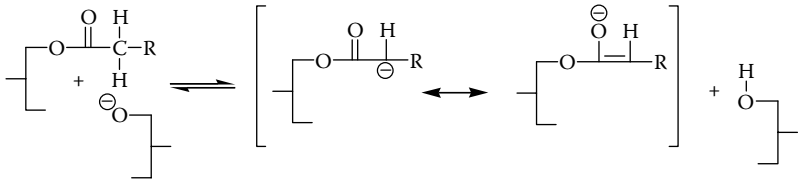


FIGURE 4.8 Enolate formation by reaction with glycerolate.

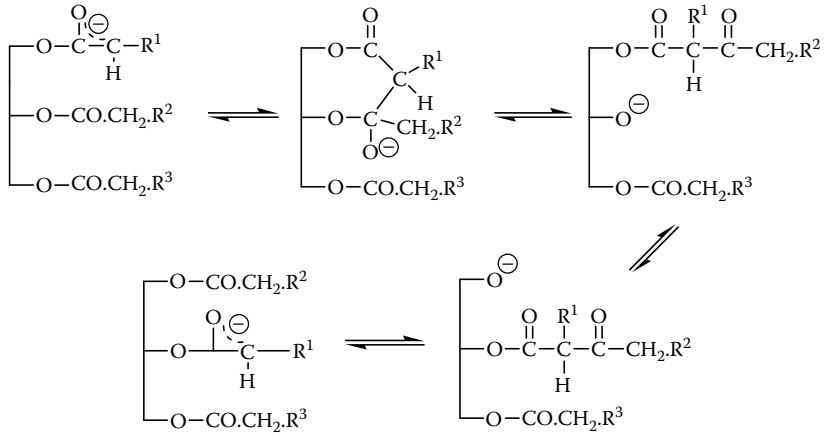


FIGURE 4.9 Intramolecular interesterification via the WEISS mechanism.

of reaction. In addition, the activity 1of the β-keto ester intermediate would be expected to be rather low due to its resonance stabilisation. Both factors seem to counteract the assumption that a β-keto ester intermediate precedes interesterification. In accordance with this, no reaction appeared to take place in an experiment where Na-ethyl acetoacetate was used as catalyst." Previously, it had also been remarked (Sreenivasan, 1978) that the infrared absorption peak mentioned in support of the β-keto ester could also originate from soaps.

4.3.2.3 LIU mechanism

The interesterification mechanism proposed by Liu (2004) operates via the Claisen condensation mechanism. What it has in common with the WEISS mechanism is that it assumes the first step to involve the abstraction of an α-hydrogen and the second step the formation of a β-keto ester by the nucleophilic attack of the enolate on an ester carbonyl under simultaneous formation of a glycerolate. As explained above, this is unlikely since Na-ethyl acetoacetate is not catalytically active (Heldal and Mørk, 1981).

On the other hand, the paper (Liu, 2004) explains the formation of long chain ketones, such as laurone (Huyghebaert et al., 1970) from β-keto esters and can be made to explain the direct formation of a fatty acid moiety when a β-keto ester reacts with water according to Figure 4.10.

In Figure 4.10, the first reaction shows the formation of the β-keto ester anion from the enolate anion (which

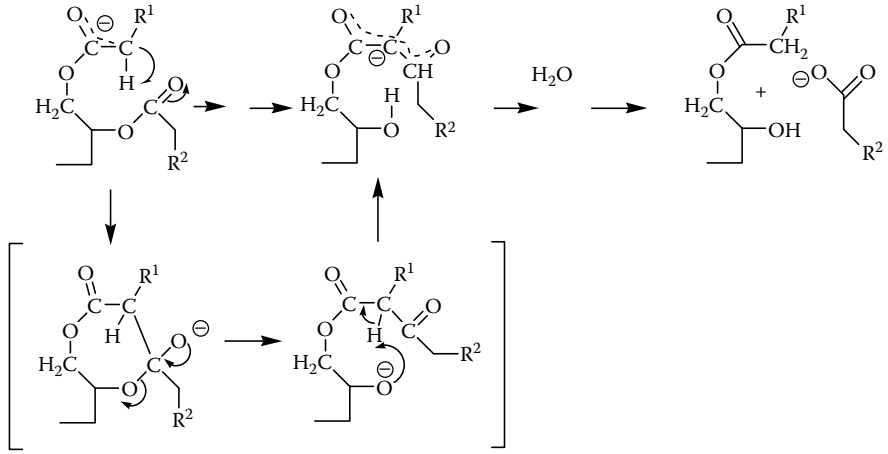


FIGURE 4.10 Formation and hydrolysis of β-keto ester.

is likely to be a two-step reaction) and the second reaction shows how, on reaction with water, this β-keto ester dissociates into diglyceride and a carboxy anion. The figure also shows the conjugated system of the β-keto ester comprising three mesomeric structures. According to the author (Liu, 2004), the ester, therefore, would absorb in the UV region and this, he claims, would account for the reddish-brown colour that appears on catalyst activation and that is used for reaction control (Liu and Lampert, 2001).

4.3.2.4 Enolate mechanism

The enolate mechanism (Dijkstra et al., 2005) can be summarized by defining the first step as the generation of an enolate anion by abstraction of an α-hydrogen by a sufficiently strong base and the second step as a reaction of this enolate anion with a hydroxyl group. If this hydroxyl group belongs to a partial glyceride, this second step will result in ester interchange and the formation of a glycerolate anion that will subsequently regenerate the enolate anion in accordance with the first step of the mechanism. The various reactions that may be involved are listed below in more detail. As an example, sodium methanolate will be used as the catalyst.

1. When added to the oil, this sodium methanolate can react in several ways:
   - Any water present will react with the sodium methanolate to form sodium hydroxide and methanol.
   - If any free fatty acids are present, they will react with sodium methanolate under formation of soaps and methanol.
   - The methanolate anion may react with partial glycerides to form a glycerolate anion and free methanol. This is an equilibrium reaction that is likely to favour the glycerolate anion.
   - The negatively charged methanolate anion can exert a nucleophilic attack on a carbonyl carbon atom of a fatty ester linkage and form a complex that then dissociates into a glycerolate anion and a FAME as illustrated in Figure 4.11.
   - The methanolate anion can also abstract a hydrogen from a fatty acid chain and, thus, form an enolate anion (and methanol) as illustrated in Figure 4.7 and proposed by Weiss et al. (1961).
2. Any glycerolate formed is then assumed to abstract a hydrogen and form an enolate anion as shown in Figure 4.8.
3. According to the enolate mechanism (Dijkstra, 2004b), the enolate anion then reacts with hydroxyl groups.
   - If the hydroxyl group is part of a partial glyceride, the reaction leads to interesterification, according to Figure 4.12.

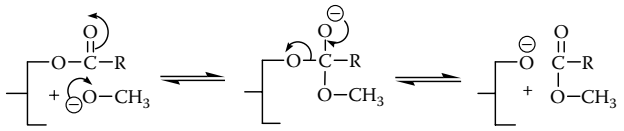


FIGURE 4.11 Formation of diacylglycerolate anion and FAME.

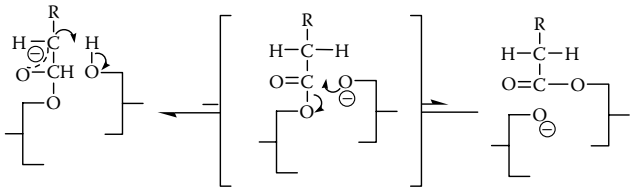


FIGURE 4.12 Intermolecular interesterification (enolate mechanism).

   - The glycerolate formed during this interesterification reaction will regenerate the enolate anion in accordance with the reaction shown in Figure 4.8. This reaction is an equilibrium reaction and, in all likelihood, it can be assumed that the constant of this equilibrium is not very different from unity. Since in a triglyceride oil, the concentration of esterified hydroxyl groups is much larger than the concentration of free hydroxyl groups, the enolate anion concentration will be larger than the glycerolate anion concentration. This is especially important for the two reactions listed below.
   - Of course, the hydroxyl group can also belong to an alcohol, such as methanol liberated in No. 1 substeps, or added as such. In that case, the reaction product is a FAME, as illustrated in Figure 4.13.
   - When the catalyst is inactivated with water, the enolate anion rather than the glycerolate anion reacts according to (Figure 4.14):
   - As shown in Figures 4.12 and 4.13, the reactions between the enolate anion and a partial glyceride or an alcohol lead to a glycerolate anion as by-product. According to the enolate mechanism, this glycerolate anion will regenerate the enolate anion in line with step No. 2 and as illustrated by Figure 4.8. On the other hand, the reaction between the enolate anion and water does not lead to the formation of the glycerolate anion since in a rapid acid-base reaction this would immediately convert to a carboxylate; it would immediately react with the free fatty acid to form a partial glyceride and a carboxyl anion.
   - According to the enolate mechanism, the enolate anion reacts only with hydroxyl groups. Pure triglycerides and linear esters, such as waxes for instance, should not react. However, when pure esters are used as a substrate, hydroxyl groups will be formed on catalyst activation because the sodium methylate will react in such a way (step

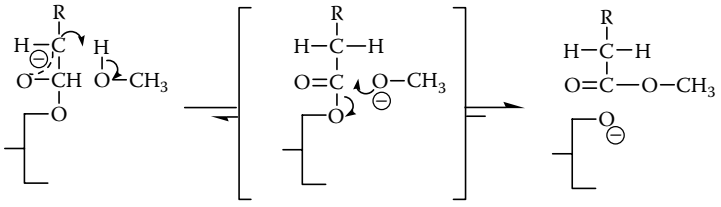


FIGURE 4.13 FAME formation by reaction of an enolate anion with methanol.

No. 1, substep 5, Figure 4.7 or Step No. 2) that some free methanol will finely result. This methanol is then available to react with the enolate to form FAME and a fatty alcoholate. This latter reaction is not as favourable as the reaction with a partial glyceride, since glycerolate will be resonance stabilised. However, when methyl palmitate and isopropyl oleate are allowed to interesterify, ester interchange is observed when sodium methylate is added and, as is to be expected, the extent of interesterification is catalyst dose dependent (see Liu, 2004, Table 1).

4. According to the enolate mechanism, the enolate anion can also form a β-keto ester anion (cf. Figure 4.9) and this β-keto ester anion is assumed to have no catalytic activity. At low temperatures, the formation of this β-keto ester anion is slow so that catalytic activity can be maintained for long periods, but with increasing temperatures, the catalytic activity increases as does the rate at which it decreases because of β-keto ester anion formation.
5. Finally, the 'enolate' mechanism as suggested (Dijkstra, 2004b) also proposes that acetone can donate a proton to an enolate anion and then abstract a proton from the α-hydrogen of a different fatty acid and, thus, act as a hydrogen transfer agent. Since acetone and its enolate are small molecules and can diffuse readily in the reaction medium, this hydrogen transfer might lead to an increase in the overall rate of interesterification (Figure 4.15).

4.3.2.5 Glycerol and caustic

In his review on esterification and interesterification, Eckey (1956) mentions that similar results as obtained by the use of sodium methanolate, such as a short reaction time and little or no darkening of the colour of the fat, "can also be obtained with other sodium alkoxides, finely dispersed metallic sodium, sodium potassium alloy (Hawley and Holman, 1956), and sodium hydride (Eckey, 1951)." He also mentions that "many other less active catalysts have been proposed for which a considerably higher reaction temperature is appropriate. … A method, which would seem to have practical possibilities, but does not appear to have much use, is to heat a fat with a small quantity of glycerol or monoglyceride and a small quantity of alkaline material, such as sodium hydroxide, sodium carbonate, or sodium soap, at temperatures in the neighborhood of 225°C." (Gooding, 1943). He also refers to a later patent (Holman et al., 1956) that describes the use of an aqueous caustic soda solution in a temperature range of 150 to 175°C and involving flash drying. When Rozendaal (1997) refers to this process, he naturally refers to Unilever patents (Keulemans and Smits, 1986; Keulemans and Rozendaal, 1988) and he also mentions the slightly lower temperature range of 125 to 140°C.

He also states that the genuine catalyst responsible for fatty acid exchange consists of the sodium or potassium compound of a diacylglycerol molecule (cf. Figure 4.11) and that "the genuine catalyst is identical for all types of pre-catalysts:" sodium methanolate, sodium metal, or the combination of glycerol and sodium hydroxide. On the

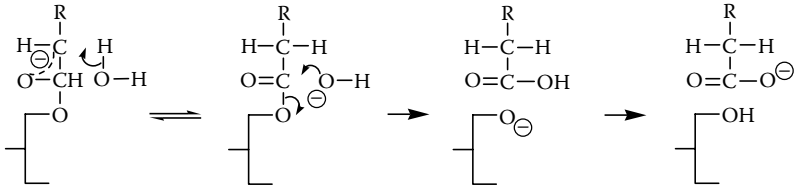


FIGURE 4.14 Inactivation/hydrolysis of the enolate anion by water.

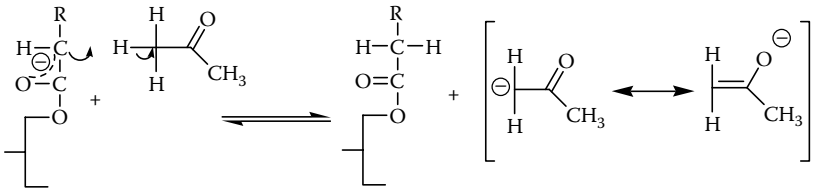


FIGURE 4.15 Acetone acting as a proton transfer agent.

other hand, he contradicts himself (Rozendaal, 1990) when reporting a difference in that the alkali metals and their alcoholates are active below 100°C, whereas "sodium glycerate, which can be formed *in situ* from sodium hydroxide and glycerol, is intermediate in activity."

Since the use of 1 wt %[4] sodium methanolate at 205°C requires more than 3 hours before the equilibrium is approached (Naudet, 1947) and the genuine catalyst formed from sodium methanolate has been found to be thermally unstable (*vide supra*), it is obvious that the interesterification mechanism is temperature dependent. Below 100°C one mechanism prevails and, as explained above, there are a number of arguments that support the enolate mechanism; at temperatures above, say, 120°C another mechanism operates, but the literature is not explicit about the mechanism concerned.

Because the thermal inactivation reaction suggested in support of the enolate mechanism involves the formation of a β-keto ester anion and it was argued that this ester would be insufficiently active for catalysing ester interchange (Heldal and Mørk, 1981), it could perhaps be that at elevated temperatures, this β-keto ester anion becomes more active and possibly sufficiently active to catalyse ester interchange. However, this cannot be the complete picture since the *in situ* condensation product of sodium hydroxide is quite active at, say, 130°C, whereas sodium methanolate catalysed interesterifications at 205°C require several hours. (Naudet, 1947)

4.3.2.6 Heterogeneous catalysis

The mechanisms listed above are all concerned with homogeneous interesterification reactions, but heterogeneous catalysts have also been reported. One such catalyst (Bayense et al., 2000) contains one or more oxides and/or oxy salts of metals of the groups IA and IIA of the Periodic System. Potassium carbonate on magnesium oxide is an example. According to the examples, the temperature required is around 250°C and although the catalyst itself may not be sensitive to water, it will catalyse hydrolysis if any water is present.

Following the development of heterogeneous catalysts for the alcoholysis of triglyceride oils (Peter et al., 2002; Peter and Weidner, 2004; 2005), the same catalyst, a zinc salt of the amino acids arginine or carnitine, was also used for the interesterification of triglyceride oils (Peter et al., 2005). The process uses large amounts of catalyst (preferably 10 to 20 wt %), a fairly high temperature (about 125°C), and takes several hours; a duration of 8 hours is mentioned in one of the examples. The application also mentions the possibility of using a catalyst slurry in a batch process with subsequent filtration and also continuous and semicontinuous processes. It does not mention anything about yield losses or catalyst life but even when assuming these to be good, it is doubtful if these advantages outweigh the disadvantages mentioned before.

4.3.3 Enzymatic interesterification

It is interesting to note that the string of publications on enzymatic interesterification of edible oils originally started in industry (Unilever (Coleman and Macrae, 1977); Fuji Oil Co (Matsuo et al., 1981)). It is only after biotechnology was envisaged as spearhead technology that governments, keen not to miss the boat, started to grant subsidies for research projects proposed under the banner of biotechnology. And indeed, the advantages of enzymatic modification of fats and oils look quite attractive on paper. To quote Akoh (1997):

- *Position-specific structured lipids — desirable fatty acids can be incorporated at specific positions of triacylglycerol.*
- *Enzymes exhibit regioselectivity (discriminate on the bond to be cleaved), enantioselectivity (optical activity), chemoselectivity (based on functional group), and fatty acid chain length specificity.*
- *Can design structured lipids on a case-by-case basis to target for specific food or therapeutic uses — custom synthesis.*
- *Fats and oils with defined structure can be produced.*
- *Novel fats and oils that cannot be made by conventional plant breeding and genetic engineering can be obtained, such as by inserting a specific acid at the sn-2 position of glycerol molecule.*
- *Mild reaction conditions.*
- *Little or no unwanted side reactions or products.*
- *Ease of product recovery.*
- *Add value to fats and oils.*
- *Improve functionality and properties of fats.*

Another reason for the sudden interest in the use of lipases (EC 3.1.1.3) may well have been their commercial availability when they started to be used in detergents, leather processing, and sewage treatment (Gandhi, 1997).

Consequently, articles in the *Journal of the American Oil Chemists' Society* that are categorised as "Biotechnology and Biocatalysis" now regularly outnumber those categorised as "Processing and Engineering Technology;" a review by Malcata et al. (1990) on the use of immobilised enzyme technology already lists over 200 references. Similarly, Gandhi when reviewing the applications of lipase in 1997 and limiting herself to English literature also arrives at over 200 references.

The earliest patent (Coleman and Macrae, 1977) is very broad and just claims enzymatic interesterification by mentioning a fatty substrate, a lipase, and a moisture content that suffices to activate the enzyme. This water content is subsequently specified from 0.2 to 1% by weight, which means that if this water were to be used for hydrolysing triglycerides, even the 0.2% by weight would generate some

[4] Industrial interesterification reactions use only 0.05 wt % sodium methylate.

3% by weight FFA (free fatty acids) and 6% by weight diglycerides. Consequently, Macrae (in Rozendaal and Macrae, 1997) advocates a careful control of the water content of the reaction mixture to achieve a balance between reaction rates and product yields.

Nevertheless, the reaction product will always require neutralisation and when it is intended as a confectionary fat (for quite some time the only product commanding a sufficiently high price to afford enzymatic interesterification), its partial glyceride content may have to be lowered. This can be done chromatographically, but also enzymatically by using the *Penicillium cyclopium* lipase (Yamaguchi et al., 1993), since this enzyme exhibits a strong specificity for partial glycerides, but again, this purification adds to the cost of the residue, which has to bear both the processing cost and the yield loss.

Therefore, it is not surprising that a subsequent patent (Matsuo et al., 1981) specifies a low water content. It also specifies the substrate in somewhat more detail as a mixture of triglycerides and either a free fatty acid or a lower alcohol ester of this fatty acid. Shortly afterwards, the same inventors (Matsuo et al., 1983) also include hydrogenation and subsequent recycling of the fatty acid moiety liberated during the enzymatic interesterification process. They aim at the production of a cocoa butter equivalent (CBE) having a high content of glycerol 1,3 dioctadecanoate 2-(9-octadecenoate), commonly referred to as SOS, by allowing high oleic sunflower oil to exchange oleic acid for stearic acid and hydrogenating and recycling the oleic acid thus liberated according to the scheme represented in Figure 4.16.

However, the above reaction equation does not take into account that several different interesterification reactions are involved and that they are all reversible and, thus, lead to various equilibria. Accordingly, the reaction between 4 mol of trioleate (OOO) and 8 mol stearic acid (S) will not lead to 4 mol of SOS, but to the equilibrium mixture consisting of only 1 mol SOS, 2 mol SOO (or rather 1 mol SOO and 1 mol OOS), 1 mol OOO, 4 mol S, and 4 mol 0. So instead of using 1 mole OOO and 2 mol S to produce 1 mol SOS, four times as much starting material is required. The yield of the target compound (SOS) is low, and further lowered by the necessary purification steps, the cost of which has to be borne by the small amount of end product.

Another method to produce fat with a high SOS content has been disclosed by Macrae et al. (see Macrae and Brench, 1983, example 1). It uses shea butter as a starting material since this wild fat already contains some 30% SOS and also some POS (P = palmitic acid). Most of these symmetrical monounsaturated triglycerides are isolated by fractionation, which also produces an olein fraction with an appreciable SOO content. By subjecting this olein fraction to an interesterification catalysed by a 1,3-specific lipase, some additional SOS is formed, which can then again be upgraded in the fractionation plant that is already being used for the shea butter fractionation.

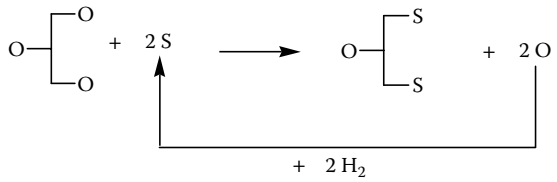


FIGURE 4.16 Formation of SOS with recycling of hydrogenated fatty acid.

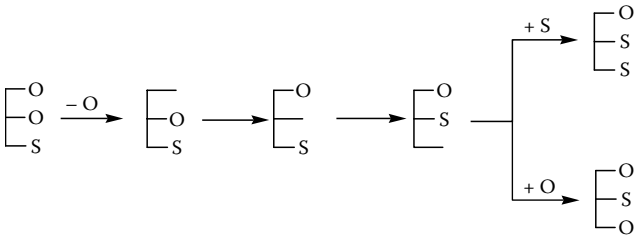


FIGURE 4.17 Loss of selectivity by isomerisation of diglycerides.

This process may have been attractive when shea butter and other wild fats were expensive and their supply was unreliable, but this has changed. Nowadays, fractionating shea butter and using the stearin as the SOS-component of CBE is significantly cheaper than attempting to obtain more SOS through enzymatic interesterification of the olein by-product.

Besides, the EU Chocolate Directive (Berger, 2003; Stewart and Kristott, 2004) restricts the permissible CBEs to fats from six crops only: palm oil, illipe butter, shea butter, sal fat, kokum gurgi fat, and mango kernel fat; this excludes fats obtained by interesterification, enzymatic, or otherwise. It is not surprising that a CBE producer concluded that "for the time being, the cost of enzymes is not competitive to other possible raw materials" (Nielsen, 2001), in spite of the development of a new immobilised lipase derived from *Thermomyces lanuginosa* that does not require water to sustain its activity (Berben et al., 2001).

It should also be noted that the specificity of the enzymatic interesterification process turns out not to be absolute in that some substitution in the 2-position is always observed. The cause for this nonspecificity is not so much the enzyme itself, but the isomerisation of intermediates which may be diglycerides as illustrated in Figure 4.17.

A different mechanism as shown in Figure 4.18 has been suggested earlier (Rozendaal and Macrae, 1997, Figure 25). It is based on the isomerisation of monoglycerides, but since it involves many more steps than the mechanism involving the isomerisation of diglycerides, the mechanism shown in Figure 4.17 is probably more likely.

Because the rate of the chemically catalysed isomerisation of the partial glycerides and the rates of the enzyme catalysed hydrolysis and esterification reactions are independent of each other, the relative extent of the isomerisation can be suppressed by increasing the rates of the enzyme catalysed reactions (Xu et al., 1998a; 1998b; Xu,

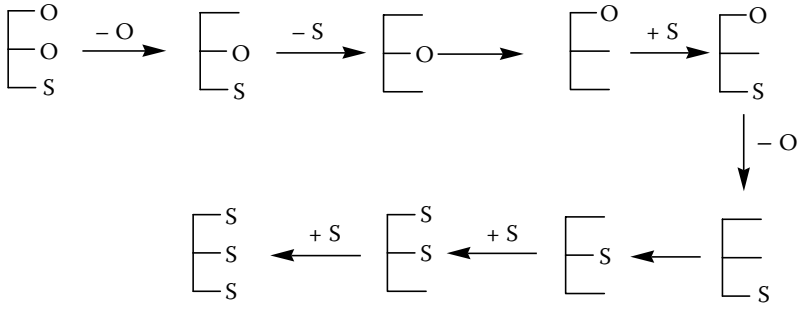


FIGURE 4.18 Loss of selectivity by isomerisation of monoglycerides.

2000a), ergo by using more enzyme as, for instance, in a bed packed with immobilised enzyme.

However, filling a packed bed with a large amount of immobilised enzyme constitutes a considerable and above all risky expenditure, since in industrial practice, how fast the enzyme loses its activity is a matter of "wait and see." There is as yet no method of predicting this rate of inactivation, let alone controlling or decreasing it (Diks, 2002). Moreover, the substrate constituent or constituents responsible for the inactivation of the catalyst may act fairly slowly so that guarding the bed packed with fresh enzyme by a precolumn packed with almost inactive enzyme is, at most, only partially effective.

Consequently, the few industrial installations operating enzymatic interesterification are regularly, but unpredictably, faced with a situation whereby in order to maintain productivity or product quality (specificity), an amount of enzyme preparation that still shows enzymatic activity has to be discarded and replaced by fresh enzyme. Although the price of enzymes has dropped to € 50/kg (Kellens, 2005) and may well continue to decrease, this replacement of enzymes is still expensive and, therefore, hardly conducive to widespread adoption of the enzymatic interesterification process.

Moreover, the statement (Husum et al., 2004) that, unlike chemical catalysis, enzymatically interesterified reaction products do not require a bleaching treatment before being deodorised, is only one side of the coin since the raw materials require extensive purification. Bleaching with very intensive mixing is claimed in a patent application (Ten Brink et al., 2005) and according to recent information (M. Kellens, personal communication), the use of fully and freshly deodorised material may also decrease the risk of a sudden loss of enzymatic activity.

Passing this material through a column of old enzyme is similarly claimed to effectively protect downstream columns from rapidly losing their activity. The interesterification product must, however, be re-deodorised to remove the FFA formed during interesterification, although this has already been reduced by replacing the *Rhizomucor miehei* enzyme (Lipozyme IM) with the *Thermomyces lanuginose* enzyme (Lipozyme TL IM) that remains active at low water concentration. It is not clear to what extent all these aspects have been taken into account in the cost comparison (Kellens, 2005) that arrives at a roughly equal price tag for the chemical and the enzymatic process.

Industrial enzymatic interesterification has been mainly limited to infant formula fat to ensure a high palmitic acid content at the 2-position of the triglyceride (Quinlan and Moore, 1993; Kavanagh, 1997) and a symmetrical monounsaturated dibehenate (BOB) used as a fat bloom inhibiting additive in chocolate (Sagi et al., 1989). In addition, some interesterification products that up until now have been made perfectly satisfactory by chemical catalysis, are now being made by enzymatic catalysis (see, for instance, Muderhwa et al., 1989), but this is not for technological reasons or to realise cost savings, but purely as a public relations exercise.

So, whereas the industrial application of enzymatic interesterification is still quite limited, the range of topics involving enzymatic interesterification covered by the literature is very wide indeed (Hayes, 2004). They cover *inter alia* characterisation and identification of the enzymes as reviewed by Beisson (2000), their immobilisation, enzyme catalysed fat splitting, alcoholysis, glycerolysis, and synthesis of triglycerides and monoglycerides (Arcos and Otero, 1996), synthesis of waxes, synthesis of "structured lipids" as reviewed by Xu (2000b), and engineering aspects (Xu, 2003). And as if the use of enzymes was not already sufficiently fanciful, these topics also include enzymatic interesterification in supercritical carbon dioxide (Jackson and King, 1997).

4.3.4 Industrial interesterification processes

4.3.4.1 Batch processes

The most common industrial interesterification process is a batch process using sodium methanolate or ethanolate as the catalyst. It employs a reaction vessel with a conical bottom that can be heated, agitated and evacuated (Sreenivasan, 1978; Laning, 1985).

Because alcoholates react with water and FFA, the oil or fat to be interesterified must be dried to a water content below 0.01% and neutralized to a FFA content below 0.05% (Laning, 1985; Kellens, 1998). In practice this is achieved by the addition of caustic soda followed by drying

and taking a sample to demonstrate alkalinity. Drying can be effectively achieved by spray drying *in vacuo,* but agitating a batch heated to 80 to 100°C or higher (Laning, 1985) under vacuum and/or sparging with nitrogen is also sufficiently effective. An amount of 0.05% by weight of catalyst generally suffices and the use of preweighed aliquots packaged in sealed plastic bags is recommended. Equilibrium is generally reached within a few minutes at temperatures around 90°C but reaction times of 30 min are common.

After this period of time, the catalyst is inactivated by the addition of water that may have been acidified. This water leads to the formation of soaps that can subsequently be removed by washing with water or by the addition of some silica hydrogel (Leake, 1997). If acidified water is used for the inactivation, the resulting FFA will be removed during the subsequent deodorisation treatment, which will also remove the FAME formed after catalyst addition. The yield loss involved in the formation and subsequent removal of FAME and FFA has been estimated by Kellens (2000) to amount to 13 times the weight of the sodium methanolate added; this corresponds to 30% of the variable costs.

According to the enolate mechanism, anhydrous catalyst inactivation would avoid a fatty acid moiety to be split off and lost. Accordingly, Holman et al. (1959) advocate the use of gaseous carbon dioxide, but the use of phosphoric acid is also mentioned (Laning, 1985) as a means to minimise process losses; Eckey used glacial acetic acid for catalyst inactivation (1949).

Instead of alkali alcoholates, alkali metals can also be used as catalysts in the randomisation process. They can be extruded into the oil to be interesterified, where they melt if the oil is sufficiently warm so that they can be easily dispersed by a homogeniser or high shear agitator (De Groot and Hilder, 1971). According to Laning (1985), "a sodium/potassium alloy, Na/K (Anon., 1955) is much faster at catalysing the interesterification reaction at lower temperatures than Na or K or either alcoholate. This is apparently due to the ability of the liquid Na/K particles to retain unreacted metal on their surfaces, while solid particles of Na or K become coated with oxides and hydroxides" (Hawley and Holman, 1956).

4.3.4.2 Continuous processes

Continuous processes only lend themselves to standard grades with a steady and sufficiently large demand. Failing this, the necessary product changes would make the continuous process more expensive than the batch process. Directed interesterification processes as used for lard in the U.S. were continuous or semicontinuous (Holman and Going, 1959). One of the processes used for sunflower seed oil in Belgium commenced continuously with drying the oil and dispersing the catalyst into the oil, activating the catalyst, then chilling the interesterifying stream while feeding this stream into one of several crystallisation vessels, but subsequently, the contents of these vessels were treated as separate batches. Their temperature was maintained and controlled by circulating the vessel contents over a scraped surface heat exchanger and crystallisation was also promoted by slowly agitating the vessels. When a batch had reacted sufficiently, the catalyst was inactivated by the addition of water that had been acidulated with phosphoric acid while the vessel contents were being transferred to a washing vessel. There the reaction mixture was heated and fully melted, the acid water was drained away, and the oil was washed with water before being dried and sent to intermediate storage.

The review by Going (1967) shows flow diagrams of continuous randomisation processes using sodium hydroxide catalyst and sodium methanolate as catalysts. These diagrams have been redrawn by Rozendaal (1997). Both diagrams show an oil heater to raise the oil temperature to 150 to 180°C and a two-stage vacuum dryer, but if sodium hydroxide is used, this catalyst is added before the oil is dried. The hot oil is then given a residence time of 2 min before being cooled, water-washed, and centrifuged to eliminate the soaps. If sodium methanolate is used as the catalyst, the oil is dried and cooled to 50°C before catalyst addition. The catalyst is slurried in a small amount of dry oil and metered into the main stream of dry oil, which is then given some residence time before the catalyst is inactivated by water addition.

The use of a sodium metal catalyst in continuous randomisation has been described by De Groot and Hilder (1971) and a flow diagram for the continuous process using sodium hydroxide and glycerol has been given by Rozendaal (1990; 1992; 1997). The latter process is still being used. In this process, a solution of caustic soda in glycerol is metered into a stream of preheated oil and the mixture is sprayed into a vacuum dryer. From this vessel, the reaction mixture is pumped through a plug flow reactor to the refining stage.

4.3.4.3 Process control

In industrial practice, control of the interesterification process amounts to verifying if the reaction has taken place. If this is not the case, the batch concerned has to be reprocessed, which means that the control method has to be fast and preferably suitable for immediate use on the shop floor. If the melting point of the interesterified product is sufficiently different from the melting point of the starting mixture, determining the melting point is a quick and easy way of establishing interesterification. If the difference is fairly small, a fast determination of the solid fat content (Rutledge et al., 1988) may have to be used. In both cases, samples should be taken before catalyst inactivation so that the reaction can be completed by the addition of extra catalyst.

A quite different approach has been described by Liu and Lampert (1999; 2001). It makes use of the reddish-brown

colour that appears on initiation of the interesterification reaction, since they claim that "there is a direct correlation between the absorbance of the reaction mixture and the degree of interesterification." Accordingly, they also claim partially interesterified mixtures, which they say have "unique functional properties compared to the physical and randomized blends." However, they are not that unique since it was shown on mathematical grounds that partially interesterified products can be regarded as mixtures of the noninteresterified starting material and the fully randomised end-product (Dijkstra, 2000).

4.3.5 Interesterification products

Several interesterification products have already been mentioned, such as the interesterified lard produced in the U.S. by randomisation (Vander Wal and Van Akkeren, 1951; Dominick et al., 1953; Mattil and Nelson, 1953; Lutton et al., 1962) or by directed interesterification (Hawley and Holman, 1956; Holman and Going, 1959), health margarines produced by directed interesterification of high linoleic acid oils (Haighton and Kattenberg, 1974; Kattenberg, 1974; De Lathauwer et al., 1980; Kogan and Pelloso, 1982) and the products made by enzymatic interesterification, such as cocoa butter equivalents (Coleman and Macrae, 1980; Macrae and Brench, 1983; Matsuo et al., 1983), fat bloom inhibitor (Sagi et al., 1989), and infant formula (Quinlan and Moore, 1993; Kavanagh, 1997). Accordingly, this section will be limited to interesterification products obtained by chemical randomisation and used, for example, as hard stock in fat blends for margarines and shortenings.

One such hardstock is based upon palm oil and/or palm stearin and a lauric oil (Fondu and Willems, 1972; Hustedt, 1976). Palm stearin itself is not suitable as a hardstock because of its high content of high melting, fully saturated triglycerides that would give the resulting spread a sticky feel in the mouth. Accordingly, the melting point of the high melting triglycerides is reduced by the introduction of shorter chain fatty acids originating from the lauric oil. Such randomised hardstocks have the additional advantage that they have not been subjected to the chemical hydrogenation process, which in health stores tends to be regarded as a positive sales argument. Their disadvantage is that their use leads to margarines with a relatively high content of saturated fatty acids. This content has been somewhat reduced by interesterifying a very hard palm stearin and a palm kernel stearin (Ullanoormadam, 2004)[5].

The disadvantage has been more effectively overcome by the use of fully hydrogenated and randomised blends of a long-chain oil like soya bean oil and a lauric oil, as had already been described by Gooding (1943). Although the hardstocks themselves are fully hydrogenated, only small amounts (8 to 15% by weight) are required to provide tub margarines with the consistency required. Accordingly, palm kernel oil has been claimed for this application by Graffelman (1971), coconut oil by Delfosse (1971), and finally babassu oil in the U.S. (Ward, 1982). Blending a relatively small amount of such fully hydrogenated hardstocks with, for instance, liquid sunflower seed oil, allows health margarines to be produced with a fat blend that has a low (around 25%) content of saturated fatty acids and a relatively high (about 55%) linoleic acid content. These figures can be improved upon by using the midfraction of such interesterification products (Schijf et al., 1983). The SFC values of such products have been tabulated by Allen (1996).

In Europe, sunflower seed oil has a health connotation, so spreads based solely on sunflower seed oil tend to command a premium. However, partially hydrogenated sunflower seed oil is β-tending, so blending this hardstock with liquid sunflower seed oil will lead to sandiness resulting from the large and high melting β-crystals. This can be effectively overcome by interesterifying the partially hydrogenated sunflower seed oil with some liquid oil and using the interesterification product as hardstock (Gander et al., 1966) to be blended with liquid sunflower seed oil. Another possibility is to interesterify the partially hydrogenated sunflower seed oil with oil that is relatively rich in palmitic acid so that the interesterification product contains more than 10% of this acid (Gercama and Schijf, 1983). However, since the hydrogenation involved is partial, the hardstock and, thus, the final product will contain some *trans* isomers, which nowadays are considered to be a disadvantage.

Accordingly, "modern" margarine fat blends tend to be *trans* isomer free (Brinkmann, 2001). They can be produced by interesterifying fully hydrogenated oil with liquid oil and using the resulting interesterification product as hardstock (Hurtová et al., 1996; List, 2004). However, when processed, such fat blends tend to be α-persistent, causing the resulting margarine to be somewhat more brittle and less unctuous (Van Duijn, 2000). This problem can be alleviated by the addition of crystallisation accelerators, such as triglycerides having fatty acid carbon chains of different chain lengths (Lansbergen et al., 1999).

Finally, fat substitutes, such as olestra (see also www.olean.com), sucrose esterified with six to eight fatty acids (Mattson and Volpenhein, 1976), caprenin (caprocaproylbehenin), salatrim (acronym for short- and long-chain acyl triglyceride molecules), and EPG (esterified propoxylated glycerin) should also be mentioned as interesterification products. They are quite different products in that Olean® is totally indigestible and was intended as an inhibitor of cholesterol absorption. It is manufactured by interesterifying FAME and sucrose (Rizzi and Taylor,

[5] The application of this patent (Sahasranamam, 2002) mentions a different inventor because the U.S. Patent and Trademark Office confused his first forename with his surname.

1976; 1978). It does not contribute to the energy content of crisps fried in this fat substitute. Sorbestrin is the sorbitol equivalent of olestra and is also synthesised by interesterifying a sugar (sorbitol) with FAME.

The EPG fat substitute is also manufactured by interesterification with FAME (Cooper, 1992). The interesterification substrate is prepared by allowing propylene oxide to react with glycerol in the presence of an alkali metal alcoholate catalyst, and the same catalyst is used for the interesterification with FAME. In a later patent (Cooper, 1994), Example 1 describes the synthesis of EPG by direct esterification and subsequent examples describe the interesterification of this product with triglycerides containing behenic acid, which is then incorporated into the EPG.

Caprenin and salatrim, on the other hand, are triglycerides made by esterifying a monoglyceride with acid anhydrides (Stipp and Kluesener, 1992) or by interesterification (Wheeler et al., 1996). Salatrim derives its low energy from its short-chain fatty acids (acetic, propionic, butyric, or a combination of all three) and the low absorption by the body of the longest of its long-chain fatty acids (C_{16} to C_{22}). Consequently its energy content is only just over half of that of fully digestible fats (Auerbach et al., 2001). Stearic and behenic MCTs (medium chain triglycerides) with C_6 to C_{10} saturated fatty acids have also been reported (Seiden, 1994). However, after initial enthusiasm, the expectations for these fat substitutes have been much reduced (Watkins, 2003).

References

Akoh, C.C. (1997), Making new structured fats by chemical reaction and enzymatic modification, *Lipid Techn.*, **9**, 61–66.

Allen, D.A. (1996), Interesterification — a vital tool for the future? *Lipid Techn.*, **8**, 11–15.

Anon. (1955), Le NACK. Le potassium remplacera-t-il le sodium? *Rev. Franç. Corps Gras*, **2**, 337–338.

Arbeitskreis "Technologien der industriellen Gewinnung und Verarbeitung von Speisefetten" (1973), Gemeinschaftsarbeiten der DGF, 57 Mitteilung. Die Umesterung von Speisefetten I, *Fette Seifen Anstrichm.*, **75**, 467–474.

Arcos, J.A. and Otero, C. (1996), Enzyme, medium, and reaction engineering to design a low-cost, selective production method for mono- and dioleylglycerols, *J. Am. Oil Chem. Soc.*, **73**, 673–682.

Artman, N.R. et al. (1968), Interesterification of glycerides, US Patent 3,376,326, assigned to The Procter & Gamble Company.

Auerbach, M.H. et al. (2001), Reduced-energy lipids, in *Structured and Modified Lipids*, Gunstone, F.D., Ed., Marcel Dekker, New York, 485–510.

Baltes, J. (1960), Reaktionsmechanismus und Kinetik der Umesterung von Fetten, *Die Nahrung*, **4**, 1–16.

Barsky, G. (1939), Method of reconstructing glyceride oils, US Patent 2,182,332, assigned to Wecoline Products Inc.

Barsky, G. (1973), Directed interesterification of glycerides of fatty acids and products thereof, US Patent 3,758,530.

Bayense, C.R. et al. (2000), Process for the interesterification of triglycerides, US Patent 6,072,064, assigned to Engelhard Corporation.

Beisson, F. et al. (2000), Methods for lipase detection and assay: a critical review, *Eur. J. Lipid Sci. Technol.*, **1**, 133–153.

Berben, P.H. et al. (2001), Interesterification with immobilized enzymes, *Paper presented at SCI Symposium "The Future of Interesterification in Oil Processing," London*.

Berger, K.G. (2003), EU Chocolate Directive discussed in May, *inform*, **14**, 511.

Braun, W.Q. (1960), Interesterification of edible fats, *J. Am. Oil Chem. Soc.*, **37**, 598–601.

Brink, H.B. ten et al. (2005), Process for the interesterification of a glyceride fat, US Patent Application Publication 2005/0019316 A1, assigned to Unilever.

Brinkmann, B. (2001), Interesterification as a tool to improve the nutritional value of fat blends for various applications, in *Proceedings of the World Conference on Oilseed Processing and Utilization*, Wilson, R.F., Ed., AOCS Press, Champaign, IL, 31-33.

Burgers, J. et al. (1965), Interesterification process, US Patent 3,170,798, assigned to The Procter & Gamble Company.

Coenen, J.W.E. (1974), Fractionnement et interestérification des corps gras dans la perspective du marché mondial des matières premières et des produits finis II Interestérification, *Rev. Franç. Corps Gras*, **21**, 403–413.

Coleman, M.H. and Macrae, A.R. (1977), Composition de matières grasse et procédé pour sa fabrication, French Patent 2 340 979, assigned to Unilever.

Coleman, M.H. and Macrae, A.R. (1980), Fat process and composition, British Patent 1 577 933, assigned to Unilever.

Cooper, C.F. (1992), Preparation of esterified propoxylated glycerin by transesterification, US Patent 5,175,323, assigned to Arco Chemical Technology, L. P.

Cooper, C.F. (1994), Process for producing a reduced calorie lipid composition, US Patent 5,298,637, assigned to Arco Chemical Technology, L. P.

De Lathauwer, R. et al. (1980), Process for the directed interesterification of a triglyceride oil or oil mixture, European Patent 0 009 207, assigned to N.V. Vandemoortele International.

Delfosse, J.K.F. (1971), Margarine fat composition, British Patent 1 244 868, assigned to Unilever.

Dijkstra, A.J. (2000), Edible oils in Europe, *inform*, **11**, 386–394.

Dijkstra, A.J. (2001), Refining and processing for shortening manufacture and other uses, in *Animal Carcass Fats*, Rossell, J.B., Ed., LFRA Limited, Leatherhead, U.K., 51–75.

Dijkstra, A.J. (2004a), Edible oil processing *quo vadis*, *Eur. J. Lipid Sci. Technol.*, **106**, 77–78.

Dijkstra, A.J. (2004b), The interesterification mechanism revisited, *Paper presented at the 3rd EuroFedLipid Congress*, Edinburgh, page 86 in the book of abstracts.

Dijkstra, A.J. et al. (2005), The base-catalyzed, low-temperature interesterification mechanism revisited, *Eur. J. Lipid Sci. Technol.*, **107**, 912–921.

Diks, R.M.M. (2002), Lipase stability in oil, *Lipid Techn.*, **14**, 10–14.

Dominick, W.E. et al. (1953), Lard crystal modification, US Patent 2,625,484, assigned to Swift & Company.

Duijn, G. van (2000), Technical aspects of *trans* reduction in margarines, *Oléagineux Corps Gras Lipides/OCL*, **7**, 95–98.

Eckey, E.W. (1945b), Process for treating fats and fatty oils, US Patent 2,378,006, assigned to The Procter & Gamble Company.

Eckey, E.W. (1945a), Process for treating fats and fatty oils, US Patent 2,378,005, assigned to The Procter & Gamble Company.

Eckey, E.W. (1948a), Modification of glycerides, US Patent 2,442,535, assigned to The Procter & Gamble Company.

Eckey, E.W. (1948b), Process for treating fats and fatty oils, US Patent 2,442,531, assigned to The Procter & Gamble Company.

Eckey, E.W. (1951), Catalytic processes for ester-ester interchange, US Patent 2,558,547.

Eckey, E.W. (1956), Esterification and Interesterification, *J. Am. Oil Chem. Soc.*, **33**, 575–579.

Eckey, E.W. and Formo, M.W. (1949), Directed interesterification in glycerides. Reaction in presence of added glycerol, *J. Am. Oil Chem. Soc.*, **26**, 207–211.

Faur, L. (1977), L'interestérification. Applications dans le domaine alimentaire, *Rev. Franç. Corps Gras*, **24**, 85–91.

Feuge, R.O. et al. (1945), Modification of vegetable oils IV. Reesterification of fatty acids with glycerol, *Oil & Soap*, **22**, 202–208.

Fondu, M.P.V. and Willems, M.A.G. (1972), Margarine fat containing randomized fat component, US Patent 3,634,100, assigned to Unilever.

Formo, M.W. (1954), Ester reactions of fatty materials, *J. Am. Oil Chem. Soc.*, **31**, 548–559.

Freeman, I.P. (1968), Interesterification. I. Change in glyceride composition during the course of interesterification, *J. Am. Oil Chem. Soc.*, **45**, 456–460.

Friedel, Ch. and Crafts, J.R. (1865). *Annals*, **133**, 207–211.

Gander, K.F. et al. (1966), Procédé de fabrication de margarine obtenue principalement à partir de l'huile de tournesol, French Patent 1.457.751, assigned to Unilever.

Gandhi, N.N. (1997), Applications of lipase, *J. Am. Oil Chem. Soc.*, **74**, 621–634.

Gercama, A. and Schijf, R. (1983), Margarine fat blend with reduced tendency to sandiness and a process for reducing the development of sandiness in fat blends, European Patent 0 084 900, assigned to Unilever.

Going, L.H. (1967), Interesterification products and processes, *J. Am. Oil Chem. Soc.*, **44**, 414A–456A.

Gooding, C.M. (1943), Preparation of mixed esters of polyhydric alcohols, US Patent 2,309,949, assigned to The Best Foods Inc.

Graffelman, H.A. (1971), Margarine fat and process for preparing same, US Patent 3,617,308, assigned to Unilever.

Groot, W.H. de and Hilder, M.H. (1971), Preparation and use of alkali metal dispersions, British Patent 1 236 233, assigned to Unilever.

Grün, A. (1922), Improvements in the manufacture of nutritious fats, British Patent 160,840, assigned to Georg Schicht, A. G.

Haighton, A.J. and Kattenberg, H.R. (1974), Interesterification process, US Patent 3,855,254, assigned to Unilever.

Hawley, H.K. and Holman, G.W. (1956), Directed interesterification as a new processing tool for lard, *J. Am. Oil Chem. Soc.*, **33**, 29–35.

Hayes, D.G. (2004), Enzyme-catalyzed modification of oilseed matrials to produce eco-friendly products, *J. Am. Oil Chem. Soc.*, **81**, 1077–1103.

Heldal, J.A. and Mørk, P.C. (1981), On the mechanism of the base catalyzed interesterification of fats, *Paper presented at the 11th Scandinavian Symposium on Lipids,* Bergen, Norway, 147–151.

Holman, G.W. and Going, L.H. (1959), Rapid directed interesterification of glycerides, US Patent 2,875,066, assigned to The Procter & Gamble Company.

Holman, G.W. et al. (1956), Catalytic interesterification, US Patent 2,738,278, assigned to The Procter & Gamble Company.

Holman, G.W. and Mills, V. (1959), Inactivation of catalyst in molecular rearrangement reactions, US Patent 2,886,578, assigned to The Procter & Gamble Company.

Hurtová, S. et al. (1996), Random interesterification of fat blends with alkali catalysts, *Fett/Lipid*, **98**, 60–65.

Hustedt, H.H. (1976), Interesterification of edible oils, *J. Am. Oil Chem. Soc.*, **53**, 390–392.

Husum, T.L. et al. (2004), Enzymatic interesterification: a trouble-free process for *trans*-free fats, *Paper presented at the 3rd EuroFedLipid Congress*, Edinburgh, page 88 of the Abstracts.

Huyghebaert, A.D. et al. (1970), La présence des cétones dans des graisses semblables au beurre, *Fette Seifen Anstrichm.*, **72**, 289–293.

Jackson, M.A. and King, J.W. (1997), Lipase-catalyzed glycerolysis of soybean oil in supercritical carbon dioxide, *J. Am. Oil Chem. Soc.*, **74**, 103–106.

Kattenberg, H.R. (1974), Beschleunigung der gelenkten Umesterung mittels periodischer, variabler Temperaturführung, *Fette Seifen Anstrichm.*, **76**, 79–82.

Kaufmann, H.P. et al. (1958), Umesterungen auf dem Fettgebiet I: Theoretische Grundlagen und Schriftum — Die Hydrier-Umesterung, *Fette Seifen Anstrichm.*, **60**, 919–930.

Kavanagh, A.R. (1997), A breakthrough in infant formula fats, *Oléagineux Corps Gras Lipides/OCL*, **4**, 165–168.

Kellens, M.J. (1998), État des lieux et évaluation des procédés de modification des matières grasses par combinaison de l'hydrogénation, de l'interestérification et du fractionnement, *Oléagineux Corps Gras Lipides/OCL*, **5**, 384–391.

Kellens, M.J. (2000), Oil modification processes, in *Edible Oil Processing*, Hamm, W. and Hamilton, R.J., Eds., Sheffield Academic Press, Sheffield, U.K., 127–173.

Kellens, M.J. (2005), Interesterification, *Paper presented at the Short Course on Edible Oil Refining and Processing*, Salt Lake City, UT.

Keulemans, C.N.M. and Rozendaal, A. (1988), Process and apparatus for the interesterification of a triglyceride oil and products therefrom, US Patent 4,790,962, assigned to Unilever.

Keulemans, C.N.M. and Smits, G. (1986), Interesterification process and apparatus, US Patent 4,585,593, assigned to Unilever.

Kogan, L. and Pelloso, T.A. (1982), Edible fat product, European Patent 0 050 418, assigned to Nabisco Brands Inc.

Laning, S.J. (1985), Chemical interesterification of palm, palm kernel and coconut oils, *J. Am. Oil Chem. Soc.*, **62**, 400–405.

Lansbergen, G.J.T. et al. (1999), Triglyceride fat crystallization, US Patent 5,888,575, assigned to Unilever.

Leake, J.R. (1997), Soap removal from interesterified oils using Sorbsil® silica adsorbents, *Poster presented at the 88th AOCS Annual Meeting & Expo,* Seattle.

List, G.R. (2004), Processing and reformulation for nutrition labelling of *trans* fatty acids, *Lipid Techn.*, **16**, 173–177.

Liu, L. (2004), How is chemical interesterification initiated: nucleophilic substitution or α–proton abstraction? *J. Am. Oil Chem. Soc.*, **81**, 331–337.

Liu, L. and Lampert, D. (1999), Monitoring chemical interesterification, *J. Am. Oil Chem. Soc.*, **76**, 783–787.

Liu, L. and Lampert, D.S. (2001), Partial interesterification of triacylglycerols, US Patent 6,238,926 B1, assigned to Cargill Incorporated.

Loon, C. van (1926), An improved process for the conversion of neutral or nearly neutral triglycerides, mutually or with other esters, British Patent 249,916.

Luddy, F.E. et al. (1955), Effect of catalytic treatment with sodium methylate on glyceride compositions and properties of lard and tallow, *J. Am. Oil Chem. Soc.*, **32**, 522–525.

Lutton, E.S. et al. (1962), Interesterification of lard, *J. Am. Oil Chem. Soc.*, **39**, 233–235.

Macrae, A.R. and Brench, A.W. (1983), Edible fat process, European Patent 0 069 599, assigned to Unilever.

Malcata, F.X. et al. (1990), Immobilized lipase reactors for modification of fats and oils — a review, *J. Am. Oil Chem. Soc.*, **67**, 890–910.

Marangoni, A.G. and Rousseau, D. (1995), Engineering triacylglycerols: the role of interesterification, *Trends Food Sci. Technol.*, **6**, 329–335.

Matsuo, T. et al. (1981), Method for enzymatic transesterification of lipid and enzyme used therein, European Patent 0 035 883, assigned to Fuji Oil Company Ltd.

Matsuo, T. et al. (1983), Method for the modification of fats and oils, European Patent 0079 986, assigned to Fuji Oil Company Ltd.

Mattil, K.F. and Nelson, D.W. (1953), Process of producing modified lard, US Patent 2,625,483, assigned to Swift & Company.

Mattson, F.H. and Volpenhein, R.A. (1976), Pharmaceutical composition for inhibiting absorption of cholesterol, US Patent 3,954,976, assigned to The Procter & Gamble Company.

Muderhwa, J. et al. (1989), Interestérification catalysée par les lipases régio-sélectives 1,3 en milieu fondu: applications à l'huile de palme at à sa fraction concrète, *Rev. Franç. Corps Gras*, **36**, 11–19.

Muller, J.J. and Kock, T.J. (1974), Interesterification of glycerides in the presence of an aliphatic ketone, US Patent 3,809,709, assigned to Unilever.

Naudet, M. (1947), *Contribution à l'étude des migrations d'acyles entre triglycerides*, Ph. D. thesis, Université de Marseilles, France.

Naudet, M. (1954), Emplois industriels de l'interestérification, *Rev. Franç. Corps Gras*, **1**, 129–136.

Naudet, M. (1976), Interestérification et estérification. Mécanismes réactionnels et conséquences sur la structure, *Rev. Franç. Corps Gras*, **23**, 387–391.

Nielsen, K. (2001), Interesterification in use for the production of confectionary fats, *Paper presented at SCI Symposium "The Future of Interesterification in Oil Processing,"* London.

Normann, W. (1924), Verfahren zur Herstellung von gemischten Glyceriden, German Patent 407180.

Normann, W. (1925), Verfahren zur Umesterung von Fettsäureestern, German Patent 417 215, assigned to Oelwerke Germania G.m.b.H.

Norris, F.A. and Mattil, K.F. (1946), Interesterification reactions of triglycerides, *Oil & Soap*, **23**, 289–291.

Peter, S.K.F. et al. (2002), Alcoholysis of triacylglycerols by heterogeneous catalysis, *Eur. J. Lipid Sci. Technol.*, **104**, 324–330.

Peter, S.K.F. and Weidner, E. (2004), Process for the transesterification of fat and/or oil by means of alcoholysis, US Patent 2004/0059143 A1, assigned to S.K.F. Peter.

Peter, S.K.F. and Weidner, E. (2005), Process for the transesterification of fat and/.or oil by means of alcoholysis, US Patent 6,933,398 B2.

Peter, S.K.F. et al. (2005), Process for the transesterification of fat and/or oil by heterogeneous catalysis, US Patent Application Publication 2005/0020843 A1, assigned to Siegfried Peter.

Quinlan, P. and Moore, S. (1993), Modification of triglycerides by lipases: process technology and its application to the production of nutritionally improved fats, *inform*, **4**, 580–585.

Rizzi, G.P. and Taylor, H.M. (1978), A solvent-free synthesis of sucrose polyesters, *J. Am. Oil Chem. Soc.*, **55**, 398–401.

Rizzi, G.P. and Taylor, H.M. (1976), Synthesis of higher polyol fatty acid polyesters, US Patent 3,,963,699, assigned to The Procter & Gamble Company.

Rousseau, D. and Marangoni, A.G. (2002), Chemical interesterification of food lipids: theory and practice, in *Food Lipids, Chemistry, Nutrition and Biotechnology*, Akoh, C.C. and Min, D.B., Eds., Marcel Dekker Inc, New York, Basel, 301–333.

Rozendaal, A. (1990), Interesterification of oils and fats, in *Edible Fats and Oils Processing: Basic Principles and Modern Practices*, Erickson, D.R., Ed., AOCS, Champaign, IL, 152–157.

Rozendaal, A. (1992), Interesterification of oils and fats, *inform*, **3**, 1232–1237.

Rozendaal, A. and Macrae, A.R. (1997), Interesterification of oils and fats, in *Lipid Technologies and Applications*, Gunstone, F.D. and Padley, F.B., Eds., Marcel Dekker, New York, 223–263.

Rutledge, D.N. et al. (1988), Contribution à l'étude d'une méthode de contrôle rapide de la qualité des margarines par RMN-IBR, *Rev. Franç. Corps Gras*, **35**, 157–162.

Sagi, N. et al. (1989), Hard butter composition containing triglycerides, and process for the production thereof, US Patent 4,839,192, assigned to Fuji Oil Company Ltd.

Sahasranamam, U.R. (2002), *Trans*-free hard structural fat for margarine blend and spreads, US Patent 2002/0001662 A1.

Schijf, R. et al. (1983), Margarine fat blend, and process for producing said fat blend, European Patent 0 089 082, assigned to Unilever.

Seiden, P. (1994), Reduced calorie fats made from triglycerides containing medium and long chain fatty acids, US Patent 5,288,512, assigned to The Procter & Gamble Company.

Sreenivasan, B. (1973), Directed-interesterified glyceridic oils having a high linoleic acid content and process for their production, US Patent 3,748,348, assigned to Unilever.

Sreenivasan, B. (1978), Interesterification of fats, *J. Am. Oil Chem. Soc.*, **55**, 796–805.

Stewart, I. and Kristott, J. (2004), European Union Chocolate Directive defines vegetable fats for chocolate, *Lipid Techn.*, **16**, 11–14.

Stipp, G.K. and Kluesener, B.W. (1992), Selective esterification of long chain fatty acid monoglycerides with medium chain fatty acid anhydrides, US Patent 5,142,072, assigned to The Procter & Gamble Company.

Ullanoormadam, S.R. (2004), *Trans*-free hard structural fat for margarine blend and spreads, US Patent 6,808,737, assigned to Premium Vegetable Oils Berhad.

Vander Wal, R.J. and Van Akkeren, L.A. (1951), Modified lard and process of producing same, US Patent 2,571,315, assigned to Armour and Company.

Ward, J. (1982), Edible fat product, US Patent 4,341,812, assigned to Nabisco Brands Inc.

Watkins, C. (2003), New developments for olestra, Ztrim and salatrim, *inform*, **14**, 611.

Weiss, T.J. et al. (1961), Reaction mechanism of sodium methoxide treatment of lard, *J. Am. Oil Chem. Soc.*, **38**, 396–399.

Wheeler, E.L. et al. (1996), Reduced calorie triglyceride mixtures, US Patent 5,552,174, assigned to Nabisco Inc.

Willems, M.A.G. and Padley, F.B. (1985), Palm oil: Quality requirements from a customer's point of view, *J. Am. Oil Chem. Soc.*, **62**, 454–459.

Xu, X. (2000a), Enzymatic production of structured lipids: process reactions and acyl migration, *inform*, **11**, 1121–1131.

Xu, X. (2000b), Production of specific-structured triacylglycerols by lipase-catalyzed reactions: a review, *Eur. J. Lipid Sci. Technol.*, 287–303.

Xu, X. (2003), Engineering of enzymatic reactions and reactors for lipid modification and synthesis, *Eur. J. Lipid Sci. Technol.*, **105**, 289–304.

Xu, X. et al. (1998a), Pilot batch production of specific-structured lipids by lipase-catalyzed interesterification: preliminary study on incorporation and acyl migration, *J. Am. Oil Chem. Soc.*, **75**, 301–308.

Xu, X. et al. (1998b), Production of specific-structured lipids by enzymatic interesterification: Elucidation of acyl migration by response surface design, *J. Am. Oil Chem. Soc.*, **75**, 1179–1186.

Yamaguchi, S. et al. (1993), Preparation of glycerides having a high content of monoglycerides with a lipase from *Penicillium cyclopium* ATCC 34613, US Patent 5,270,188, assigned to Amano Pharmaceutical Co., Ltd.

4.4 Fractionation

As has been explained in more detail in Section 3.8, the term "dewaxing" will be used to describe processes for the removal of waxes from edible oils, and the term "winterisation" will be avoided. The term "fractionation" is used to describe fractional crystallisation processes of triglycerides and, thus, includes the removal of high melting triglycerides from cottonseed oil (Lee, 1939) and brush-hydrogenated soya bean oil, processes which in the U.S. are called "winterization" and for which a solvent fractionation process has been developed (Going et al., 1969).

In their review on cottonseed oil, O'Brien and Wan (2001) describe the fractionation process as a 19th century French development to make whale oil substitutes, but in his 2004 EuroFedLipid Technology Award Address (published in 2005), Dr. R. E. Timms attributes the invention of the fractionation process to Mège-Mouriès (French patent 86.480[6]) as an essential element of the invention of margarine. His oleomargarine was tallow based and to ensure that it was not sticky, he prescribed that the tallow should be fractionated and the liquid, olein fraction used for margarine. According to this patent (Mège 1869), the olein is prepared by pressing partially solidified tallow after wrapping it in cloths.

The fractionation process has two main stages, the first one being the crystallisation stage. Crystals grow when the temperature of the molten fat or its solution is lowered, and their solubility at the final or separation temperature determines the triglyceride composition of the crystals formed as well as of their mother liquor. Accordingly, the olein composition is determined by the separation temperature.

The stearin composition, on the other hand, is only partially determined by the separation temperature, since the separation efficiency has a much larger influence (Hamm, 2005). This separation efficiency can be expressed in different ways, such as the liquid to solids ratio in the filter cake (Hamm, 1986) or the liquid oil content in weight percent of the filter cake (Timms, 2005). The separation efficiency itself is affected by the crystal morphology, the extent of inclusion of olein within the crystals and especially by the amount of interstitial olein, or the amount of olein left between the crystals after the separation stage. Accordingly, the separation process strongly affects the stearin properties (Table 4.11).

Less olein between the crystals means more filtrate and less filter cake. Therefore, the separation efficiency also affects the yield of both fractions. Similarly, the use of a solvent reduces the interstitial olein by sheer dilution, and subsequent washing of the filter cake with clean solvent reduces the interstitial olein even further. Solvent fractionation thus leads to very high separation efficiencies that manifest themselves as a "selective" fractionation with a high olein yield and a large difference between the properties of both fractions.

4.4.1 Separation processes

Because the efficiency of the separation of the fat crystals from their mother liquor has such an impact on the properties and yields of the resulting fractions, the various separation processes will be discussed before the crystallisation processes will be discussed. It has been mentioned (O'Brien and Wan, 2001) that clear oil can be obtained by decanting the top layer from an outside holding tank that has cooled off during winter, but this

[6] The patent was kindly sent to the author by Dr. Timms.

TABLE 4.11 Yield (%) and IV of palm stearin

Year	Company	Process	Yield of Stearin	IV of Stearin
Mid 1960s	Unilever	Continuous acetone tube crystalliser, belt filter, washing with pure solvent	10–11	~8
Mid 1970s	Bernardini	Batch dry crystalliser, drum filter	37–40	44–48
Early 1980s	De Smet	Batch dry crystalliser, drum filter	37–40	44–48
Mid 1970s	Tirtiaux	Batch dry crystalliser, belt filter	28–32	39–42
Mid 1970s	Alfa Laval	Batch crystalliser, detergent centrifugation (Lanza/Lipofrac process)	17–23	25–34
1983	Vandemoortele	Batch dry crystalliser, decanter	<10[a]	~30
Late 1980s	De Smet	Batch dry crystalliser, membrane filter press	22–23	32–33
2000	De Smet	Batch dry crystalliser, membrane filter press, 50 mm chamber width, 6 bar pressure	~24	~34
2000	De Smet	Batch dry crystalliser, membrane filter press, 25 mm chamber width, 30 bar pressure	~17	~30

[a] The separation temperature in this instance (Example 1 in US Patent 4,542,036) is higher than in most other instances listed in the table

separation process has obvious disadvantages. "So the stearine can also be pressed or centrifuged out leaving a clear salad oil" (Wrenn, 1998). The centrifuge used is a solid bowl separator (Ayres, 1957) and like the filter press, it is located in a cold room.

4.4.1.1 Vacuum filters

Because the plate and frame filter press is quite labour intensive, a vacuum drum filter was used instead and in 1974 this was apparently the main continuous filter system in operation (Coenen, 1974; see also Bernardini and Bernardini, 1975, Figure 8). However, horizontal vacuum belt filters have the advantage over vacuum drum filters that they can be washed and, therefore, do not clog. Accordingly, for the separation stage involving solvents, Pannevis Gas Tight moving belt filters were introduced in the 1970s (see: www.larox.com/products/pannevis). Their design incorporates a sealed housing with a continuously recycled nitrogen atmosphere at slight overpressure.

In the absence of solvents, much cheaper equipment suffices, such as the Florentine filter made by Fractionnement Tirtiaux SA (Tirtiaux, 1976). The first installation of this kind dates from 1959 and was used for edible tallow; butter oil fractionation followed in 1973 (Ricci-Rossi and Deffense, 1984). This is the filter that started to be used on a large scale in the dry fractionation of palm oil in the 70s. A slight vacuum maintained under the moving stainless steel belt ensures that the filter cake is sucked as dry as possible, and in the lower part of the filter housing, the belt is heated to melt any material that might clog a filter belt hole, and then cooled to prevent the crystal slurry feed heating up and melting. The slurry feed rate, filter belt speed, and the belt slope can be varied to accommodate the filtration characteristics of the slurry.

4.4.1.2 Centrifugal separators

In the detergent fractionation process, also referred to as the Lanza process (Anon., 1907) or the Lipofrac® process (Seugé and Vinconneau, 1975), water containing a salt and a wetting agent is added to the crystallising melt with the result that the fat crystals move into the aqueous phase. This phase can then be separated from the olein in a normal, stacked disk centrifugal separator as used in degumming and neutralisation processes. The separation efficiency of the detergent process employing centrifugal separators is better than observed with vacuum filters. It is, however, no better than observed with membrane filters, and since the variable cost of the latter is much lower than that of the detergent process, they have made the detergent fractionation process obsolete (Hamm, 1995).

Another type of stacked disk centrifugal separator is used in the dry fractionation process presented by Wilp in 2000. In this piece of equipment, a number of evenly spaced nozzles are mounted in the outer rim of the bowl. During operation, crystals collect inside the rim and are extruded through the nozzles by the centrifugal force. Since the speed of rotation is kept constant, this force is constant so the rate of extrusion (stearin production) is also constant.

If this rate of extrusion is lower than the rate of supply of compacted crystals, olein will also be extruded and if it is larger, the nozzles cannot cope with the crystal supply so that crystals leave the separator in the olein stream. Only when the feed rate of the separator is such that nozzle capacity and crystal supply are a perfect match, will the fractions not be contaminated. Adjusting the feed rate to reach this situation and maintain it is quite complicated and causes the equipment to be under utilised. At best, the separation efficiency of the nozzle separator is in between those of a vacuum belt filter and a membrane press so it has little to offer; only a few installations have been sold as a consequence.

4.4.1.3 Conical screen-scroll centrifuges

In their patent (Maes and Dijkstra, 1983; 1985) describing a process to separate solids from oils, the inventors mention several types of equipment including a decanter. However,

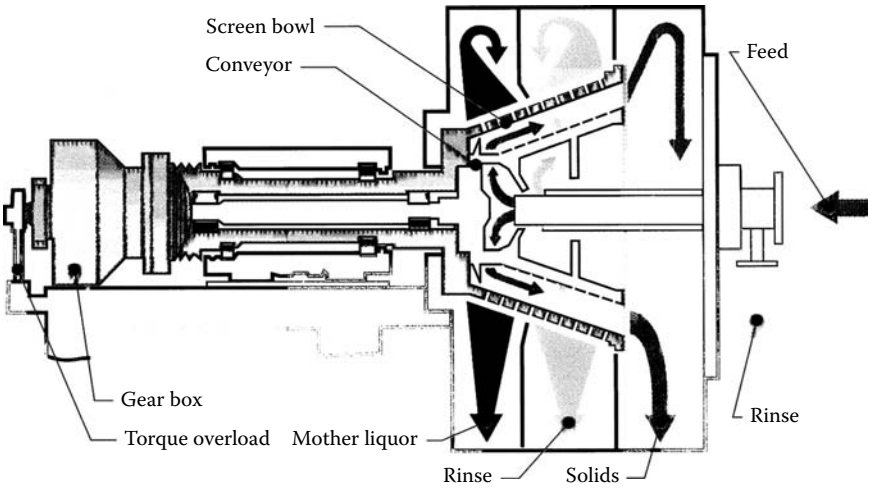


FIGURE 4.19 Cross section of conical screen-scroll centrifuge.

the industrial realisation of their process uses the conical sieve centrifuge provided with a co-rotating scroll, such as the SuperScreen®, formerly manufactured by The Sharples Corp. (later Alfa-Laval and then Tetra-Laval) and the Conturbex® made by Siebtechnik GmbH (Mülheim an der Ruhr, Germany); the latter is now the only supplier of such equipment. A cross section of this type of equipment is given in Figure 4.19.

Screen centrifuges are used industrially for the manufacture of cocoa butter equivalents and other confectionery fats (Dijkstra, 1998; 1999) because they have the advantage that they can replace the interstitial olein by atmosphere and that the olein can drain away through the screen while the stearin crystals move and thus roll over the screen and expose different sides to the screen. The high centrifugal force drives the olein out from in between the crystals and this can lead to very high separation efficiency, higher than when the crystals are compacted, as happens in a hydraulic or membrane filter press (Deffense, 2000; 2001).

The separation process using a screen centrifuge has a disadvantage in that it is quite sensitive to the feed characteristics. If the slurry feed contains some small crystals besides the normal, larger crystals, these small crystals may cause partial blockage of the screen. This partial blockage causes the screen to become less permeable so that olein flows over the screen instead of passing through it. This results in poorer separation efficiency. This can be counteracted by lowering the feed rate, but that is, of course, not a long-term solution.

So, the screen centrifuge can be recommended as a superior means of separation for partially crystallised melts containing crystals of reasonably uniform size; a good example is partially hydrogenated soya bean oil that is rich in *trans* isomers and used as a starting material for a cocoa butter replacement fat. The use of the screen centrifuge then also represents an advantage through the relatively low investment and low space requirement involved. The low liquid oil content of a filter cake obtained by centrifugal laboratory filtration has been confirmed by Breeding and Marshall (1995).

There could perhaps also be scope for a hybrid process employing a crystallisation from the melt and a separation of the partially crystallised fat with a screen centrifuge and using the X-proof version of this equipment simultaneously to wash the filter cake with a solvent. This solvent would remove residual interstitial olein by diluting it and, thus, improve the separation efficiency of the process (von Rappard and Plonis, 1979). The solvent volume involved would be small so that the cost of distillation and solvent losses would also be low. This process should lead to the same stearin composition as obtained by solvent fractionation proper since "the presence of a solvent does not affect the partition of triglycerides between the liquid and solid phases" (Hamm, 1986). However, it has not been evaluated in industrial practice.

4.4.1.4 Hydraulic presses

Presses have been used in the edible oils industry for a long time (Mège, 1869), and are still used, for instance, to squeeze oil out of ground walnuts (Dijkstra, 2003) or olive paste. In fractionation they can also be used to squeeze an olein out of a partially crystallised fat, such as palm kernel oil. To this end, the palm kernel oil is allowed to solidify in blocks that are then wrapped in canvas bags and stacked in the hydraulic press (Rossell, 1985). After pressing, the stearin is removed from the bags by hand, or the bags containing the stearin are heated to melt the stearin (see also Dieffenbacher, 1986). The use of a hydraulic press is labour intensive and its large-scale use has been discontinued in Europe since at least 1986 (Timms, 1986).

4.4.1.5 Membrane filter presses

A far less labour intensive process for the dry fractionation of palm kernel oil was developed in Japan

by Fuji Oil Co. (Higuchi et al., 1989). It employs a special filter apparatus that also has a pressing function in the form of a membrane. Molten or partially solidified oil is pumped into a chamber of the filter apparatus, whose chamber has a filter cloth on one side and an impermeable membrane on the other. The filter cloth has first been sealed with molten feed material that is then solidified in the cloth to retain the subsequent feed of partially crystallised oil. Cold water is passed behind the membrane and causes the oil to crystallise further. Raising the pressure in this cold water circuit then squeezes the crystallised oil and releases its olein. After pressing, the filter press is opened and the stearin filter cake is recovered.

Subsequently, Yoneda et al. (1997; 2000a) developed a less labour-intensive process that starts with a stationary crystallisation of the fat after this has been evenly distributed into trays as a partially crystallised mixture of the fat and its olein (Yoneda et al., 2000b). After this stationary crystallisation, trays are emptied and the partially solidified blocks are crushed so that a pumpable paste results. This paste is then transferred to a membrane filter press and separated into a stearin and an olein that is partially recycled. To avoid contact with circulating air during the partial crystallisation, a crystalliser was developed permitting a much faster crystallisation and avoiding the need for olein recycling (Hendrix and Kellens, 2003a; 2003b).

The use of membrane presses was further pursued by Krupp Maschinentechnik GmbH (Willner et al., 1989; 1991; Willner and Weber, 1994) in the Statofrac® process, wherein a partially crystallised melt is fed to a membrane press and gradually fills this press with fat crystals while recovering olein as a filtrate. Pressure is applied after the chambers have been filled so that more olein is squeezed out and a dry filter cake results. For pressures up to 5 bar, air can be used, but safety considerations demand that a liquid is used for higher pressures (see also Gibon and Tirtiaux, 2002).

Membrane presses are nowadays fully automated and the duration of the operation cycle elements has been listed in Table 4.12. In comparison with early membrane presses, the life expectancy of the membranes has been greatly extended, so their variable costs are lower than those incurred by the detergent fractionation process, which the membrane press has ousted. Consequently, new fractionation plants mostly comprise membrane presses for the separation stage (Tan, 2005).

4.4.1.6 Decanters

Decanters are mentioned in the patent (Maes and Dijkstra, 1983) that also describes the use of the conical sieve centrifuge. However, their performance was found to be inferior to that of the screen centrifuges when the patented process was industrialised. Accordingly, the latter were installed for the production of confectionery fats. In a recent presentation, though, Deffense (2005) reports that experiments with a decanter have shown this to be far less sensitive to the presence of small crystals than the screen centrifuge and that a very good separation and dry cake can be obtained even at throughputs of 15 tonnes per hour provided the slurry feed system meets certain as yet undisclosed requirements. In comparison with a membrane filter press, the "Sedicanter®" occupies far less space and is much cheaper.

TABLE 4.12 Duration of membrane filtration cycle elements

Operation	Duration [min]
Slurry feed	8–10
Squeezing	12–15
Core blowing	<1
Filtrate blowing	<2
Opening/closing	2–3
Total cycle	25–30
Cycles day (excluding washing)	37–40
Washing with olein (once a day)	45–60

Source: Tan, I. (2005), Membrane filter press for edible oil applications, Paper presented at SCI Symposium: Fractionation — Current status and future prospects in a low-trans world, Ghent.

4.4.2 Industrial fractionation processes

Fractionation can occur spontaneously during storage or transport and this forms the basis of the dry fractionation process. This process is the oldest process type and thanks to steadily improved separation methods it has become competitive on product quality grounds with other, more expensive processes, such as solvent and detergent fractionation, which thereby have become obsolete; thus, the discussion of these processes will be brief.

4.4.2.1 Solvent fractionation processes

Solvent fractionation was advocated quite early on for fatty acids using the difference in the solubility of their salts in ethanol (Schlenker, 1934), using aqueous methanol as a solvent (Myers and Muckerheide, 1942). It has also been applied to glyceride oils using, for instance, Skellysolve B (described as "a commercial petroleum naphtha consisting essentially of hexane") to "modify peanut oil to simulate olive oil" (Bailey et al., 1943) or Skelly Solve A ("a lower boiling naphtha") for a multistage milk fat fractionation (Henderson and Jack, 1944). Acetone was also used for fatty acid fractionation (Swern et al., 1945).

Quite a few solvents have been suggested for the solvent fractionation of glyceride oils: propane (Passino and Meyers, 1949; Lacey and Leaders, 1951); isopropyl acetate, methyl isobutyl ketone, or ethyl acetate (Muckerheide, 1950); acetone-hexane mixtures (Boucher and Skau, 1951; Skau, 1954); dichloroethane (Loury, 1956); aqueous ethanol (Subrahmanyam and Achaya, 1958); low molecular weight ketones, acetates, and ethers (Rubin et al., 1959);

nitroparaffins (Andrikides, 1960); methyl formate (Blaney, 1973); isopropanol (Koslowsky, 1973; Koslowsky, 1976); trichloro-trifluoro-ethane (Anon., 1974); or a whole range of azeotropic mixtures, such as acetone/methanol, acetone/methyl acetate, acetone/*n*-pentane, acetone/*n*-hexane, ethanol/cyclohexane, ethanol/*n*-hexane, ethanol/*n*-heptane, and ethanol/*iso*-propyl acetate. Ethanol/methyl cyclohexane, ethanol/methyl ethyl ketone, ethanol/*n*-pentane, and ethanol/*n*-propyl acetate (Luddy and Longhi, 1983) and even supercritical gases at pressures ranging from 100 to 400 bar (Biernoth and Merk, 1985).

When industrial solvent fractionation processes, which should be regarded as just scaled-up laboratory experiments, were reviewed in 1976 by two Durkee employees (Thomas and Paulicka, 1976), they mentioned only acetone (Youngs and Sallans, 1955; Ault and Morris, 1956) as being used by Unilever, hexane as used in plants built by Bernardini (Bernardini and Bernardini, 1971; 1975) and 2-nitropropane (Kawada and Matsui, 1970) used by Durkee. In comparison with the dry fractionation process, the various solvent fractionation processes present the following advantages (Harris, 2005):

- The crystallisation is faster so that fewer vessels are needed.
- In fact, crystallisation can be affected in a scraped surface tubular heat exchanger.
- The presence of a solvent allows more material to be crystallised before the slurry can no longer be handled. So solvent fractionation can do in one step what dry fractionation may require several steps to achieve.
- The solvent dilutes the interstitial olein and enables excellent separation efficiencies to be achieved.
- These efficiencies can be improved even further by washing the filter cake with fresh solvent.

These advantages seldom outweigh the disadvantages listed below:

- Working with flammable solvents necessitates extensive safety precautions that entail a heavier investment.
- Cooling a dilute solution requires more energy than cooling just the solute.
- Cooling it to a lower temperature increases the energy requirements even further, but evaporating the solvent (Smorenburg, 1972) and subsequent vapour compression (Grundmann, 1984) can save on refrigeration costs.
- Separating the solutes (olein and stearin) from their solutions involves distillation and this also costs energy. However, this argument cannot be held against miscella fractionation (Cavanagh, 1968) because this only postpones solvent evaporation.
- Acetone may need drying by fractional distillation before being recycled.
- There will always be a loss of solvent.

So, in 1986, when Hamm asked the question: "With or without solvent?" he still saw some instances where solvent fractionation could be justified. Less than 10 years later, when discussing trends in edible oil fractionation (Hamm, 1995), he concentrated on dry fractionation and potential future alternatives and hardly mentioned solvent fractionation. As mentioned at the end of Section 4.4.1.1, there could be the possibility of using the conical screen-scroll centrifuge in a mixed dry-solvent process employing crystallisation from the melt and washing the filter cake with a solvent.

4.4.2.2 Detergent fractionation processes

In the 1950s, the detergent fractionation process became extensively employed in the oleochemical industry, since it permits a separation between oleic acid and stearic acid. Such a separation is not possible by fractional distillation since the two acids have close molecular weights and thus close boiling points. It can be done by fractional crystallisation and then pressing, but this leaves a substantial amount of olein in the press cake. Detergent fractionation ensures a lower olein content of the stearin fraction and replaced the pressing system.

In the detergent fractionation process as developed for fatty acid fractionation (Stein and Hartmann, 1957), the fatty acids are melted and added to an aqueous solution of an electrolyte, such as sodium sulfate and a surface active material such as alkyl sulfonates, fatty alkyl sulfates or alkylbenzyl sulfonates (Coenen, 1974). The amount of water is about the same as or greater than the amount of fatty acids, which means that more crystalliser capacity is needed in the detergent fractionation process than in a dry process. Given that water has a higher specific heat than fatty acids, the cooling energy requirements are also substantially higher in the detergent fractionation process.

When this mixture is then cooled, the highest melting fatty acids will eventually crystallise, but this crystallisation process probably differs from the crystallisation of fatty acids in bulk in the same way as observed between cream and anhydrous milk fat (AMF). It takes longer for cream to crystallise than AMF since each and every fat globule must generate its own nuclei (see also Section 7.2.1). This has also been observed for an emulsion of a solution of fully hydrogenated palm oil in sunflower seed oil (Kloek et al., 2000).

In the detergent fractionation process, the gentle agitation and the surface active agent ensure that the crystals formed are wetted and stay in the water phase, whereas the fatty acids that are still molten form a separate, oily phase. Subjecting this three-phase mixture to centrifugal acceleration causes this oily phase to float so that it can be collected as such from a centrifugal separator, while the fatty acid crystals are collected together with the other, aqueous phase.

Heating the aqueous phase thus collected will cause the crystals to melt so that they can also be recovered as the stearin fraction. As shown by Hamm (1986), the ratio of liquid to solid in the stearin fraction resulting from the Lipofrac® process varies between 0.5 and 0.8; this corresponds to a solid content range of 56 to 67%, which is much higher than that of the press cake. The warm aqueous phase is recycled and the molten stearin fraction is washed with water to fully remove the surface active agent[7].

Accordingly, the Lipofrac® process consumes the surface-active agent, which then causes an effluent problem. This consumption and the effluent treatment both cost money. As calculated by Hamm (1986), the Lipofrac® process has a cost disadvantage in comparison with the filtration process using membrane filter presses. This disadvantage was independent of the stearin value since both processes lead to about the same solids content in the stearin fraction (cf. Table 4.9). It is not surprising that this membrane filter press process has ousted the Lipofrac® process, for which at one stage more than 70 plants had been sold with a total installed capacity estimated at about 1,000,000 tonnes per annum (Haraldsson, 1979).

4.4.2.3 The Sorbex® process

A poster presented during the AOCS World Conference on "Emerging Technologies" (Gembicki et al., 1986) described the Sorbex® process, a separation process that can perhaps most easily be described as fully automated and continuous column chromatography. At that time, the most important application of Sorbex® was the so-called Parex® process used to isolate *p*-xylene from a mixture of xylene isomers. The poster mentioned that 26 Parex® plants were then in operation with an annual capacity of 6 billion pounds (2.7 million tonnes).

Earlier work by UOT (Neuzil and deRosset, 1977) shows that unsaturated fatty acid esters can be separated from saturated ones by preferentially adsorbing the former by using specified zeolites. This work was extended by The Proctor & Gamble Company to triglycerides using ion exchange resins containing silver ions and a solvent mixture of 25% hexane and 75% ethyl acetate (Logan and Lubsen, 1981), surface aluminated silica gel adsorbents (Logan and King, 1981), or permutite adsorbents (Logan, 1981).

The UOP poster (Gembicki et al., 1986) showed that on a laboratory scale, monoglycerides can be extracted from a mixture of partial glycerides and that an acid oil can be neutralised by the extraction of oleic acid. It showed the laboratory separation of three different fatty acids (palmitic acid, stearic acid, and oleic acid) and concluded that, in principle, this could lead to the production of a pure stearic acid extract with an oleic acid palmitic acid mixture as raffinate, or an oleic acid raffinate by extracting palmitic acid and stearic acid simultaneously.

As can be expected of the petrochemical industry, the Sorbex® process is heavily protected by patents. One of these patents (Cleary et al., 1985) covers the separation of oleic acid from a mixture also comprising linoleic acid and possibly rosin acids as resulting from tall oil. This patent refers to quite a number of earlier patents dealing with various Sorbex® aspects, such as the flow system, the rotary valve, adsorbents, etc. A subsequent patent (Cleary, 1986) is concerned with the separation of different saturated fatty acids, such as myristic acid and lauric acid. These techniques have also been used to develop a production process for CBE (Ou, 1990) by the isolation of monounsaturated triglycerides from feed streams, such as palm olein or shea butter, while using silver-exchanged aluminosilicates as the adsorbent. The solvents used as the desorbent are acetone and *n*-heptane and, thus, food grade.

So, here is a simple process for the production of CBEs from raw materials already used for this purpose while using food grade solvents. Moreover, the process can be used for other separations, such as, for instance, the removal of partial glycerides from confectionery fats; this could well improve their crystallisation behaviour and SFC profile. In addition, this possibility does not require an upfront investment in a separation plant since UOP could offer a toll conversion service. It must, however, be mentioned that the process conditions have not yet been optimised and that a detailed costing of the process has not been published. Much later, a supercritical variant of the process was reported (Denet et al., 2000).

4.4.2.4 Dry fractionation processes

Dry fractionation processes are the oldest and cheapest, and because their performance has been steadily improved by the development of improved separation techniques, they are also predominant. There are several types of dry fractionation process characterised by the way the melt is agitated.

In the most simple process, the melt is not agitated at all and the molten fat is allowed to crystallise in trays and form semisolid blocks that can then be wrapped in bags and pressed hydraulically (Rossell, 1985); crystallisation in a membrane filter press (Higuchi et al., 1989), or in trays after the fat has been mixed with some olein from a previous fractionation (Yoneda et al., 2000b) or in a specially designed crystalliser (Hendrix and Kellens, 2003b) are other possibilities. This type of process is used for oils and fats, such as coconut oil and palm kernel oil, that form a crystal network rather than isolated crystals on cooling.

In the dry fractionation processes employing agitation during the crystallisation stage, two approaches can be

[7] Given the large number of centrifugal separators required, it is quite understandable that the Lipofrac® process has been strongly promoted by Alfa Laval.

discerned. There is the approach as exemplified by Fractionnement Tirtiaux SA (Deffense, 1998) that aims at generating crystals that drain well on filtration, an approach that has been described by Dijkstra (1999) as "slowly, slowly catch a monkey" as opposed to the "sledgehammer" approach as exemplified by DeSmet-Ballestra that relies mainly on membrane filtration to attain separation efficiency. This difference in approach calls for a discussion of the crystallisation processes in dry fractionation.

4.4.3 Crystallisers and crystallisation

Fat crystallisation has been studied in two totally different environments. There is the fast crystallisation as encountered in scraped surface heat exchangers during the production of, for instance, margarine. As will be explained in more detail in Section 7.2.4, this leads to the formation of the α-polymorph that then recrystallises into small crystals of the β′-polymorph, which form a crystal network that retains the liquid oil without exudation and can also stabilise an emulsion.

The other environment deals with slow crystallisation as encountered, for example,e in the dry fractionation process. As explained by several authors (Sambuc, 1964; Puri, 1980; Van Putte and Bakker, 1987; Timms, 1991; Grall and Hartel, 1992; Timms, 1997; Herrera and Hartel, 2000) and the publications they refer to, this process starts with the formation of crystal nuclei, which then grow to form visible and subsequently filterable crystals. In practice, spontaneous or homogenous nucleation rarely occurs in fats. Instead heterogeneous nucleation takes place on solid particles, such as dust, the walls of the container, or foreign molecules (Timms, 1991) (see also Section 7.2.1). Such particles may be added deliberately to act as seeding crystals (von Rappard and Plonis, 1980); they may be added (Iida et al., 1981) to an oil that has preferably been heated to some 20 to 30°C above its melting point to erase any crystal "memory."

Dieffenbacher (1986) goes even a step further and seeds palm oil with β-crystals for the first fractionation, and when aiming for a palm midfraction by fractionating the olein, he seeds this olein with β′-crystals. A different approach was taken by Maes et al. (1995) who added palm oil to the crystallising palm olein to ensure a steady supply of trisaturated triglycerides that would crystallise and act as nuclei.

Easy filtration requires crystals of near uniform size. Accordingly, the crystallisation stage should focus on making existing nuclei grow and avoiding the emergence of new nuclei. This can in practice often be realised by avoiding excessive supersaturation by cooling slowly. Cooling too fast will lead to an increase in supersaturation and a sudden surge in crystal growth causing the batch temperature to rise. It may well be that this surge coincides with the formation of a large number of additional nuclei and their subsequent development into fine crystals; this is to be avoided (Tirtiaux and Gibon, 1996).

The emergence of new nuclei through secondary nucleation can also result from agitation (Timms, 1997) and this may well be the reason why it is so difficult to scale-up laboratory experiments to pilot plant or industrial scale. In a recent development[8], fast agitator movement through the crystallising melt is avoided so that all agitator parts move at substantially the same linear speed. Highly uniform crystals result so that crystal slurries with an SFC of 30% can still be handled. This means that a palm olein with an IV of 63 can be produced in a single fractionation step. A most unexpected and surprising aspect of this development is that the agitator no longer assures temperature and SFC homogeneity throughout the crystalliser. Lack of this homogeneity has always been thought to be detrimental to proper crystal growth, but, apparently, this is not the case.

Large vessels are ideal for slow cooling because their cooling surface to volume ratio is small. Ensuring temperature homogeneity within the batch being cooled, which has until recently (*vide supra*) been considered as essential, necessitates a small temperature difference between cooling surface and vessel contents and this also makes for slow cooling. Finally, secondary nucleation resulting from fast agitation is to be avoided and this causes the rate of agitation to be slow during the crystallisation stage; it lowers heat transfer and thus also leads to slow cooling.

Early crystallisers (for figures, see Timms, 1997) tended to be cylindrical vessels with spiral cooling coils and a central multispeed agitator (Deffense, 1985). This agitator will run at high speed when the batch has to be heated or cooled as fast as possible, but when secondary nucleation has to be avoided, its speed is reduced. The agitator blades effectively prevent the crystals from settling during the crystallisation stage, but when the crystalliser is emptied and the liquid level falls below the lowest agitator blade, the remaining crystals may settle and allow the supernatant olein to drain away without being transferred themselves. Crystals may also remain on the coils and in between the coils and the wall, so that the crystalliser is far from emptied. The type of crystalliser that can also be used as hydrogenation vessel (Athanassiadis, 1993) and is fitted with sets of horizontal cooling coils, probably also suffers from the same problem of incomplete emptying.

These difficulties have been at least partially overcome by using vertical cooling tubes or cooling baffles (for figures, see Timms, 1997). In addition, these vertical cooling elements also prevent the batch from just being swirled

[8] The patent application describing this development will be published in the second half of 2007. Those interested should look in a database such as be.espacenet.com under "Advanced search" with De Smet Engineering as applicant and Kellens as inventor.

around and, thus, effect proper agitation, which facilitates crystallisation by making this less diffusion controlled.

If a relatively fast crystallisation is required, the above crystallisers are not suitable since their cooling surface is small in comparison with their content. This drawback has been overcome by the concentric crystalliser consisting of concentric, annular-shaped subvessels that provide a far larger surface for a given crystalliser volume (see Kellens and Hendrix, 2000, Figure 11.8). The subvessels are agitated by a common agitator, but this has the possible disadvantage that the agitator speed is much higher in the outer vessel than in the vessel close to the agitator axis. A simplified flow sheet of a dry fractionation plant incorporating such concentric crystallisers is represented in Figure 4.20; this flow sheet also illustrates how a membrane press is cleaned with hot olein.

Another crystalliser vessel with a special agitator that has also been illustrated in (Kellens and Hendrix, 2000) is the STAR-type (Stirring Area) crystalliser (Weber et al., 1998). In this crystalliser, eccentrically rotating cooling coils move through the melt and a much higher heat transfer coefficient of 300 W/m^2K is reported as a result. This allows faster cooling and in addition, a higher stearin yield and a higher olein IV are also claimed as advantages.

A major problem in dry fractionation is that for some reason or other, the crystallisation process lacks predictability. Doing ostensibly the same may lead to different results. This is probably why dry fractionation has been proclaimed an art (Tirtiaux, 1990). Fortunately, the fractionation process is reversible in that the starting material can always be reconstituted at little cost by putting the faulty products together again. In this respect, the fractionation process has an advantage over the nonreversible hydrogenation and interesterification processes.

Understandably, research efforts have been devoted to finding out what factors affect dry fractionation in general and its crystallisation in particular. This effort has focused on minor constituents, probably because it was noted quite early on that small amounts of a whole range of compounds exerted a large influence on the crystallisation process. In 1953, Mattil added magnesium or barium carboxylate of paraffin wax-substituted phenoxy acetic acid to cotton seed oil, white grease, and crude sperm oil and noted large effects on their crystallisation behaviour. Similarly, Baur drastically reduced the filtration time when fractionating partially hydrogenated soya bean by adding only 0.005 wt % of glucose pentapalmitate (Baur, 1962).

Partial glycerides occur naturally in oils and fats; they can be the result of partial hydrolysis, but also occur as a residual by-product of triglyceride biosynthesis. Unbruised palm fruit with an FFA of only 0.32% nevertheless contained 5.66% diglycerides (Goh and Timms, 1985). On fractionation, these diglycerides end up in both fractions. During the detergent fractionation process of palm oil, the diglycerides hardly crystallise and are concentrated in the olein (Goh and Timms, 1985). On dry fractionation, dipalmitin (measured as C_{32}) has a preference for the stearin fraction, whereas the more unsaturated diglycerides are concentrated in the olein (Siew and Ng, 1995).

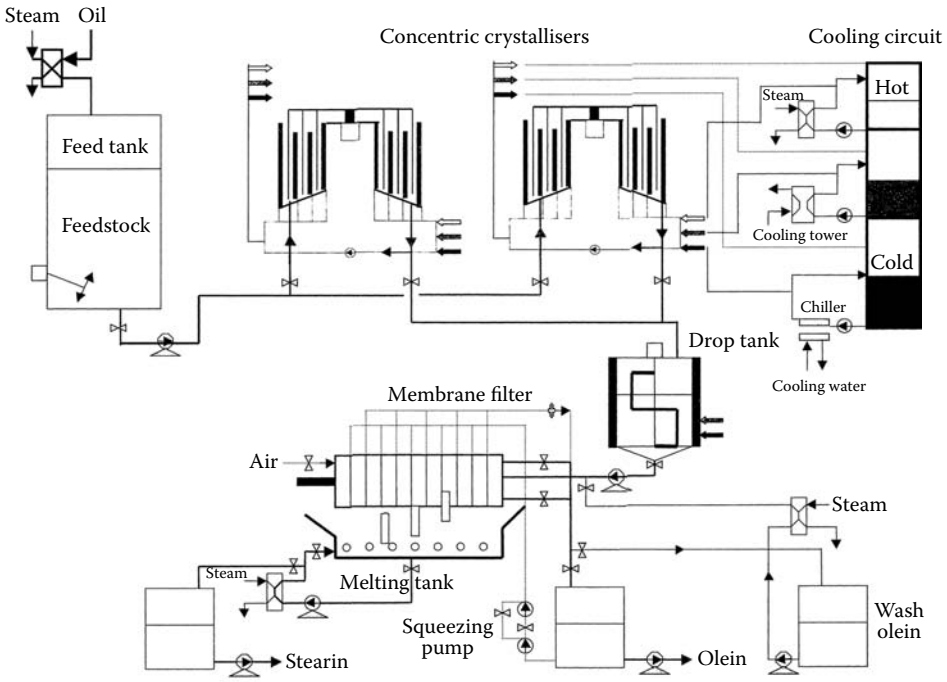


FIGURE 4.20 Simplified flow sheet of a dry fractionation plant.

The effect of such partial glycerides on crystallisation has also been investigated. In 1987, it was concluded that diglycerides do not affect the crystallisation of palm oil (Martinenghi et al., 1987). But subsequently, Smith and Povey used trilaurin as a model system and demonstrated that whereas FFA or monoglycerides hardly affect the trilaurin crystallisation, diglycerides can significantly reduce the rate of crystallisation. Maximum inhibition occurs when the chain length of the diglycerides matches the lauric acid moieties in the triglycerides (Smith and Povey, 1997).

Wright et al. (2000) isolated partial glycerides from anhydrous milk fat (AMF) and studied their effect on AMF crystallisation. They noted that the partial glycerides delay the onset of crystallisation. Subsequently, it was shown that it is only the *sn*-1,2-diglycerides that are responsible for these effects (Wright and Marangoni, 2002). When using specific diglycerides (distearin and diolein) and monoglycerides (monostearin and monoolein) instead of the partial glycerides isolated from AMF, Foubert et al. showed that their solubilities determine what effect they have on nucleation and crystal growth (2004).

Phospholipids are also minor oil constituents present in crude oil and removed from the oil by refining. Their effect has been studied by Smith (2000) who observed that even small amounts (0.05 to 0.50%) of PE, PI, or lysoPE when added to palm oil, trilaurin, or tristearin lead to much larger spherulites that are more uniform in size. He concludes that nucleation is retarded and that crystal growth is modified. On laboratory filtration, the olein yield is markedly higher than the control sample. When similar amounts of PC were added to the oil, the resulting spherulites were again larger than the control, but there was no yield increase in olein. When nonfractionated phospholipids (i.e., soya bean lecithin, 45% PC) are added to AMF, longer induction times are observed, indicating a decrease in crystal growth rate (Vanhoutte et al., 2002).

It, therefore, can be concluded that some insight has been developed into how certain compounds affect the crystallisation process. However, the stage where the fractionation process is fully understood and can be adequately controlled has not yet been reached. Doing the same as last time and hoping for the best may still be the best guide for process control.

A more drastic approach would entail a thorough purification of the raw material to be used in the fractionation process. This purification would comprise the elimination of partial glycerides, since they are known to affect both nucleation and crystal growth. This elimination could be by re-esterification or by removal or a combination of both (Long, 2004). It would unavoidably lead to a yield loss and, thus, incur an expense. Since it is not known to what extent this yield loss would be offset by a faster fractionation process and/or improved fraction quality, this approach has not been pursued in practice.

4.4.4 Fractionation products

The nomenclature of the various fractions resulting from a multistep, cascade fractionation has not yet been standardised, except that the filtrate is always referred to as an "olein." The filter cake may be a "stearin" but can also be referred to as a "mid-fraction." Terms like "very hard stearin," "hard stearin" and "superolein" (Deffense, 1993) tell us little about the process sequence by which these products have been obtained. This sequence can be shown by a letter sequence, such as, for example "OOS," which indicates that the fraction concerned is a stearin (S) resulting from a fractionation of an olein (O) that itself was the olein fraction of the raw material (Tirtiaux, 1990). As suggested by Breitschuh and Flöter (2002), inclusion of the raw material and the filtration temperature characterises the fraction in even more detail. Accordingly, they suggest for instance the term "su$MF_{26}O_{18}S$" for the stearin that was obtained at 18°C from an olein resulting from the fractionation of summer milk fat at 26°C.

4.4.4.1 Animal products

As mentioned above (Mège-Mouriès), edible tallow was the first product to be fractionated on an industrial scale to provide the fat component for margarine. The subsequent introduction of the hydrogenation process formed another source of hardstock, so other outlets for tallow were needed. An economic evaluation showed that the confectionery industries could be quite attractive (Taylor et al., 1976). Accordingly, Luddy et al. developed a solvent fractionation batch process using acetone to isolate a tallow fraction that was compatible with cocoa butter over a wide range (Luddy et al., 1973). Subsequently, this process formed the basis of a continuous process (Kozempel et al., 1981). However, the EU Chocolate Directive (Berger, 2003; Stewart and Kristott, 2004) prohibits the use of tallow in chocolate, so it is unlikely that this process will ever be used.

Nevertheless, the low price of tallow makes it an attractive raw material for other food applications, such as puff pastry margarines, a means to harden shortenings, fats for deep frying, and even as a salad oil ingredient as illustrated in Table 4.13 (Gibon and Tirtiaux, 2002). The fractions listed have probably been obtained by using a membrane filter press, which leads to less stearin with a lower iodine value and a slightly higher melting point than stearin obtained by vacuum filtration, as illustrated by Table 4.14. For yields and melting points of various fractions obtained by membrane filter pressing, see (Levembach, 2001).

With respect to the fractionation of lard, the literature reports turbidity measurements of crystallising lard with only few data on the properties of the fractions (Wang and Lin, 1995), the solvent fractionation of interesterified lard (Chobanov and Topalova, 1982) and some data on dry fractionation (Kokken, 1991) that have been summarised in Table 4.15.

TABLE 4.13 Solid Fat Contents [%] of beef tallow fractions

Temperature [°C]	5	10	15	20	25	30	35	40
Beef tallow (BT)	64	56	45	34	23	15	9	4
BT-S	77	75	68	60	49	38	28	18
BT-O	37	21	6	1	0			
BT-SS	90	88	85	82	76	70	65	55
BT-SO	68	60	49	38	30	21	12	6
BT-OS	56	45	26	8	2	0		
BT-OO	31	12	0					
BT-OOS	49	36	15	0				
BT-OOO	15	1	0					

Source: Gibon, V. and Tirtiaux, A. (2002), *Lipid Techn.*, **14**, 33–36.

TABLE 4.14 Properties of beef tallow fractions

Stearin Separation Method	Yield [%]	Iodine Value	Melting Point [°C]
Co-produced olein with drop point of 37–38°C			
BT-S (Vacuum filter)	28–33	37.5	51.0
BT-S (Membrane filter press)	22.5	30.5	53.2
BT-O	77.5	50	39.0
BT-OS	37.5	44	41.8
BT-OO	40.0	57	19.5
Co-produced olein with drop point of 20–22°C			
BT-S (Vacuum filter)	58–63	40.1	48.3
BT-S (Membrane filter press)	36	32	48.8
BT-O	64	55.5	22.0
BT-OS	29	49	31.0
BT-OO	35	59	18.5

Source: Kokken, M.J. (1991), *Rev. Franç. Corps Gras,* **38**, 367–376.

TABLE 4.15 Properties of fractions of lard and interesterified lard

Product	Yield [%]	Iodine Value	Drop Point [°C]	Cloud Point [°C]
Lard		62	30	
Interesterified lard (IL)			38	
IL-O (Vacuum filter)	45	68		8
IL-S (vacuum filter)	55	55	44	
IL-OO (Membrane filter)	40	70–72		4–5

The few data reported in the recent literature (Gibon and Tirtiaux, 2002) on chicken fat fractions have bee summarised in Table 4.16.

Because Kaylegian and Lindsay published a book on milk fat fractionation in 1995, the present discussion will concentrate on what has been published subsequently, such as Table 4.17 giving SFC values for various milk fat fractions. The SFC values for the AMF differ, but it

TABLE 4.16 Properties of chicken fat fractions

Temperature [°C]	Olein		Stearin	
	Yield [%]	Iodine Value	Yield [%]	Iodine Value
10	75	78.2	25	56.2
4	59	81.2	41	59.8
0	50	84.0	50	61.1

Source: Gibon, V. and Tirtiaux, A. (2002), *Lipid Techn.*, **14**, 33–36.

should not be concluded that the high values pertain to winter butter and the low ones to summer butter, since for winter milk fat, a value of 67% has been quoted at 5°C (Breitschuh and Flöter, 2002). Given the relatively low values for the oldest stearin MF-S, it is likely that that was separated by vacuum filtration and that the more recent values originate from membrane filter pressing.

This latter separation method has also led to an increased olein yield permitting a third stage olein fractionation (OOO). Another method of increasing the final olein (OOO) yield is to recycle the stearin fraction (OOS) resulting from the third stage fractionation to the first olein (O) and encourage its higher melting constituents to leave the recycling system as part of the OS fraction (Deffense, 1995). The crystallisation of milk fat and its fractions has been studied by Van Aken et al. (1999) who concluded that milk fat comprises three groups of triglycerides that crystallise independently of each other in the β′-polymorph and also at different rates. Subsequently, it was found that these groups show solid phase immiscibility (Breitschuh and Flöter, 2002).

4.4.4.2 Vegetable products

In the fractionation of vegetable oils and fats, three main domains can be distinguished. There is salad oil production in the U.S. by fractionating cottonseed oil or brush-hydrogenated soya bean oil. Secondly, there is bulk fractionation of palm oil to produce palm olein with a sufficiently low cloud point, combined with finding suitable outlets for the inevitable stearin. Thirdly, there is the production of confectionery fats based upon lauric oils like palm kernel oil, upon *trans* isomer-rich hydrogenated

TABLE 4.17 Solid Fat Contents [%] of anhydrous milk fat fractions (Gibon and Tirtiaux, 2002) and in brackets (Deffense, 1987)

Temperature [°C]	5	10	15	20	25	30	35	40
Anhydrous Milk Fat (MF)	56	46	31	16	9	3	0	
	(51.3)	(42.0)	(28.7)	(15.0)	(8.6)	(3.9)		
MF-S	84	79	73	63	53	42	29	15
	(67.9)	(62.9)	(54.6)	(41.1)	(30.5)	(20.6)	(11.0)	(3.1)
MF-O	47	32	17	2	0			
	(39.9)	(23.7)	(7.7)					
MF-OS	76	68	59	37	13	2	0	
	(57.4)	(46.5)	(30.8)	(10.2)	(3.1)			
MF-OO	31	14	2	0				
MF-OSS	86	84	78	67	43	5	0	
MF-OSO	69	58	41	20	2	0		
MF-OOS	60	48	26	5	0			
MF-OOO	5	0						

Source: Gibon, V. and Tirtiaux, A. (2002), *Lipid Techn.*, **14**, 33–36, and Deffense, E.M.J. (1987), *Fat Sci. Technol.*, **89**, 502–507.

TABLE 4.18 Yields and properties during fractionation of partially hydrogenated soybean oil

Product	SFC	Stearin Yield [%]	Iodine Value	Solid Fat Content [%] at [°C]				
				0	10	20	30	40
Feedstock	22.5		86.0	49.6	39.1	16.3	2.5	
Olein,	0.0		92.5	7.0	5.0			
Stearin, vacuum	51.0	44.5	77.8	66.2	60.7	37.9	11.2	
Stearin, 5 bar	64.6	35.4	74.5	72.7	66.4	49.9	21.2	
Stearin, 15 bar	69.9	30.1	71.2	79.1	74.5	59.0	27.3	
Stearin, 25 bar	72.0	28.0	69.6	84.2	80.0	63.5	29.2	1.2

Source: Kellens, M.J. and Hendrix, M. (2000), in *Introduction to Oils and Fats Technology*, O'Brien, R.D., Farr, W.E., and Wan, P.J., Eds., AOCS Press, Champaign, IL, 194–207.

nonlauric oils, and finally upon fats with a high content of symmetrical monounsaturated triglycerides (SMUT); this last category may involve palm oil as well, since palm midfraction (for instance, OSS, formerly referred to as a palm midstearin) can be made to a high SMUT content and, thus, become compatible with cocoa butter.

Kellens and Hendrix (2000) have provided recent data on the fractionation of soya bean oil that had been hydrogenated to an iodine value of 86; their data are summarised in Table 4.18. This table also illustrates that the use of a membrane filter press leads to a higher SFC of the filter cake than when using a vacuum filter and that the pressure applied also affects the olein content of the cake and, thus, its iodine value and SFC.

Lauric fats like palm kernel oil and coconut oil form a specific group of vegetable oils characterised by a low iodine value and nevertheless a low melting point because of their high content of lauric and myristic acids. If they are hydrogenated, their oleic and linoleic acids are then converted into stearic acid that raises the melting point. Fractionation concentrates triglycerides with unsaturated fatty acids into the olein fraction so that the melting point of this fraction is raised much more on hydrogenation than of the stearin, as illustrated by Table 4.19.

The next table, Table 4.20 illustrates how the various products differ in solid fat content and shows why hydrogenated palm kernel stearin is a highly suitable cocoa butter substitute. It has in common with cocoa butter that the SFC at 30°C is still quite high whereas at 35°C, it is very low, giving it a cool taste. Its mouth feel can be improved by randomisation (Timms, 1986). Because the triglyceride compositions of hydrogenated palm kernel oil and cocoa butter are very different, the products are noncompatible, meaning that they lower each other's SFC when mixed. Consequently, chocolate substitutes based

TABLE 4.19 Iodine values and melting points of lauric oils and derivatives

	Palm Kernel Oil (PK)		Coconut Oil (CN)	
	Iodine value	Melting point [°C]	Iodine value	Melting point [°C]
Nonfractionated	17.5	28	8.5	24
Stearin S	7	32	4	30
Hydrogenated S	4	31	1.5	32
Hydrogenated S	0.4	35		
Olein O	24.5	23.5	10	
Hydrogenated O	1.0	41		

Source: Rossell, J.B. (1985), *J. Am. Oil Chem. Soc.*, **62**, 385–390, and Jayaraman, G. and Thiagarajan, T. (2001), Specialty fats from lauric oils, in *Proceedings of the World Conference on Oilseed Processing and Utilization*, Wilson, R.F., Ed., AOCS Press, Champaign IL, 67–73.

TABLE 4.20 Iodine values, solid fat contents, and melting points of lauric oils and derivatives in comparison with cocoa butter

Product	Iodine Value	SFC [%] at Temperature [°C]					Melting Point [°C]
		20	25	30	35	40	
Palm kernel oil (PK)	17.5	44	20	0			28
PK-S	7	82	68	29	0		32
Hydrogenated PK-S	4	87	70	24	1	0	31
Hydrogenated PK-S	0.4	95	90	50	5	1	35
Cocoa butter	34.5	76	70	45	0		34
Hydrogenated PK-O	1.0	73		27	14	6	41
Coconut oil (CN)	8.5	36	0				24
CN-S	4	84	53	2	0		30
Hydrogenated CN-S	1.5	92	57	8	2		32

Source: Rossell, J.B. (1985), *J. Am. Oil Chem. Soc.*, **62**, 385–390.

TABLE 4.21 Fatty acid compositions of the various palm oil fractions

Product	IV	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0
Palm oil (PO)	50.0	1.1	43.7	0.2	4.4	39.9	9.7	.02	0.4
PO-S	31.3	1.3	60.8	0.1	5.4	26.1	5.4	0.1	0.4
PO-O	55.6	1.0	38.8	0.2	4.1	43.8	10.8	0.2	0.4
PO-SS	16.2	1.1	77.4	0.1	4.7	13.4	2.9	0.0	0.3
PO-SO	38.7	1.3	54.0	0.3	5.0	31.3	7.2	0.1	0.3
PO-OS	44.4	0.8	47.9	0.1	5.2	37.8	7.3	0.1	0.3
PO-OO	63.8	1.1	33.0	0.3	3.6	46.4	14.2	0.3	0.3
PO-OSS	35.9	0.8	54.3	0.0	5.5	34.5	4.1	0.0	0.3
PO-OOO	69.5	1.0	28.8	0.3	2.5	52.0	14.6	0.4	0.2

Source: Deffense, E.M.J. (1995), *Lipid Technol.*, **7**, 34–38

on hydrogenated palm kernel stearin should utilise a fat-free cocoa powder (Padley, 1997).

Cocoa butter replacers, which are partially miscible with cocoa butter, can also be made by fractionation by using a *trans* isomer-rich, partially hydrogenated vegetable oil that has a sufficiently high palmitic acid content. If rapeseed oil or soya bean oil is used, blending with some palm olein prior to the hydrogenation process is recommended (Hughes and Smith, 1994). For some applications, the olein fraction obtained from the hydrogenation product can be used. For more demanding applications, the olein has to be fractionated again to yield a midfraction.

Confectionery fats rich in SMUT, also known as cocoa butter equivalents (CBEs), should preferably use a starting material that already has a substantial SMUT content. Examples of such fats are: shea butter, sal fat, and mango kernel fat; in fact, the wild fats that can be included into chocolate according to the EU Chocolate Directive. This Directive also includes palm oil, kokum gurgi fat, and illipe butter (Kaufmann and Thieme, 1954), but these fats differ from the examples just mentioned in that palm oil serves a whole number of purposes other than confectionery fats, and that kokum fat and illipe butter do not need any fractionation.

There are also wild fats with a substantial SMUT content that are not included in the Directive. Mowrah fat (Sinnema, 1962), malabar tallow, *Allanblackia,* and Chinese vegetable tallow are examples of these fats (Padley, 1997).

In general, the stearin fraction of the wild fats listed above can be used as CBEs, but midfractions may be preferred. Shea butter needs a pretreatment to remove the somewhat gummy polyterpenes present in this fat. In a solvent fractionation plant, this can be done by a treatment with acetone, but a dry fractionation removing only a small amount of triglycerides as stearin is also effective, provided a filter aid is used.

Whereas the SMUTs in cocoa butter are predominantly POS, in the wild fats listed above SOS (1,3-distearyl-2-oleyl glycerol) dominates except for illipe butter where it is POS. Mixing the higher melting SOS with the lower melting POP leads to a CBE that is fully compatible with cocoa butter, and adjusting the mixing ratio in the CBE allows chocolate properties to be fine-tuned (Soeters et al., 1981). This POP can be cheaply provided by a palm midfraction as obtained (Best et al., 1960; 1961), but nowadays, dry fraction is preferred for cost reasons.

In fact, there can be several palm midfractions, since the stearin (OS) obtained from the first olein (O) can be fractionated to provide a stearin fraction (OSS) and an olein fraction (OSO) that can be recycled together with the stearin fraction (OOS) by mixing with the first olein (O), as illustrated in Figure 4.21 (Deffense, 1995). The fatty acid compositions of the various fractions shown in Figure 4.21 have been listed in Table 4.21. They clearly show that saturated fatty acids are concentrated in the stearins and that the unsaturated fatty acids gather in the

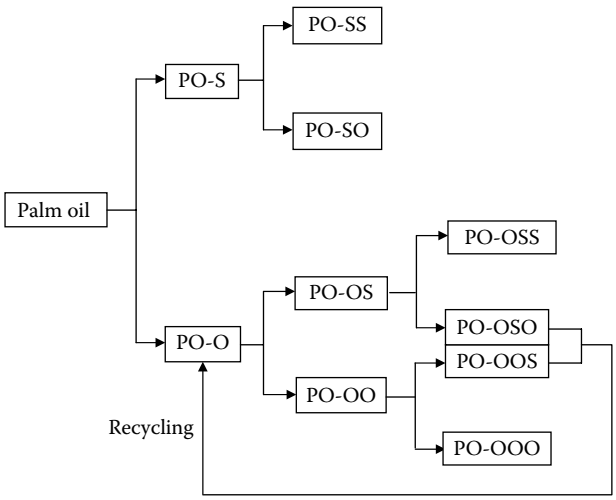


FIGURE 4.21 Multiple dry fractionation of palm oil.

olein fractions. They also show that the midfractions (OS) and (OSS) have a low linoleic acid content and that their oleic acid content is close to one-third as to be expected for a SMUT.

References

Aken, G.A. van et al. (1999), Composition and crystallisation of milk fat fractions, *J. Am. Oil Chem. Soc.*, **76**, 1323–1331.

Andrikides, A.L. (1960), Séparation et purification des acides gras, des glycérides et des alcools gras, French Patent 1.251.738, assigned to Norco Products Company.

Anon. (1907), Verfahren zur Zerlegung des aus Fetten und fetten Ölen gewonnenen Fettsäuregemisches in Ölsäure und feste Fettsäuren, German Patent 191238, assigned to Fratelli Lanza.

Anon. (1974), Fractionnement de corps gras à l'aide du trifluorotrichloro-éthane, French Patent 2.208.403, assigned to Société pour l'Équipement des Industries Chimiques Speichim.

Athanassiadis, A. (1993), Procédé et installation de traitement d'une matière grasse, en particulier alimentaire, Belgian Patent 1005617 A3.

Ault, W.C. and Morris, S.G. (1956), Lard oil and oleo oil, US Patent 2,729,659, assigned to USA, Secretary of Agriculture.

Ayres, A.U. (1957), Process of centrifugally separating glycerides from oil, US Patent 2,807,411, assigned to The Sharples Corporation.

Bailey, A.E. et al. (1943), Modifications of vegetable oils. I. New products by fractional crystallization of glycerides from petroleum naphtha, *Oil & Soap*, **20**, 129–132.

Baur, F.J. (1962), Crystallization process, US Patent 3.059,008, assigned to The Procter & Gamble Company.

Berger, K.G. (2003), EU Chocolate Directive discussed in May, *inform*, **14**, 511.

Bernardini, E. and Bernardini, M. (1975), Palm oil fractionation and refining using the C.M.B. process, *Oléagineux*, **30**, 121–128.

Bernardini, M. and Bernardini, E. (1971), Fractionnement, frigélisation et décirage des huiles et graisses alimentaires, *Rev. Franç. Corps Gras*, **18**, 439–443.

Best, R.L. et al. (1961), Cocoa butter substitutes and products containing them, US Patent 2,975,060, assigned to Unilever.

Best, R.L. et al. (1960), Improvements relating to cocoa butter substitutes, British Patent 827,172, assigned to Unilever.

Biernoth, G. and Merk, W. (1985), Fractionation of butterfat using a liquefied gas or a gas in the supercritical state, US Patent 4,504,503, assigned to Unilever.

Blaney, T.L. (1973), Method of separating fatty acids, US Patent 3,755,389, assigned to The Procter & Gamble Company.

Boucher, R.E. and Skau, E.L. (1951), Phase relations pertaining to the solvent winterization of cottonseed oil in hexane and in acetone-hexane mixtures, *J. Am. Oil Chem. Soc.*, **28**, 48–3-487.

Breeding, C.J. and Marshall, R.T. (1995), Crystallization of butter oil and separation by filter-centrifugation, *J. Am. Oil Chem. Soc.*, **72**, 449–453.

Breitschuh, B. and Flöter, E. (2002), Solid immiscibility in milk fat fractions and methods to determine their occurrence, *Eur. J. Lipid Sci. Technol.*, **104**, 713–719.

Cavanagh, G.C. (1968), Method of separating components of oleaginous mixtures by fractional crystallization, British Patent 1,120,456, assigned to Ranchers Cotton Oil.

Chobanov, D.G. and Topalova, M.R. (1982), Solvent fractionation of tallow and interesterified lard, *Grasas Aceites*, **33**, 19–25.

Cleary, M.T. (1986), Process for separating saturated fatty acids from each other, US Patent 4,578,223, assigned to UOP Inc.

Cleary, M.T. et al. (1985), Process for separating oleic acid from linoleic acid, US Patent 4,529,551, assigned to UOP Inc.

Coenen, J.W.E. (1974), Fractionnement et interestérification des corps gras dans la perspective du marché mondial des matières premières et des produits finis I - Fractionnement, *Rev. Franç. Corps Gras*, **21**, 343–349.

Deffense, E.M.J. (1985), Fractionation of palm oil, *J. Am. Oil Chem. Soc.*, **62**, 376–385.

Deffense, E.M.J. (1987), Multi-step butteroil fractionation and spreadable butter, *Fat Sci. Technol.*, **89**, 502–507.

Deffense, E.M.J. (1993), Milk fat fractionation today: A review, *J. Am. Oil Chem. Soc.*, **70**, 1193–1201.

Deffense, E.M.J. (1995), Dry multiple fractionation: trends in products and applications, *Lipid Technol.*, **7**, 34–38.

Deffense, E.M.J. (1998), Dry fractionation and selectivity, *Oléagineux Corps Gras Lipides/OCL*, **5**, 391–395.

Deffense, E.M.J. (2000), Dry fractionation technology in 2000, *Eur. J. Lipid Sci. Technol.*, **1**, 234–236.

Deffense, E.M.J. (2001), Fractionation of animal carcass fats into hard and soft fats, in *Animal Carcass Fats*, Rossell, J.B., Ed., Leatherhead Publishing, Leatherhead, U.K., 149–173.

Deffense, E.M.J. (2005), Fractionation of fish, milk fat and other oils, *Paper presented at SCI Symposium: Fractionation — Current status and future prospects in a low-trans world, Ghent* (available on www.soci.org).

Denet, F. et al. (2000), Industrial chromatographic separation of fats, *Eur. J. Lipid Sci. Technol.*, **1**, 236–-240.

Dieffenbacher, A. (1986), Fat fractionation, US Patent 4,594,194, assigned to Nestec S.A.

Dijkstra, A.J. (1998), Alternatives to hydrogenation, *SCI Lecture Paper Series*, available from www.soci.org.

Dijkstra, A.J. (1999), L'expérience d'un utilisateur, *Paper presented at the AFECG Journée de Printemps* "*Le fractionnement des corps gras,*" Paris.

Dijkstra, A.J. (2003), My visit to a walnut oil mill, *inform*, **14**, 755–756.

Foubert, I. et al. (2004), Temperature and concentration dependent effect of partial glycerides on milk fat crystallisation, *Eur. J. Lipid Sci. Technol.*, **106**, 531–539.

Gembicki, S.A. et al. (1986), Sorbex separations: a key to new product opportunities, in *World Conference on Emerging Technologies in the Fats and Oils Industry*, Baldwin, A.R., Ed., American Oil Chemists' Society, 380–382.

Gibon, V. and Tirtiaux, A. (2002), Latest trends in dry fractionation, *Lipid Technol.*, **14**, 33–36.

Goh, E.M. and Timms, R.E. (1985), Determination of mono- and diglycerides in palm oil, olein and stearin, *J. Am. Oil Chem. Soc.* , **63**, 730–734.

Going, L.H. et al. (1969), Continuous solvent winterization of partially hydrogenated soybean oil, US Patent 3,450,277, assigned to The Procter & Gamble Company.

Grall, D.S. and Hartel, R.W. (1992), Kinetics of butterfat crystallisation, *J. Am. Oil Chem. Soc.*, **69**, 741–747.

Grundmann, J. (1984), Neue Entwicklungen auf dem Gebiet der Kristallisation, *Fette Seifen Anstrichm.*, **96**, 500–505.

Hamm, W. (1986), Fractionation — with or without solvents? *Fette Seifen Anstrichm.*, **88**, 533–537.

Hamm, W. (1995), Trends in edible oil fractionation, *Trends Food Sci. Technol.*, **6**, 121–126.

Hamm, W. (2005), Entrainment — are we making progress? *Paper presented at SCI Symposium: Fractionation — Current status and future prospects in a low-trans world,* Ghent (availble on www.soci.org).

Haraldsson, G. (1979), Pretreatment and fractionation of industrial fats, *Riv. Ital. Sostanze Grasse*, **56**, 325–331.

Harris, J. (2005), Why solvent fractionation? *Paper presented at SCI Symposium: Fractionation — Current status and future prospects in a low-trans world,* Ghent, available from www.soci.org.

Henderson, J.L. and Jack, E.L. (1944), The fractionation of milk fat from a solvent at low temperatures, *Oil & Soap*, **21**, 90–92.

Hendrix, M. and Kellens, M.J. (2003b), Process and installation for dry fractionation, European Patent 1 281 749 A1, assigned to De Smet Engineering N.V.

Hendrix, M. and Kellens, M.J. (2003a), Process and installation for dry fractionation, US Patent Application 2003/047290 A1, assigned to De Smet Engineering N.V.

Herrera, M.L. and Hartel, R.W. (2000), Effect of processing conditions on crystallisation kinetics of a milk fat model system, *J. Am. Oil Chem. Soc.*, **77**, 1177–1187.

Higuchi, K. et al. (1989), Method for dry fractionation of fatty material, US Patent 4,795,569, assigned to Fuji Oil Company Ltd.

Hughes, A.D. and Smith, K.W. (1994), Fats rich in *trans*-acids, World Patent 94/24882, assigned to Loders Croklaan B.V.

Iida, M. et al. (1981), Method for fractionating an oil or fat to separate the high melting point components thereof, US Patent 4,265,826, assigned to Ajinomoto Company Inc.

Jayaraman, G. and Thiagarajan, T. (2001), Specialty fats from lauric oils, in *Proceedings of the World Conference on Oilseed Processing and Utilization*, Wilson, R.F., Ed., AOCS Press, Champaign, IL, 67–73.

Kaufmann, H.P. and Thieme, J.G. (1954), Was ist "Illipé-Butter"? *Fette Seifen Anstrichm.*, **56**, 1001–1002.

Kawada, T. and Matsui, N. (1970), Process for the fractionation of oils and fats, US Patent 3,541,123, assigned to Kao Soap Co.Ltd.

Kaylegian, K.E. and Lindsay, R.C. (1995a), *Handbook of Milkfat Fractionation Technology and Applications*, AOCS Press, Champaign, IL.

Kellens, M.J. and Hendrix, M. (2000), Fractionation, in *Introduction to Oils and Fats Technology*, O'Brien, R.D., Farr, W.E., and Wan, P.J., Eds., AOCS Press, Champaign, IL, 194–207.

Kloek, W. et al. (2000), Nucleation kinetics of emulsified triglyceride mixtures, *J. Am. Oil Chem. Soc.*, **77**, 643–652.

Kokken, M.J. (1991), Obtention et emploi des corps gras fractionnés à sec (corps gras végétaux, matières grasses laitières), *Rev. Franç. Corps Gras*, **38**, 367–376.

Koslowsky, L. (1973), Salad oil and edible fats from palm oil by a new fractional crystallisation method in iso-propyl alcohol, *Grasas Aceites*, **24**, 295–298.

Koslowsky, L. (1976), Procédé pour séparer des huiles et graisses animales et végétales en une fraction liquide moins saturée et une fraction soldie plus saturée, French Patent 2.300.129, assigned to H.L.S. Ltd.

Kozempel, M.F. et al. (1981), Development of a continuous process to obtain a confectionary fat from tallow: final status, *J. Am. Oil Chem. Soc.*, **58**, 921–925.

Lacey, F.E. and Leaders, W.M. (1951), Solvent fractionation of fatty materials, US Patent 2,552,797, assigned to Swift & Company.

Lee, A.P. (1939), The winterizing of cottonseed oil, *Oil & Soap*, **16**, 148–150.

Levembach, M. (2001), Refined beef tallow in Argentina: production and applications, in *Proceedings of the World Conference on Oilseed Processing and Utilization*, Wilson, R.F., Ed., AOCS Press, Champaign, IL, 64–66.

Logan, T.J. (1981), Fractionation of triglyceride mixtures, US Patent 4,277,412, assigned to The Procter & Gamble Company.

Logan, T.J. and King, R.M. (1981), Fractionation of triglyceride mixture, US Patent 4,284,580, assigned to The Procter & Gamble Company.

Logan, T.J. and Lubsen, T.A. (1981), Fractionation of triglyceride mixtures, US Patent 4,297,292, assigned to The Procter & Gamble Company.

Long, K. (2004), A special RBD olein with low diglyceride content, *Paper presented at the 3rd EuroFedLipid Congress,* Edinburgh, page 126 of the abstracts.

Loury, M. (1956), Étude d'un procédé de séparation des acides gras concrets et fluides du suif par cristallisation dans le dichloréthane, *Rev. Franç. Corps Gras*, **3**, 30–34.

Luddy, F.E. et al. (1973), Development of edible tallow fractions for specialty fat uses, *J. Am. Oil Chem. Soc.*, **50**, 240–244.

Luddy, F.E. and Longhi, S. (1983), Azeotropic fat fractionation, European Patent 0.132 506, assigned to Société des Produits Nestlé S.A.

Maes, P.J. and Dijkstra, A.J. (1983), Process for separating solids from oils, European Patent 0 088 949, assigned to N.V. Vandemoortele International.

Maes, P.J. and Dijkstra, A.J. (1985), Process for separating solids from oils, US Patent 4,542,036, assigned to N.V. Vandemoortele International.

Maes, P.J. et al. (1995), Method for dry fractionation of fatty substances, European Patent 0 651 046, assigned to N.V. Vandemoortele International.

Martinenghi, G.B. et al. (1987), Sulla lavorazione dell'olio di palma VIII - Influenza di pretrattamenti sul frazionamento, *Industri Alimentari*, 349–353.

Mattil, K.F. (1953), Treatment of glyceride oils and the resulting product, US Patent 2,631,156, assigned to Swift & Company.

Mège, H. (1869), Production de certains corps gras d'origine animale, French Patent 86 480.

Muckerheide, V.J. (1950), Method of separating glycerides, US Patent 2,514,608, assigned to Emery Industries Inc.

Myers, L.D. and Muckerheide, V.J. (1942), Method of separating fatty acids, US Patent 2,293,676, assigned to Emery Industries Inc.

Neuzil, R.W. and deRosset, A.J. (1977), Process for separating esters of fatty acids by selective adsorption, US Patent 4,049,688, assigned to UOP Inc.

O'Brien, R.D. and Wan, P.J. (2001), Cottonseed oil: processing and utilization, in *Proceedings of the World Conference on Oilseed Processing and Utilization*, Wilson, R.F., Ed., AOCS Press, Champaign, IL, 90–144.

Ou, J.D. (1990), Process for separating triglycerides and regenerating absorbent used in said separation process, US Patent 4,961,881, assigned to UOP Inc.

Padley, F.B. (1997), Chocolate and confectionary fats, in *Lipid Technologies and Applications*, Gunstone, F.D. and Padley, F.B., Eds., Marcel Dekker, New York, 391–432.

Passino, H.J. and Meyers, J.M. (1949), Fractionation of oleaginous materials, US Patent 2,467,906, assigned to The M.W. Kellogg Company.

Puri, P.S. (1980), Winterization of oils and fats, *J. Am. Oil Chem. Soc.*, **57**, 848A–850A.

Putte, K.P.A.M. van and Bakker, B.H. (1987), Crystallization kinetics of palm oil, *J. Am. Oil Chem. Soc.*, **64**, 1138–1143.

Rappard, G. von and Plonis, G.F. (1979), Fett mit einem hohen Gehalt an 1,3-Dipalmitoyl-2-oleyl-glycerin, Verfahren zu seiner Gewinnung und seine Verwendung, German Patent 27 47 765, assigned to Walter Rau Lebensmittelwerke.

Rappard, G. von and Plonis, G.F. (1980), Process for recovering triglycerides, British Patent 2 048 928, assigned to Walter Rau Lebensmittelwerke.

Ricci-Rossi, G. and Deffense, E.M.J. (1984), Erfahrungen mit der Fraktionierung von Fetten nach dem Tirtiaux-Verfahren, *Fette Seifen Anstrichm.*, **86**, 500–505.

Rossell, J.B. (1985), Fractionation of lauric oils, *J. Am. Oil Chem. Soc.*, **62**, 385–390.

Rubin, L.J. et al. (1959), Method for the fractional crystallisation of cottonseed oil, US Patent 2,910,363, assigned to Canada Packers Limited.

Sambuc, E. (1964), Bases théoriques du fractionnement des glycérides A — Fractionnement par cristallisation, *Rev. Franç. Corps Gras*, **11**, 319–325.

Schlenker, E. (1934), Process for the approximate separation of solid and liquid fatty acids, US Patent 1,948,683.

Seugé, J.P. and Vinconneau, H.F. (1975), Le procédé Lipofrac Alfa-Laval pour le fractionnement et la frigélisation en continu des graisses comestibles, *Oléagineux*, **30**, 25–30.

Siew, W.-L. and Ng, W.-L. (1995), Partition coefficient of diglycerides in crystallization of palm oil, *J. Am. Oil Chem. Soc.*, **72**, 591–595.

Sinnema, Y.A. (1962), Process for the preparation of a cocoa butter substitute as well as for the preparation of chocolate and the shaped products thus obtained, US Patent 3.070,445, assigned to N.V. Twincon.

Skau, E.L. (1954), Process for modifying glyceride fats and oils, US Patent 2,684,377, assigned to USA, Secretary of Agriculture.

Smith, P.R. (2000), The effects of phospholipids on crystallisation and crystal habit in triglycerides, *Eur. J. Lipid Sci. Technol.*, **1**, 122–127.

Smith, P.R. and Povey, M.J.W. (1997), The effect of partial glycerides on trilaurin crystallization, *J. Am. Oil Chem. Soc.*, **74**, 169–171.

Smorenburg, J.J. (1972), Werkwijze voor het fractioneren van vetzuren en/of esters van vetzuren, Dutch Patent 7103763, assigned to Stork Amsterdam N.V.

Soeters, C.J. et al. (1981), Hard fat replacer and chocolate containing same, US Patent 4,283,436, assigned to Unilever.

Stein, W. and Hartmann, H. (1957), Separation of high molecular organic compounds, US Patent 2,800,493, assigned to Henkel & Cie. G.m.b.H.

Stewart, I. and Kristott, J. (2004), European Union Chocolate Directive defines vegetable fats for chocolate, *Lipid Technol.*, **16**, 11–14.

Subrahmanyam, V.V.R. and Achaya, K.T. (1958), Crystallization of Indian beef tallow fatty acids from aqueous ethanols, *J. Am. Oil Chem. Soc.*, **35**, 467–469.

Swern, D. et al. (1945), Fractionation of tallow fatty acids. Preparation of purified oleic acid and an inedible olive oil substitute, *Oil & Soap*, **22**, 302–304.

Tan, I. (2005), Membrane filter press for edible oil applications, *Paper presented at SCI Symposium: Fractionation — Current status and future prospects in a low-trans world,* Ghent.

Taylor, H.H. et al. (1976), Substitutability of fractionated beef tallow for other fats in the food and confectionary industries: an economic evaluation, *J. Am. Oil Chem. Soc.*, **53**, 491–495.

Thomas, A.E. and Paulicka, F.R. (1976), Solvent fractionated fats, *Chem. Ind.*, 774–770.

Timms, R.E. (1986), Processing of palm kernel oil, *Fette Seifen Anstrichm.*, **88**, 294–300.

Timms, R.E. (1991), Crystallisation of fats, *Chem. Ind.*, 342–345.

Timms, R.E. (1997), Fractionation, in *Lipid Technologies and Applications*, Gunstone, F.D. and Padley, F.B., Eds., Marcel Dekker, New York, 199–222.

Timms, R.E. (2005), Fractional crystallisation — the fat modification process for the 21st century, *Eur. J. Lipid Sci. Technol.*, **107**, 48–57.

Tirtiaux, A. (1990), Dry fractionation: a technique and an art, in *Edible Fats and Oils Processing: Basic Principles and Modern Practices*, Erickson, D.R., Ed., American Oil Chemists' Society, Champaign, IL, 136–141.

Tirtiaux, A. and Gibon, V. (1996), Dry fractionation: the boost goes on, *Paper presented at the AOCS World Conference,* Istanbul.

Tirtiaux, F. (1976), Le fractionnement industriel des corps gras par crystallisation dirigée - procédé Tirtiaux, *Oléagineux*, **31**, 279–285.

Vanhoutte, B. et al. (2002), Effect of phospholipids on isothermal crystallisation and fractionation of milk fat, *Eur. J. Lipid Sci. Technol.*, **104**, 738–744.

Wang, F.-S. and Lin, C.-W. (1995), Turbidimetry for crystalline fractionation of lard, *J. Am. Oil Chem. Soc.*, **72**, 585–589.

Weber, K. et al. (1998), Fat crystallizers with stirring surfaces: theory and practice, *Oléagineux Corps Gras Lipides/OCL*, **5**, 381–384.

Willner, T. et al. (1989), Herstellung von Kakaobutterersatz durch fraktionierte Speiseölkristallisation, *Fat Sci. Technol.*, **91**, 586–592.

Willner, T. et al. (1991), Einsatzmöglichkeiten der Hochdruckpressfiltration und Verfahren zur Erzeugung druckstabiler Fettkristalle, *Fat Sci. Technol.*, **93**, 588–595.

Willner, T. and Weber, K. (1994), High-pressure dry fractionation for confectionary fat production, *Lipid Technol.*, **6**, 57–60.

Wilp, Ch. (2000), Dry fractionation of fats and oils by means of centrifugation, *Paper presented at the 91st AOCS Annual Meeting & Expo, San Diego.*

Wrenn, L.B. (1998), Cottonseed oil's rise to prominence, *inform*, **9**, 100–106.

Wright, A.J. et al. (2000), The effect of minor components on milk fat crystallization, *J. Am. Oil Chem. Soc.*, **77**, 463–475.

Wright, A.J. and Marangoni, A.G. (2002), Effect of DAG on milk fat TAG crystalization, *J. Am. Oil Chem. Soc.*, **79**, 395–402.

Yoneda, S. et al. (1997), Process for dry fractionation of fats and oils, European Patent Application 0 798 369 A2, assigned to Fuji Oil Company Ltd.

Yoneda, S. et al. (2000a), Apparatus for dry fractionation of fats and oils, US Patent 6,060,028, assigned to Fuji Oil Company Ltd.

Yoneda, S. et al. (2000b), Method of dry fractionating fat and stationary crystallization apparatus, EP Patent 1 028 159, assigned to Fuji Oil Company Ltd.

Youngs, C.G. and Sallans, H.R. (1955), Acetone as a selective solvent for vegetable oils, *J. Am. Oil Chem. Soc.*, **32**, 397–400.

4.5 Food grade emulsifiers

The lipophilic character of fatty acids and their ready availability make them an ideal constituent of surfactants and emulsifiers. Moreover, some natural products, such as phospholipids, already fulfill an amphipathic role in nature so that their utilisation only requires isolation, purification, and, perhaps, some modification. This is a definite advantage for potential food additives. Food additives containing fatty acid moieties are listed in Table 4.22, together with some of their typical applications.

4.5.1 Lecithin and lecithin-based products

Nowadays, lecithin is produced by water degumming crude vegetable oils, especially soya bean oil, but the first commercial lecithin was egg-based and thus expensive. As recounted by Wendel (2000a; 2000b), the first soy-based lecithin was produced in Europe by the Hanseatische- Mühlenwerke AG, a company founded in Hamburg by Bollmann who had developed a process to hydrate phosphatides by passing live steam through crude oil obtained by solvent extraction (Bollmann, 1923) and separating the gums by using a stacked disc centrifugal separator (Pardun, 1988). Instead of a centrifugal separator, a decanter can also be used to isolate the gums (A.L. Zambone, personal communication). The gums obtained contain some 35 to 50% phosphatides or rather 'acetone insolubles (AI), and 15% triglyceride oil with water accounting for the remainder.

Originally they were dried in a special Bollmann drier comprising a circular grid made from tubes conveying the heating medium, and rotating around a horizontal axis inside a vessel with a semispherical bottom, at a batch temperature of 70 to 80°C and a pressure of 25 to 50 mbar (Pardun, 1988). This drying process also improves the taste in that bitter constituents distil over with the water or added alcohol (Bollmann, 1930).

Another process invented by Bollmann aims at improving the lecithin colour by treating it with hydrogen peroxide (1931). According to Flider (1985), mixing 0.3 to 1.5% hydrogen peroxide (30%) with the gums to be dried is more effective than degumming the oil with water containing this amount of hydrogen peroxide. Double-bleached lecithin is produced by adding 0.3 to 0.5% of benzoyl peroxide to the gums that already contain some hydrogen peroxide or to the dried gums (single-bleached lecithin). The amount of bleaching agent required to meet a lecithin colour specification depends very much on the colour of the gums to be bleached. Lightly coloured gums may meet the colour specification for double-bleached lecithin after having been treated with hydrogen peroxide only and double-bleaching dark gums may still not lead to a sufficiently light colour.

Lightly coloured lecithin can also be obtained (Marmor and Moyer, 1949) by treating the miscella with an adsorbent, removing the adsorbent by filtration, recovering the crude bleached oil by removing the solvent by evaporation, adding water to hydrate the phosphatides, and separating the hydrated phosphatides from the degummed oil. Since lecithin darkens on heating, temperature control is essential in that its storage temperature should be below 60°C. Higher temperatures during drying are acceptable provided the time involved is short. The cause of the darkening could be a Maillard reaction between the free amino group of the PE and the sugars present in lecithin.

These sugars have been investigated by Scholfield et al. (1952) who determined that soy lecithin contains 7.2% by weight free sugars (3.1% saccharose; 0.7% raffinose; 3.4%

TABLE 4.22 Food emulsifiers containing fatty acids

Product	EC No.	US/FDA 21 CFR	Typical Applications
Lecithin	E322	§ 182.1400 GRAS	o/w and w/o emulsions, instant products, chocolate
Polysorbate 20	E432		Bread, ice cream desserts, biscuits and wafers
Polysorbate 80	E433	§ 172.840	o/w emulsions, dairy and bakery products
Polysorbate 40	E434		Bread, ice cream, bakery products
Polysorbate 60	E435	§ 172.836	o/w emulsions, dairy and bakery products
Polysorbate 65	E436	§ 172.838	o/w emulsions, dairy and bakery products
Ammonium phosphatidate	E442		Chocolate
Na-,K- and Ca-soaps	E470a	§ 172.863	Co-emulsifiers
Mg-soaps	E470b		
Mono- and diglycerides	E471	§ 182.1505 GRAS	Spreads, ice cream, coffee whiteners
Acetic acid esters of monodiglycerides	E472a	§ 172.828	Cakes, toppings
Lactic acid esters of monodiglycerides	E472b	§ 172.852	Cakes, toppings
Citric acid esters of monodiglycerides	E472c	§ 184.832	o/w and w/o emulsions, spreads, meat products
Tartaric acid esters of monodiglycerides [a]	E472d		
Mono- and diacetyl-tartaric acid esters of monodiglycerides	E472e	§ 182.4101	Bread improvers, rolls, buns, sauces, o/w emulsions, coffee whiteners
Acetic acid esters of tartaric acid esters of monodiglycerides [a]	E472f		Bread improvers
Fatty acid esters of sucrose	E473	§ 172.859	o/w emulsions, bakery products, dessert products
Sucroglycerides	E474		
Fatty acid esters of polyglycerol	E475	§ 172.854	o/w emulsions, bakery products, dessert products
Polyglycerol polyricinoleate	E476		Low-fat spreads, margarines and chocolate
Fatty acid esters of propylene glycol	E477	§ 172.85	Cakes, dessert products, toppings
Thermally oxidised soybean oil interacted with monodiglycerides	E479b		
Succinic esters of monodiglycerides		§ 172.830	Bread improvers
Ethoxylated monodiglycerides		§ 172.834	Bread improvers
Sodium stearoyl lactate	E481	§ 172.846	Bread improvers (antistaling)
Stearoyl lactic acid		§ 172.848	
Calcium stearoyl lactate	E482	§ 172.844	o/w emulsions, bread improvers
Stearyl tartrate [a]	E483		
Sorbitan monostearate	E491	§ 172.842	Confectionary products
Sorbitan tristearate	E492		Recrystallisation inhibitor

[a] Are no longer listed in www.emulsifiers.org

Sources: Als, G. and Krog, N. (1991), in *World Conference on Oleochemicals into the 21st Century*, Applewhite, T.H., Ed., AOCS Press, Champaign, IL, 67–73; Krog, N. (1994), in *The Lipid Handbook*, Gunstone, F.D., Harwood, J.L., and Padley, F.B., Eds., Chapman & Hall, 296–304; Krog, N. (1997), in *Lipid Technologies and Applications*, Gunstone, F.D. and Padley, F.B., Eds., Marcel Dekker, New York, 521–534; Stauffer, C.E. (2002), in *Fats in Food Technology*, Rajah, K.K., Ed., Sheffield Academic Press, Sheffield, U.K., 228–274; and www.emulsifiers.org

o/w = oil-in-water

w/o = water-in-oil

stachyose) and 1.5% bound sugars consisting of galactose, mannose, and arabinose. They can be removed by dissolving the lecithin in a hydrocarbon like hexane and extracting the hexane solution with a lower alcohol, such as methanol, ethanol, or isopropanol (Scholfield and Dutton, 1955) or by mixing a suspension of active yeast into the gums (Eichberg, 1961).

Because these sugars do not dissolve in acetone, they contribute to the AI fraction of lecithin, which also comprises glycolipids (6.7% (Schneider, 1997a)). Consequently, the factor that is often used to convert phosphorus content to AI is larger than the weighted molecular weight of the phosphatides that is close to 25. As pointed out by Pardun (1964), the ratio of the % AI to % P is not a constant, but depends upon the acid value of the lecithin. In old soya beans, some phosphatides will have been hydrolysed and the hydrolysis products are no longer insoluble in acetone, so a high ratio of 33 to 34 is, therefore, indicative of a good quality lecithin.

Lecithin specification parameters as presented in Table 4.23 also include its moisture content. In 1964, Pardun compared several different methods for water determination: *Karl Fischer* titration, azeotropic distillation with toluene, and 3 hours' drying in a vacuum oven at 60, 80,

TABLE 4.23 Specification of fluid natural soya lecithin

Acetone insolubles, min (%)	62
Moisture, max (%)	1
Hexane insolubles, max (%)	0.3
Acid value, max (mg KOH/g)	32
Colour, Gardner, max of 5% dilution	10
Viscosity, max (Pa.s at 25°C)	1500

Source: National Soybean Processors' Association, Yearbook and Trading Rules, 1978–1979.

or 100°C. He observed that the oven method at 80°C and the *Karl Fischer* method gave the most reliable results and recommends the latter because it is faster and also allows low water contents (>1%) to be determined.

Water has to be removed from the gums to prevent microbial spoilage and, nowadays, wiped film evaporators (Van der Piepen, 1962) are commonly used for this purpose. They operate under vacuum at an absolute pressure of 30 to 40 mbar and a temperature of 110 to 130°C with a short residence time of only 1 to 2 min (Pardun, 1988); these evaporators operate continuously. As illustrated in Figure 4.22, the viscosity of the product varies over a wide range during the drying operation. It increases about fourfold when the water content is decreased to 8% and, during subsequent drying, it decreases to below its original level (Van Nieuwenhuyzen, 1976).

Subsequently, the AI can be adjusted by the addition of liquid oil and the product viscosity can be controlled by the addition of FFA (Wiesehahn, 1940) or a liquid monoglyceride (Werly, 1957). It can also be converted into a free flowing powder by mixing with a large amount of dextrose (Neiman, 1955) or smaller amounts of fructose or glucose (Davis and Fellow, 1961). Subsequently, Davis (1967a) discovered that the addition of divalent cations, as provided, for instance, by calcium chloride, calcium acetate, magnesium chloride, etc., also fluidises the lecithin.

Another specification parameter concerns the hexane insolubles (HI), which were formerly determined as benzene insolubles (Pardun, 1964). When the hydrated gums are separated from the oil, they sweep the oil and collect all particulate matter, such as meal particles and also iron particles (Dijkstra and Van Opstal, 1989). In order to produce less cloudy and more brilliant lecithin, a filtration stage is necessary. In principle, this filtration can be performed at the miscella stage, or the oil can be filtered prior to water degumming, and it is even possible to filter the highly viscous lecithin. According to Brian (1976), miscella filtration is the preferred (cheapest) method and the use of 60 mesh filter leaves and the addition of 0.05% by weight of filter aid, based upon the weight of oil in the miscella, is recommended.

The maximum acid value of lecithin has been specified to guard against the use of old and damaged beans that could lead to a poor quality product, and to detect the addition of FFA to lower the viscosity of the lecithin. Since

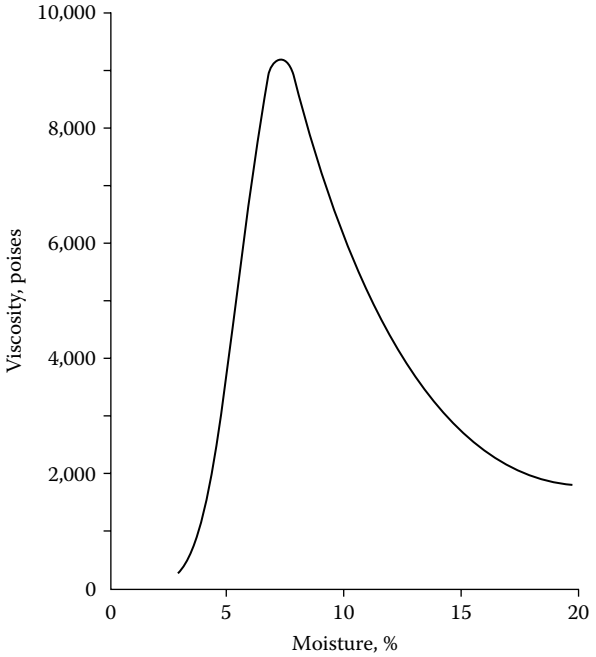


FIGURE 4.22 Viscosity of lecithin sludge. (From Nieuwenhuyzen, W. van (1976), *J. Am. Oil Chem. Soc.*, **53**, 425–427. With permission.)

quantifying two separate properties with a single measurement is bound to lead to ambiguity, a separate acid value determination of the acetone soluble fraction is recommended. This gives a clear indication of any addition of FFA, since amongst the samples that were analysed, the acid value increases from an average value of 7.6 to a range of 22.5 to 56.9 (Pardun, 1964).

The soya bean lecithin specification given in Table 4.23 is based on the assumption that the lecithin has been obtained by water degumming crude soya bean oil. It is concerned with properties rather than composition. In the next table (Table 4.24), the composition of lecithin from various agricultural sources is compared. Among those listed, soya bean lecithin is the only one that has been specified. If the other lecithins are commercialised at all, this will be more on an *ad hoc* basis.

The literature mentions properties and compositions of other lecithin products, but a word of caution is in order here. In 1940, Halden was of the opinion that the phosphate group could be attached to the α-position or the β-position of the glycerol backbone, and when Cherry et al. reviewed lecithin chemistry in 1981, they still made a distinction between the optically active α-form and the optically inactive β-form. Another warning concerns the PS content. Older literature, especially in the U.S., may report much higher values than would result from modern analytical methods (see, for instance, Cherry et al., 1981a, Table VII). Compositional data have been reported for rapeseed lecithin (Persmark, 1968), cottonseed lecithin (Vijayalakshmi et al., 1969; Cherry et al., 1981a), phosphatides from various safflower cultivars (Burkhardt,

TABLE 4.24 Phospholipid compositions (in weight %) of various lecithins as determined by ^{31}P-NMR

Phospholipid	Soya	Rape	Sunflower	Corn	Egg
Phosphatidyl choline	21.9	24.6	25.4	30.4	74.0
Phosphatidyl ethanolamine	13.6	22.1	11.0	3.2	19.1
Phosphatidyl inositol	12.0	14.7	19.4	16.3	0.4
Phosphatidyl serine			0.8	1.0	
Phosphatidyl glycerol and diphosphatidyl glycerol	2.3		1.2	1.4	0.6
Phosphatidic acid	5.8		3.3	9.4	
N-acyl phosphatidyl ethanolamine	2.8		1.0	2.6	
Sphingomyelin					2.5
Lyso-phospholipids	2.9	19.4		5.4	2.9
Others	3.6	19.2			0.5

Source: Schneider, M. (1997a), *Lipid Technol.*, **9**, 109–116.

TABLE 4.25 Phosphatide composition (by TLC) of lecithin resulting from different flake pretreatments

	Normal Soybean Flakes		
Phosphatide	(Pardun, 1962a)	(Kock, 1983)	Pretreated Soybean Flakes (Kock, 1983)
Phosphatidylcholine (PC)	35	33	46
Lysophosphatidylcholine (LPC)		2	2
Phosphatidylethanolamine (PE)	13	30	23
Phosphatidylinositol (PI)	33	14	8
Phosphatidic acid (PA)		17	19
Others	19	4	2

1971), from palm oil (Goh et al., 1982) and palm-pressed fibre (Choo et al., 2004), from rice bran oil (Adhikari and Adhikari, 1986), from high erucic acid rapeseed oil (Solomon, 1973) and canola (Przybylski and Eskin, 1991; Sosada et al., 1992; Lange et al., 1994), and also from sunflower seed oil (Holló et al., 1993).

Because the soya bean lecithin specification in Table 4.22 silently assumes that the lecithin has been produced by the water degumming process, it, therefore, should exclude lecithin obtained by any other route. If, for instance, the crude soya bean oil quality were such that water degumming did not lower the residual phosphorus level below 200 ppm (the degummed oil specification limit) and the use of acidified water were to bring the degummed oil within specification, the resulting lecithin would be outside the specification. This also applies to acid degumming processes that aim to lower the residual phosphorus contents to values that are far lower than just 200 ppm, such as the Superdegumming process (Ringers and Segers, 1977) or the organic refining process (Copeland and Belcher, 2001), especially since the dried gums of these acid degumming processes also contain the actual degumming acid. Besides, gums originating from sunflower seed oil and separated at low temperatures may contain a variable amount of waxes. Gums resulting from acid refining processes, such as TOP (Dijkstra and Van Opstal, 1986), contain even more additive residues.

This does not mean that such lecithin is useless, but only that its usefulness must be assessed and that a specification for such a product must be agreed upon between the supplier and the user. This applies for instance, to lecithin obtained by water degumming Alcon oil, that is soya bean oil obtained by extracting soya bean flakes that have undergone a live steam treatment before being extracted (Kock, 1978). Table 4.25 (Kock, 1983) illustrates that this pretreatment has a significant effect upon the phosphatide composition of the lecithin and, thus, upon its properties. Applications demanding an increased PC content and/or a decreased PE content could profitably use this different grade, which would merit a specification of its own.

4.5.1.1 De-oiled lecithin

Lecithin as specified in Table 4.23 is a viscous and sticky fluid and, therefore, difficult to dispense. For industrial applications, this does not present insurmountable problems, but it is an impediment for therapeutic applications. Buer (1936) described a process in a patent having a priority date of 1931, whereby the lecithin is thoroughly dried, then mixed with 5 to 6% of alcohol, which causes the lecithin to become plastic and allows it to be portioned into pastilles or tablets. Because they are hygroscopic, the portions are coated with beeswax or a high melting fat. The same problem had already been tackled by Rewald (1933) in a different way in that he de-oiled lecithin with acetone or ethyl acetate (see also Aneja et al., 1971, Ref. 11). Soon after, German patent 653.878, assigned to Noblee & Thörl, was published and this patent also describes the use of acetone or ethyl acetate for de-oiling purposes. Instead of using the dried gums as the

TABLE 4.26 Phosphatide enrichment (%) of 20 g of lecithin in two-solvent systems

Hydrocarbon		Acetone (ml) Containing:			Phosphatide Enrichment (%) When Using Acetone With:		
Type	Amount (ml)	1%	2%	5%	1%	2%	5%
		Water			Water		
n-pentane	10	22	24	23	98	98	97
	20	43	40	39	96	94	98
	30	51	53	47	88	96	99
n-hexane	10	25	27	27	96	100	98
	20	48	44	45	97	97	98
	30	57	57	47	93	99	96
techn. hexane	10	26	26	30	92	100	96
	20	50	44	40	98	99	99
	30	55	59	47	99	97	95

Source: Pardun, H. (1962a), *Fette Seifen Anstrichm.*, **64**, 536–541.

starting material, it uses the gums that are obtained during water degumming. However, this means that the solvent has to be rectified, which adds to the processing costs.

According to Eichberg (1939), the resulting oil-free lecithin is a waxy substance that is expensive to produce, so that demand for this type of product comes chiefly from the pharmaceutical sector. The chocolate industry prefers to use a mixture of oil-free lecithin and cocoa butter, which is cheaper to produce because acetone removal from this mixture is easier than from lecithin alone. Another way to obtain a product that is easy to handle is by mixing oil-free lecithin with some 6% fatty acid esters (Wittcoff, 1949). This provides a liquid that can easily be poured.

As pointed out by Pardun (1962a), the use of acetone as de-oiling solvent has many disadvantages. A large amount is required (15 to 30 times the amount of lecithin being de-oiled) and removing the last traces is awkward. Moreover, a further drawback is the formation of acetone by-products, such as mesityl oxide, diacetone alcohol, phorone, etc., which may only be present in small quantities, but are undesirable because of their toxicity and their distinctive odour. Accordingly, he invented a process (Pardun, 1962b) using two solvents, one of which is a good solvent for oil but does not dissolve phosphatides, whereas the other dissolves the phosphatides. Specified solvent pairs include: acetone/cyclohexane, methyl acetate/dichloroethylene, acetone/*n*-pentane, and acetone/*n*-heptane and, as illustrated by Table 4.26, the addition of a small (2 to 5%) amount of water improves the phosphatide enrichment. Thanks to this process, the amount of acetone needed per kg lecithin could be reduced by a factor of 10.

In the early 1980s, de-oiling lecithin with supercritical gases was suggested (Heigel and Hüschens, 1981) and with supercritical carbon dioxide in particular (Stahl and Quirin, 1985), but as pointed out subsequently (Weidner et al., 1993), this process has the disadvantage of operating at a pressure of 35 MPa and, besides, the carbon dioxide to lecithin ratio exceeds 20.

These disadvantages were somewhat reduced by a process employing a mixture of carbon dioxide and propane (Peter and Weidner, 1985; Peter et al., 1987) since this operates at a pressure of 8 MPa and employs less than 5 kg solvent per kg lecithin. However, a superior de-oiling process (Weidner et al., 1993) uses just propane, in an amount of some 4 kg per kg lecithin and at a pressure of only 3 to 4 MPa. The lecithin yield is >99% of theory and the residual oil content of the de-oiled lecithin is <<1%. According to Schneider (1997a), products processed with compressed gas technology have reached the market.

The Low Pressure Gas Extraction (LPGE) process (Weidner et al., 1993) is based on the discovery that the lecithin/oil/propane system is a two-phase system and the observation that the composition of the phases is strongly temperature dependent. Accordingly, the use of an extraction column with a temperature gradient (bottom temperature 40 to 55°C; top temperature 20 to 30°C higher), into which lecithin is fed about halfway up, permits a good separation. The top product is a solution of triglyceride oil with low residual phosphatides in liquid propane that can easily be separated into its constituent parts by releasing the pressure and/or an increase in temperature. Similarly, the bottom product yields a de-oiled lecithin powder on pressure release[9].

Lecithin can also be de-oiled by dissolving it in hexane and subjecting the solution to membrane filtration (Hutton and Guymon, 2000). The membrane will also retain colouring compounds, so bleaching of the hexane solution is recommended if a lightly coloured de-oiled lecithin is required.

4.5.1.2 Chemically modified lecithin products

Because lecithin is a natural product that can be used in foods, it is a good starting material for a range of

[9] If the hexane insoluble of this lecithin has to be reduced, filtration of its propane solution before pressure release could constitute a relative simple means because of the low viscosity of the solution and its relatively low volume.

surfactants and emulsifiers with different properties. Besides, modifying the chemical structure of lecithin may also permit a stricter control of these properties and has the additional advantage over fractionation processes of not leading to by-products.

4.5.1.2.1 Hydroxylation

By introducing hydroxyl groups into the fatty acid chains, they become more hydrophilic. These hydroxyl groups are introduced by making use of the reactivity of the double bonds present in the fatty acid moieties in the lecithin. When hydrogen peroxide and an acid, such as acetic acid or lactic acid, are mixed together, peracids will be formed and when these peracids are allowed to react with a double bond, they will convert this double bond into an oxide; this oxide can then react further to form esters that may finally yield vicinal diols. Because a C-O linkage is broken when the ester is formed, its fission is always accompanied by a *Walden*-inversion and the diol formed will have a *trans* configuration.

Since the triglyceride oil present in lecithin will also react with the peracids, Julian (1953) claims a composition of matter in which both the triglycerides and the phospholipids have been hydroxylated and one of the vicinal hydroxyl groups is esterified. Accordingly, the acid has a dual role in that it acts as a catalyst for the formation of the oxide and as a reagent in the ester formation. If the acid is lactic acid, its ester will still have a free hydroxyl group and, thus, contribute to its hydrophilicity. Consequently, the hydroxylated lecithin is easily emulsified in water and a very effective emulsifier. Instead of esterifying the vicinal hydroxyl groups with an organic acid, they can also be esterified with inorganic compounds, such as sulfur trioxide and phosphorus pentoxide (Hennessy and Moshy, 1957) and, thus, increase hydrophilicity even further.

4.5.1.2.2 Hydrogenation

Soon after Normann invented a process for hydrogenating triglyceride oils (1903), this invention was applied to egg lecithin to improve its stability. The first patent claiming this application (J.D. Riedel AG, 1913) prescribes that the lecithin be dissolved, and employs a platinum catalyst. Then a lecithin emulsion was also found to be a suitable substrate provided bile salts were present (J.D. Riedel AG, 1914b), but this proviso was subsequently dropped (J.D. Riedel AG, 1914a). Later, nickel catalysts were also found to be effective when used with lecithin solutions in organic solvents, such as cyclohexane, but excluding alcohols (J.D. Riedel AG, 1924b); it was later discovered that alcohols also work (J.D. Riedel AG, 1924a).

However, the phosphatides inactivate the nickel catalyst (Drozdowski and Zajac, 1977), so that additional amounts of catalyst have to be added during the course of the hydrogenation. Later, high pressures (up to 15 MPa) were used to promote the nickel catalysed hydrogenation (Jacini, 1959), but even so, the iodine value of the reaction products are well above zero. For some applications this may be desirable (Dailey et al., 1959), but as shown by Davis (1962), experimental conditions that lead to almost full hydrogenation of soya bean oil (Example 3) do not fully saturate the lecithin. The "improved method" involving chlorinated solvents (Cole, 1959) does not achieve full saturation either. Perhaps the idea mentioned in Section 4.2.6.1 could lead to full saturation since the use of hydrazine as a hydrogenation agent does not involve a catalyst and thereby avoids its inactivation.

4.5.1.2.3 Acylation

Most acids can be made to form an amide with the free amino group in PE by allowing this phosphatide to react with their anhydride. Accordingly, Davis (1967b) gives examples with acetic acid anhydride, succinic anhydride, maleic anhydride, phthalic anhydride, and diacetyltartaric anhydride. In industrial practice though, acetic acid anhydride is the only reagent used. The acetylation process is quite simple in that it can use the gums resulting from the water degumming process as the starting material (Eichberg, 1967). It can even use crude oil as the starting material by degumming this oil with an aqueous solution of acetic acid anhydride and isolating the gums and drying them (Seaberg and Hayes, 1974).

In theory, the acetic acid anhydride used for the acetylation could also react with free hydroxyl groups, but in practice, the reaction with free amino groups is much faster. Acetylation is also faster than hydrolysis of the anhydride. Consequently, ester formation and hydrolysis only result from an appreciable excess of anhydride over free amino groups (Pardun, 1969).

Lecithin or its precursor products are acetylated to modify the lecithin properties or to facilitate fractionation. According to Eichberg (1967), acetylation makes lecithin disperse more readily in water and a product that disperses even better is lecithin that has been both acetylated and hydroxylated (Szuhaj and Yaste, 1976); it can be advantageously used as a pan release agent (Szuhaj, 1975). Several fractionation processes involving acetylation have been described (Aneja, 1970; Aneja et al., 1971; Günther, 1984), but it is unlikely that they are in use industrially. Acetylation with acetic acid anhydride may also precede partial hydrolysis, whereby the acetic acid liberated during the acetylation acts as hydrolysis catalyst (Pardun, 1971a).

4.5.1.2.4 Partial hydrolysis

As mentioned in several reviews (e.g., Pardun, 1988), acids, bases, and enzymes can catalyse the partial hydrolysis of lecithin, but the reaction can also proceed at near neutral pH. Acids and bases have in common that they operate rather indiscriminately, whereas enzymes show specificity. Accordingly, a distinction has to be made between the various phospholipases as illustrated in Figure 4.23.

This figure shows that phospholipase A_1 (E.C. 3.1.1.32) specifically catalyses the hydrolysis at the *sn*-1 position,

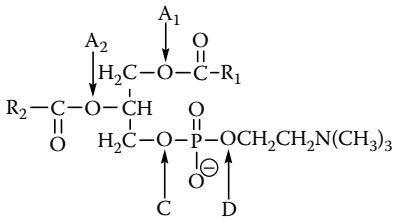


FIGURE 4.23 Phospholipase specificities.

whereas phospholipase A_2 (E.C. 3.1.1.4) does the same for the *sn*-2 position. In the literature, a phospholipase B (E.C.3.1.1.5) is also mentioned; this lipase is assumed to be nonspecific and, thus, to catalyse the hydrolysis at both *sn-1* and *sn*-2 positions. Phospholipase C (E.C.3.1.4.3) catalyses diglyceride formation and phospholipase D (E.C.3.1.4.4) catalyses the hydrolysis that leads to PA-formation by splitting off the characteristic group, choline, ethanolamine, etc.

For the acid catalysed hydrolysis of phosphatides, Davis (1971)[10] uses hydrochloric acid to reduce the pH of his gums to within a range of 2 to 4. Other inorganic acids like sulfuric acid and phosphoric acid are also claimed. In Europe, Pardun (1971c) uses sulfurous or sulfuric acid to catalyse the hydrolysis.

When describing his alkaline partial hydrolysis process, Davis (1970) refers to a much earlier patent (Schwieger, 1935) claiming a process including the step of adding at least 10% of solid alkali hydroxide to the phosphatide containing no more than 50% of water. Accordingly, Davis (1970) limits not only the amount of his basic material to the stoichiometric amount required to reduce the acid value of the phosphatide to zero, but also the extent of hydrolysis/saponification. In another patent (Mulder, 1969), a fruit coating based on partially hydrolysed lecithin is described. For this particular application, ammonia is apparently the preferred alkali.

It is not clear whether acid and/or alkali catalysed phospholipid hydrolysis processes are used industrially because this hydrolysis process is one the few examples in the edible oils and fats industry where enzymes are used on a commercial scale. The fact that the enzyme concerned is a by-product of insulin production and, therefore, has no cost price of its own may well be at least partially responsible for this situation. The first patent describing enzymatic hydrolysis of phosphatides (Klenk et al., 1962) still used snake venom and aimed at high value-added products for therapeutic use.

However, the enzyme used (Pardun, 1972a; 1972b) is this by-product, which is described as technical pancreatin, or in the main claim as "an enzyme preparation containing both lipase and phospholipase A." Because this porcine pancreatic phospholipase is relatively cheap, it is not recuperated. The main application of the enzymatically hydrolysed lecithin is as an emulsifier in calf milk replacers. This enzyme was also used in the enzymatic degumming process Enzymax® (Aalrust et al., 1993) until it was replaced by a microbial phospholipase (Clausen, 2001); these degumming processes are discussed in Section 3.4.

On a laboratory scale, Haas et al. (1994) studied the activity of various lipases with respect to PC. Not all lipases were found to be active and the activity of the active ones depends on the polarity of the solvent used. Treating lecithin dissolved in alcohol (Sarney et al., 1994) with an immobilised lipase leads to lysophosphatides and fatty acid esters. Membrane reactors were studied (Morgado et al., 1996) in an attempt to achieve savings by retaining the enzyme (porcine pancreatic phospholipase).

4.5.1.2.5 Transesterification

Lecithin products can also be modified by transesterification, and this reaction can involve the fatty acid moieties as well as the phosphate ester groups. Because of the inherent specificity of this reaction, it is advantageously catalysed by enzymes, such as phospholipases A and D, and, thus, limited to laboratory scale.

For the exchange of fatty acid moieties at the sn-1 position of phospholipids, a 1,3-specific lipase can be used. Svensson et al. (1992) used a *Rhizopus arrhizus* (now referred to as *R oryzae*) lipase, which they immobilised by adsorption onto a polypropylene support. They achieved a 100% incorporation of heptadecanoic acid almost exclusively in the *sn*-1 position of egg lysoPC. Using microbial lipases originating from *Candida cylindracea* and *Rhizopus delemar* ensured the incorporation of highly unsaturated fatty acids from sardine oil into soya bean lecithin (Totani and Hara, 1991), but also led to extensive hydrolysis. Similarly, Mustranta et al. (1994) studied commercial lipase preparations from *Aspergillus niger* and *Rhizomucor miehei* and observed that these preparations can modify lecithin by catalysing the incorporation of a different fatty acid. *Rhizopus* species have been tested (Hara and Nakashima, 1996) and were found to exhibit a good transesterification performance.

Exchanging the fatty acid moiety at the *sn*-2 position requires a phospholipase A_2 such as porcine pancrease *(Sus scrofa)* and further exchange at the *sn*-1 position can then be affected using a lipase from *Rhizopus oryzae,* which is commercially available in immobilised form (Doig and

[10] In Example 6 of this patent (Davis, 1971), a most interesting phenomenon is observed concerning residual phosphorus contents after degumming. A nondegummed soybean oil containing 576 ppm P contains 95 ppm after water degumming and 38 ppm after degumming with dilute hydrochloric acid (pH = 3.70) at 70°C. When the temperature is lowered to 30°C before the gums are separated, the residual phosphorus content drops to only 1.5 ppm. This observation is identical to the surprising finding that forms the basis of the Unilever SuperDegumming patent (Ringers and Segers, 1977) where the Davis patent (1971) is not cited as a reference, presumably because the patents concerned belong to different classes.

Diks, 2003a). Another commercially available immobilised lipase from *Rhizomucor meihei* has also been used for the outer position (Adlercreutz and Wehtje, 2004).

For the transesterification of the phosphate head group, which is also referred to as transphosphatidylation (Ulbrich-Hofmann, 2003), phospholipase D is the appropriate catalyst. It allows the PC content of lecithin to be increased without the use of fractionation solvents (Juneja et al., 1989). It permits phosphatidylglycerol (PG, a surfactant) to be synthesized from PC (Wang et al., 1997) using the supernatant of a *Pseudomonas* fermentation and, similarly, it permits PS to be made from lecithin, provided a lecithin adsorbent, such as calcium sulphate, is present (Iwasaki et al., 2003). The enzyme used in this latter reaction was prepared from recombinant strains of *Escherichia coli* bearing the phospholipase D gene of *Streptomyces antibioticus*. By using a commercial phospholipase D, Doig and Diks (2003b) prepared a whole range of transphosphorylation products with concentrations of 70 to 80% of the target component.

4.5.1.3 Lecithin fractions

In 1988 Pardun published a table with selling prices in German marks per kg (1 DEM = € 0.50) for lecithin and lecithin-based products. This table is reproduced below as Table 4.27. Although the prices are out of date and the introduction of de-oiling with propane (Peter et al., 1987) has reduced the cost of de-oiling, more recent prices will also cover a wide range. Consequently, the volume of the more sophisticated products is quite small and the market is limited to pharmaceutical applications, such as liposomes and cosmetics (Schneider, 1997b).

Accordingly, most fractionation patents stem from the pharmaceutical industry. But then, the food industry discovered that the de-oiled, alcohol-soluble fraction of lecithin (Mattikow, 1953), or a ratio of PC to PE in excess of 4, causes margarine to become less prone to spattering (Wieske et al., 1968). So a fractionation plant was built (Liebing and Lau, 1976; Pardun, 1988) to process 1000 tonnes of lecithin *per annum*. In this plant, the lecithin is first of all mixed with 5 to 10% technical monoglycerides to make it more fluid (Pardun, 1971b). Then the mixture is treated in a counter-current extractor with ethanol (90%) in a ratio of some 1:3 (weight/volume). The liquid, light phase is enriched in PC. It is clarified and then mixed with neutral oil to provide a fluid PC fraction after evaporation of the solvent.

TABLE 4.27 Prices for lecithin products (August 1987)

Product	Price Ex-Works (DEM/kg)[a]
Straight soybean lecithin	1.00
Soybean lecithin enriched in PC	9.00
De-oiled soybean lecithin	16.00
Soybean lecithin, 45% PC	70.00
Soybean lecithin, 70% PC	400.00
Soybean lecithin, 95% PC	550.00

[a] German currency at the time was the Deutschmark (DEM) with a value of 0.50.

Source: Pardun, H. (1988), *Die Pflanzenlecithine*, Verlag für chemische Industrie H. Ziolkowsky KG, Augsburg.

This fraction is very suitable for the production of margarine with a reduced tendency to spatter, but for pharmaceutical applications, it is quite useless. Accordingly, the process invented by Günther (1985) aims to produce a PC fraction with a low oil content. It is based on his discovery that the addition of 10 to 20% of water to the alcoholic extraction medium causes most of the oil to form a separate phase that can be easily eliminated from the system. Fractions with little oil and with a PC content of up to 85% can be obtained in this way and the yield based upon the crude phosphatide starting material is also improved.

Higher PC contents can be attained by selective adsorption from an alcoholic solution by alumina, magnesia, or activated carbon (Meyer et al., 1960), removing the spent adsorbent and evaporating the treated solution to dryness. Betzing (1970; 1980) on the other hand, aims at a complete adsorption of all phosphatides onto alumina followed by selective elution. Silica gel can also be used in this kind of process, with toluene as a solvent (Reichling, 1969) or alcohol (Günther, 1984).

So, extracting PC and purifying the extract can lead to a high PC to PE ratio. In theory, this can also be attained by extracting PE from lecithin. This has been realised by, first of all, acetylating the PE and then deprotonating the acetylation product (APE) by using a nitrogen base like ammonia or diethylamine and finally extracting the product with acetone or methyl acetate (Aneja, 1970). The acetone-insoluble PC remains in the extraction residue, but the dissociated APE is soluble in acetone and extracted. It was discovered that protonation also worked (Aneja et al., 1971; Aneja and Chadha, 1974).

The use of pH to affect the solubilities of the various phospholipids also forms the basis of another fractionation process (Dijkstra and De Kock, 1990). Adjusting the pH to at least 8 causes PC and PE to dissolve in the alcoholic extraction solvent, and adjusting it to well below 5, will suppress the dissociation of PA, as a result of which it becomes soluble in the extraction solvent. In addition, the patent also uses the difference in solubilities of phosphatide salts for fractionation purposes. When operating with supercritical carbon dioxide as an extraction solvent, De Kock introduced alcohol as an entrainer and again adjusted the pH to below 4 or preferably between 1 and 2 in order to solubilise and extract the PA from the starting material (De Kock, 1991).

4.5.2 Monoglycerides and derivatives

Monoglycerides and their derivatives are synthetic products. After their synthesis they may undergo some form of purification as, for instance, molecular distillation in the case of monoglycerides, but more often

the reactor contents are cooled and sold as such. They may be sold under a chemical name, such as "lactic acid esters of mono- and diglycerides" (E472b), but this may give a false impression of chemical purity which may be further reinforced by the use of molecular models in sales brochures and journal articles.

Lactic acid contains both carboxyl and hydroxyl groups while monoglycerides contain ester and hydroxyl groups, and when the two are allowed to react together many different reactions can take place. Hydroxyl groups can form ether linkages, the water then liberated can hydrolyse ester bonds, new ester bonds can be formed, etc. As explained by Lauridsen (1976), column chromatography followed by GLC-MS-analysis of the various fractions shows that the most polar fraction does not even contain any fatty acid moieties, but consists of a homologous series of glycerol lactates from mono- up to at least hexalactates. Using a mixture of tartaric acid (two carboxyl and two hydroxyl groups), acetic acid (one carboxyl group), and monoglycerides (2 hydroxyl groups and an ester group) will lead to reaction products containing even more different compounds and isomers.

4.5.2.1 Monoglycerides

Actually, this number of reaction products is even larger because of the glycerolysis equilibrium. According to this equilibrium, pure monoglycerides will form a mixture of free glycerol and mono-, di-, and triglycerides provided a suitable catalyst is present. As is to be expected, the presence of less suitable catalysts simply means that the glycerolysis equilibrium is reached somewhat more slowly. Therefore, keeping monoglycerides in the molten state for extended periods is not recommended (Lauridsen, 1976).

When describing the production of a low-calorie cocoa butter substitute (caprenin), Stipp and Kluesener (1992) listed the following different manufacturing processes for monoglycerides:

1. *Esterification or transesterification of isopropylidene glycerol*. Isopropylidene glycerol is the condensation product of acetone and glycerol and has only one (primary) hydroxyl group left. When this adduct or its precursors is/are heated in the presence of a triglyceride oil and a catalyst, such as *p*-toluenesulfonic acid, the free hydroxyl group is esterified by ester interchange. This ester interchange causes the triglyceride oil to lose fatty acid moieties and form a monoglyceride itself, which is then protected against further degradation by reaction with acetone (Reiser and Isbell, 1971). The resulting fatty acid esters of isopropylidene glycerol are converted into monoglycerides by treating them with an aqueous solution of sodium bisulfate.
2. According to Dalby (1966), glycidol (oxiranemethanol) can be made to react with organic acids whereby the free hydroxyl group is esterified and the epoxy group is simultaneously hydrolysed to a vicinal diol (Figure 4.24). The use of solvents, such as aromatic hydrocarbons, such as toluene and ketones, has been described by Kleemann et al. (1978).
3. *Esterification of glycerol with fatty acids.* In 1948, Wocasek and Koch reported that fluorides catalyse the direct esterification of fatty acids with glycerol. Zinc, cobalt, and cobalt fluorides were found to be outstanding. Perhaps this inspired Miller and Mais (1970) to use anhydrous hydrogen fluoride as a catalyst. They used far more than necessary, since the preferred range is 90 to 110 mol HF per mol of acid. Perhaps this is the reason why they obtained quite pure monoglycerides.
4. *Enzymatic hydrolysis of triglycerides.* The use of a 1,3-specific lipase leads to the formation of β-monoglycerides and as long as this reaction product does not isomerise, it will not be further hydrolysed (Holmberg and Osterberg, 1988). In addition to the enzymatic hydrolysis, Bornscheuer (1995) also describes the enzymatic esterification.
5. *Enzymatic esterification of glycerol.* The lipase from *Penicillium cyclopium* (Yamaguchi et al., 1993) has a strong preference for partial glycerides. It can be used to purify a glyceride mixture by hydrolysing the partial glycerides, but it can also be used to synthesize partial glycerides from free fatty acids and glycerol without forming triglycerides. Accordingly, reasonably pure monoglycerides can be formed and their diglyceride content can be controlled by choosing the appropriate reaction conditions. Use of the lipase from *Rhizopus* sp. leads to the production of pure diglycerides at 40°C, but at 20°C, monoglycerides are the main product (Weiss, 1990).
6. *Glycerolysis of triglyceride oils.* This is the process used in industry and accordingly, a fair amount has been published about it when it was still being developed; nowadays, biotechnological processes attract more attention.

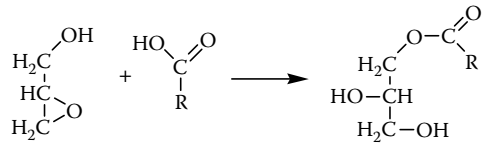


FIGURE 4.24 Monoglyceride synthesis from glycidol and FFA.

Just as the hydrolysis of triglycerides takes place in the organic phase and hardly at all in the aqueous phase or at the interphase (Lascaray, 1939), the glycerolysis of triglycerides also takes place in the phase formed by the starting materials (triglycerides and glycerol) and their reaction products, the partial glycerides. Accordingly, equilibrium will be established between the various reaction partners and its composition depends solely on the proportions of fatty acid and glycerol moieties present.

These proportions will change as the glycerolysis proceeds, since glycerol is more soluble in partial glycerides than in triglycerides. Besides, the solubility of glycerol depends very much on the temperature of the reaction mixture (Feuge and Bailey, 1946).

Feuge and Bailey (1946) also proposed calculating the composition of the homogenous reaction mixture by assuming random distribution of fatty acid and hydroxyl moieties, but this assumption was refuted (Demarcq, 1956) on the grounds that Feuge and Bailey had overlooked the presence of β-monoglycerides in the reaction mixture. Soon after, Brandner and Birkmeier (1960; 1964) introduced the partial glyceride isomers into the calculation of the reaction mixture composition, and also made a distinction between primary and secondary hydroxyl groups or esters. Analytical difficulties, such as the isomerisation of monoglycerides during the determination of the 1-isomer (Köhler et al., 1993) and the accurate determination of the diglyceride isomers prevented them from fully validating their approach, but subsequent literature data (De Groot, 1974; Yang et al., 2004) appear to support this approach.

Accordingly, the free energy difference favouring the esterification of the primary hydroxyl over the secondary hydroxyl is some 4 kJ/mol, and this value is hardly affected by which other groups are present in the glycerides. This means that the K values introduced by Brandner and Birkmeier can be calculated as a function of temperature, and that the composition of the glycerolysis reaction mixture, including the isomer distribution of the partial glycerides in this mixture, then also can be calculated. Weighting factors to be used in this calculation have been listed in Table 4.28.

This calculation is limited to the liquid phase. If partial glycerides are partially solidified and an isomerisation catalyst is present, a kind of "directed isomerisation" can lead to increased amounts of the least soluble component. In this way, reasonably pure symmetrical diglycerides can be prepared (Harwood, 1972; De Groot, 1974; Volpenhein, 1980). Commercial interest in these symmetrical diglycerides stems from their being precursors for synthetic symmetrical monounsaturated triglycerides to be used as cocoa butter equivalents.

Monoglycerides stored at ambient temperature will also isomerise, as a result of which the 1-monoglyceride content increases to 95 to 97% (Krog, 2001). Because the solubility of glycerol in the glycerolysis reaction mixture increases with the temperature, the maximum monoglyceride content of this mixture also increases as the temperature rises. At 245°C it reaches some 55% by weight based on the fatty matter in the equilibrium mixture (Noureddini and Medikonduru, 1997). Aiming at a higher content by increasing the temperature is hardly possible because this will lead to side-reactions and colour formation. Nevertheless, a high (270 to 295°C) temperature process has been described (Alsop and Krems, 1963) in which this temperature is maintained for a maximum of 3 min. This leads to an equilibrium containing at least 85% monoglycerides, and less than 15% diglycerides. The product is then quench-cooled to below 100°C to freeze the equilibrium. Two liquid phases are then formed that can easily be separated.

As indicated by Sonntag (1982), who also provides a summary of the pertinent patent literature, higher contents have also been achieved by the use of a solvent in which both glycerol and glycerides will dissolve. The solvents he mentions are phenol or cresol (Hilditch and Rigg, 1937), dioxane (Richardson, 1941; Richardson and Eckey, 1941), and pyridine (Mattil and Sims, 1952; Franzke and Kretzschmann, 1963; see also Emtir et al., 1995; Cheng et al., 2005).

Another solvent mentioned by Sonntag is carbon dioxide and he quotes an Indian patent (Kochhar and Bhatnagar, 1962) claiming its use and up to 90% monoglycerides in the reaction mixture. Among the advantages he lists are the use of water as the catalyst that obviates the need to add a catalyst, which then has to be neutralised and removed, and the ease of solvent removal. Accordingly, he cannot but express his surprise that no greater use is made of this process. Therefore, but quite some time later, Temelli et al. (1996) studied the glycerolysis reaction in supercritical carbon dioxide in a systematic manner. Using water as catalyst leads to FFA formation and the maximum monoglyceride content they observe is always <50% by weight.

Current industrial monoglyceride production processes using the glycerolysis equilibrium employ a basic catalyst,

TABLE 4.28 Weighting factors to be used in calculating glycerolysis equilibrium composition

Reaction Partner	Number of Primary Ester Bonds Per Reaction Partner	Coefficients	
		Per Isomer	Per Group
triglyceride	2		α^2
1,3-diglyceride	2	α^2	α^2
1,2-diglycerides	1	α	$2\,\alpha$
total diglycerides			$\alpha^2 + 2\,\alpha$
1-monoglycerides	1	α	$2\,\alpha$
2-monoglyceride	0	1	1
total monoglycerides			$2\,\alpha + 1$
glycerol	0		1

such as anhydrous caustic soda (0.01.0.2%), which can easily be dissolved in the glycerol. Older literature may recommend calcium oxide because this catalyst was felt to lead to monoglycerides with improved colour. However, the use of rayon grade sodium hydroxide with low iron content also leads to a light colour. Moreover, the catalyst has to be inactivated before the temperature is lowered and this is commonly done by adding an acid. Since calcium phosphate salts are more difficult to remove by filtration than sodium salts, the use of calcium oxide has become obsolete (Hasenhuettl, 2000).

Lowering the temperature reduces the solubility and, thus, the concentration of the glycerol in the reaction mixture, and if this were still catalytically active, the reaction would revert and decrease the monoglyceride yield. An acid, such as phosphoric acid, is commonly used to inactivate the catalyst. A first amount of glycerol can then be removed as liquid glycerol by settling, but residual amounts have to be removed by water washing, which entails a concentration step, or by vacuum distillation (for a complete flowsheet, see Kuhrt, 1953).

Mixtures of mono- and diglycerides with 40 and 60% monoglycerides are articles of commerce, but for certain applications, higher monoglyceride concentrations are deemed to be desirable. Consequently, the monoglycerides have to be isolated and a number of processes have been suggested for this purpose. The process used industrially since the early 1960s employs molecular distillation at a temperature of some 200°C. A totally different approach is taken in the continuous adsorption/desorption process (Sorbex®, as developed by UOP) presented as an emerging technology (Gembicki et al., 1986). In addition to the isolation of monoglycerides from a mixture with diglycerides, this poster also showed separation of oleic acid from triolein and the separation of various fatty acids from each other.

Liquid–liquid extraction systems have also been studied from quite early on. Goldsmith (1953) dissolves the partial glycerides in an alkane and extracts this solution preferably in counter-current mode with an aqueous alcohol, but immediate extraction of the partial glycerides with aqueous methanol is also possible (Kuhrt and Welch, 1955). Miller (1956) proposed the use of subcritical propane as an extraction solvent and used the temperature as a means to control the solubility of the various components. The process has probably not been used industrially since soon after molecular distillation was introduced.

In Example 2 of their patent (1982), Peter et al. describe a separation process for the separation of the glycerolysis reaction mixture using carbon dioxide at a pressure of 13.5 MPa and acetone as an entrainer, in which they obtain 95% pure monoglycerides. The sump product contains only 5% monoglycerides, which contributes to an excellent yield. Subsequently, the use of mixtures of carbon dioxide and a hydrocarbon, such as propane, was described (Peter et al., 1992) and finally the extraction process was switched to hydrocarbons alone (Peter et al., 1995).

A hypothetical plant using a different extraction system has been described by Kolstad et al. (1999). In this plant, the glycerol is removed from the glycerolysis reaction mixture by cooling, decantation, and finally steam stripping. This glycerol-free mixture is mixed with triglycerides and then subjected to a counter-current extraction system employing aqueous alcohol (this solvent was also used by Feuge and Gros, 1950). This generates an extract that is enriched in monoglycerides but still contains 12% by weight of diglycerides and almost 7% triglycerides. Accordingly, it is again mixed with triglycerides and extracted with aqueous alcohol, which produces a second extract that is sufficiently free of diglycerides. Alcohol is removed from the raffinate by water washing and from the extract by distillation so that the washing water can be combined with the distillate and used again. As in the other processes mentioned above, the di- and triglycerides are recycled into the glycerolysis reaction mixture.

Monoglycerides can be used as such, but can also serve as a starting material for substituted monoglycerides. If the intended application requires that the fatty acids in the monoglycerides be saturated, it is common to hydrogenate their starting materials, presumably because a saturated feedstock is less sensitive to the high temperature glycerolysis process. Triglycerides are the preferred starting material since this calls for one process step fewer than using fatty acids in the direct esterification of glycerol (Hasenhuettl, 2000).

4.5.2.2 Substituted monoglycerides

Whereas commercial monoglycerides can be defined by their diglyceride content, their fatty acid composition and, if necessary, by their isomer ratio, substituted monoglycerides cannot be characterised that clearly. Controlling the properties of the starting materials, adhering to set process conditions and hoping for the best, are the most effective ways of arriving at products that perform as they should, that is to say as the previous batch did and as the customer expects them to behave. The advice reading: "Meters or scales used to dispense reactants should be accurate in order to produce consistent quality surfactants" (Hasenhuettl, 2000) is highly characteristic of this industry.

Substituted monoglycerides are very much proprietary products and lactic acid esters of monoglycerides produced by one company may well perform quite differently from the same product originating from another manufacturer. The products being proprietary also means that little has been published about the manufacturing processes. Reverse engineering, or deducing how to make a product by analysing it, is virtually impossible for substituted monoglycerides. Moreover, the performance know-how is also proprietary.

Substituted monoglycerides will be discussed in the order in which they have been listed in Table 4.22, except that acetoglycerides will be discussed separately in the next section.

4.5.2.2.1 Lactic acid esters of monodiglycerides

These surfactants are usually made by allowing 15 to 35% lactic acid to react with (distilled) monoglycerides (Krog, 2001) under conditions that are conducive to water removal, say a temperature of 150 to 170°C and an absolute pressure of 30 mbar. However, before being removed, the water may hydrolyse the ester bond in monoglycerides under formation of free fatty acids. These may then form esters with the hydroxyl group of lactic acid moieties, form diglycerides, etc. (Stauffer, 2002). Accordingly, the final product composition depends very much on the rates of water formation and water removal (Lauridsen, 1976).

4.5.2.2.2 Citric acid esters of monodiglycerides

According to Krog (1997; 2001) these products "are produced by reacting citric acid in an amount of 12 to 20% by weight of the final product with monodiglycerides or distilled monoglycerides." A patent example (Lauridsen and Kristensen, 1971, Example 8) gives more details: "125 g of anhydrous citric acid were reacted for ½ hour at 130°C and 20 mmHg with 500 g of fully hardened lard monoglyceride (90% α-monoglyceride); the mole proportion of acid to glyceride was 0.43:1. The product formed hereby had acid number 131 and was not soluble in oil. It was caused to react further at 20 mmHg, first at 160°C for 2 hours and subsequently at 180°C for 1 hour, whereby a product was obtained which had acid number 18. It had considerably higher viscosity than the intermediate product and therefore a higher molecular weight."

4.5.2.2.3 Tartaric acid esters of monodiglycerides

Although allowed in Europe, this substituted monoglyceride is hardly used. It can presumably be manufactured by reacting monoglycerides with tartaric acid, just as citric acid esters of monoglycerides are made by reacting these monoglycerides with citric acid.

4.5.2.2.4 Mono- and diacetyl tartaric acid esters of monodiglycerides

Because diacetyl tartaric acid esters of monoglycerides (DATEMs) are the most important substituted monoglycerides in volume and commercial value (Krog, 2001), they received somewhat more attention in the literature than, for instance, the nonacetylated product mentioned above. Moreover, there are/were two products containing acetyl, tartaric acid, fatty acid, and glycerol moieties with different E-numbers (E472e and E472f); this may stem from the way they were manufactured.

Such a product can be made by first of all reacting tartaric acid with acetic acid anhydride and removing the by-product acetic acid, so that diacetyl tartaric acid anhydride is formed, and then allowing this product to react with monoglycerides (Lauridsen and Kristensen, 1971). This would lead to a totally different product composition than that which results when monoglycerides, tartaric acid, and acetic acid or acetic acid anhydride are allowed to react, even if the molar ratios of the various moieties were the same in both instances.

4.5.2.2.5 Succinic esters of monodiglycerides

Allowing monoglycerides to react with succinic acid anhydride leads to the formation of the various positional isomers of this substituted monoglyceride (Stauffer, 2002). A bland tasting product can be prepared by maintaining the temperature of the reaction mixture above the melting point of the monoglycerides but below 119°C, i.e., the melting point of succinic acid anhydride (Freund, 1966). The product consists mainly of the half-ester of succinic acid and of monoacylated glycerol.

4.5.2.2.6 Ethoxylated monodiglycerides

Ethoxylated monoglycerides are produced by the ethoxylation (treatment with ethylene oxide gas under pressure) of monoglycerides under alkaline catalysis (Lauridsen, 1976). In monoglycerides, the 1-hydroxy group is somewhat more reactive than the 2-hydroxy group, but both will form ether linkages with the polymerised ethylene oxide, though not to the same extent. To quote Stauffer (2002) once again: "The exact distribution of polymer chain lengths and distribution between and positions are functions of reaction conditions (e.g., catalyst type and concentration, gas pressure, temperature, agitation, and length of reaction time). Additionally, of course, any diglyceride present may also be ethoxylated.

4.5.2.2.7 Malic acid esters of mono- and diglycerides

These emulsifiers have been developed as an anti-spattering agent for margarines (Houben and Jonker, 1961a ; 1961b), but no EC number has been allotted to this type of emulsifier. In all likelihood, no number has been applied for because in the early 1960s emulsifiers of this type were linked to the development of skin rash and blisters on epidemiological grounds. Although subsequent research failed to establish a direct causal relationship between emulsifier and symptoms, nevertheless, its use in food is not recommended.

4.5.2.3 Acetoglycerides

Acetylated monoglycerides or "acetoglycerides" are used as α-tending emulsifiers, but because of their film-forming properties, they also have totally different applications: coatings for fruits, cheese (Woldhuis et al., 1990; Mikkelsen et al., 1995), and sausages (Seguin, 1984). They were described for the first time in a patent granted to Normann (1925) who interesterified tristearin with triacetin (1,2,3-propanetriol triacetate) by heating the mixture for 72 hours to 200 to 250°C. He noted that

the reaction product was no longer brittle but more wax-like.

At about the same time, Schwartz (1925) listed various different syntheses for acetoglycerides based on coconut oil:

- Heating glycerol, glacial acetic acid, and coconut oil, removing the excess acetic acid by distillation, and raising the temperature to 180 to 200°C while stripping out the water formed.
- As above, but with some sulfuric acid as a catalyst, that permits a lower working temperature. This catalyst can be neutralised with sodium acetate.
- Acidolysis of coconut oil by glacial acetic acid under pressure, removal of any free acetic acid followed by the esterification of the free coconut fatty acids by partial acetins at 180 to 200°C, with a stream of nitrogen to remove water as it is formed.
- Sulfuric acid catalysed acidolysis of coconut oil by glacial acetic acid followed by the addition of glycerol, which may also be added at the start of the reaction, and removal of the volatiles.
- Heating coconut oil with triacetin to 180 to 200°C.
- Heating this reaction mixture after addition of a catalytic amount of sulfuric acid and washing out this acid with water.

When it was found that acetoglycerides with only one long-chain fatty acid had practical advantages, a process employing molecular distillation was developed to isolate such acetoglycerides from an interesterification mixture of a triglyceride with three long chains and triacetin (Baur, 1952). When aiming at acetoglycerides with only one long fatty acid chain per molecule, which also contain free hydroxyl groups, glycerol was included in the interesterification mixture; isolation of the intended product was again by molecular distillation (Baur, 1954; Embree and Brokaw, 1956).

Also around that time, Feuge et al. (1956) introduced acylating agents, such as acetic acid anhydride, which they allowed to react with monoglycerides. The extent of acetylation can be controlled through the amount of anhydride, and the purity of the monoglycerides determines what proportion of glycerides contains two long-chain fatty acids. The acetylation reaction takes place at 120°C (Krog, 2001) and the free acetic acid formed is removed by vacuum distillation/stripping. Because the interesterification reaction mixture produced by mixing long-chain triglycerides, acetic acid, and glycerol allows more glycerol to dissolve than is possible in the glycerolysis process for the production of monoglycerides, and since this solubility improves with the concentration of free hydroxyl groups, this interesterification mixture can attain higher contents of glycerides with only one long fatty acid chain. Consequently, this led to a simple process for the production of acetoglycerides with a low content of triglycerides with more than one long-chain fatty acid and with a controlled content of free hydroxyl groups (Dijkstra et al., 2006).

4.5.3 Other fatty emulsifiers and surfactants

The food grade surfactants and emulsifiers listed in Table 4.22 also comprise products that are neither lecithin- nor monoglyceride-based. These products will be discussed in this section in the order in which they have been listed in Table 4.22 and in groups of related compounds.

4.5.3.1 Polysorbates (E432-437) and sorbitan stearates (E491-492)

These two groups of surfactants are discussed together in the present subsection since they are both sorbitan-based. Sorbitan is a dehydration product of sorbitol (D-glucitol) and this latter product is produced industrially by high pressure (14 MPa) hydrogenation of an aqueous solution of glucose at some 180°C, while using a nickel phosphate catalyst (Capik and Wright, 1972). On dehydration, various isomers are formed, such as 1,4-, 1,5- and 2,5-sorbitan and isosorbide, a compound with two rings (Bognolo, 1997).

Polysorbates form a group of surfactants that can be described as polyoxyethylene sorbitan esters of fatty acids. Each of the sorbitan isomers has two or four free hydroxyl groups left, so that on esterification with fatty acids and subsequent etherification with ethylene oxide, a vast number of different compounds results. Sorbitan esters are often referred to by their trademark name Span®. They share a common nomenclature with the sorbitan esters (referred to as Tween®), which is summarised in Table 4.29.

Sorbitan monoesters, such as the monostearate (E491), are produced by heating sorbitol with, for example, stearic acid (Brown, 1943) at elevated temperatures (up to 265°C). The inner ether bond and the ester bond are formed more or less simultaneously, whereby acids preferentially catalyse the self-condensation leading to the cyclic ethers that are characteristic of sorbitans; a caustic catalyst can then be used for the esterification (Stockburger, 1981). Using a large proportion of fatty acids with respect to the sorbitol leads to an increased degree of esterification and eventually the formation of triacylsorbitan (Krog, 1997). The ethoxylated polysorbates are prepared either by reacting a preformed polyethylene glycol with a fatty ester of sorbitan, but preferably by reacting this ester with ethylene oxide (Griffin, 1945).

4.5.3.2 Ammonium phosphatide

Adding lecithin to molten chocolate reduces its viscosity and allows moulded goods to be made with thinner walls

TABLE 4.29 System of nomenclature for sorbitan esters

Product Description	Number
sorbitan monolaurate	20
sorbitan monopalmitate	40
sorbitan monostearate	60
sorbitan tristearate	65
sorbitan mono-oleate	80

and less chocolate to be used in enrobing biscuits. Alternatively, it permits savings on cocoa butter in the chocolate itself. However, soya bean lecithin may give chocolate an off-taste. Therefore, a kind of synthetic phosphatide has been developed. It is made by partial glycerolysis of a fat or oil, allowing the partial glycerides (mainly diglycerides) to react with phosphorus pentoxide, and neutralisation of the phosphoric ester with ammonia (Harris and Bradford, 1966). The product is often referred to as YN.

4.5.3.3 Soaps (E470a–470b)

Various soaps, such as the sodium, potassium, and calcium salts of fatty acids (E470a) and their magnesium salts (E470b), may be used in foods. They can be made in a saponification process, but then the reaction mixture also contains glycerol. Producing them by allowing a base to react with a fatty acid has the advantage that the acids can be purified beforehand.

4.5.3.4 Fatty acid esters of sucrose (E473) and sucroglycerides (E474)

Fatty acid polyesters of sucrose containing six or more fatty acid moieties are also known as Olestra®, a fat replacer invented by Mattson (1976), for which Rizzi and Taylor invented a method of synthesis (1976). This method involves heating saccharose with FAME and an alkali met al soap to ensure homogeneity and a more strongly basic catalyst, such as alkali met al alcoholates. Since soap removal from the final product was found to be difficult, an improved process entails gradual soap removal by centrifuge (Grechke et al., 1992). Accordingly, this latter process is highly suitable for the production of partially esterified sucrose, containing only one to three fatty acid moieties. Solvents like DMSO can also be used, but it is difficult to remove this kind of solvent completely from the product (Krog, 2001).

According to Stauffer (2002), fatty acid esters of sucrose are made by preparing an emulsion of FAME and sucrose syrup and dehydrating this emulsion under vacuum and at elevated temperatures. After the addition of an alkaline catalyst, the temperature is raised to 150°C while the methanol is distilled off and, finally, the reaction mixture is cooled and purified.

Sucroglycerides are made by transesterification of triglycerides and sucrose in the presence of an alkaline catalyst (Hasenhuettl, 2000); they are a mixture of fatty acid esters of sucrose and monoglycerides (Krog, 1997).

4.5.3.5 Fatty acid esters of polyglycerol (E475)

These emulsifiers are made by first polycondensing glycerol while using an alkaline catalyst (Babayan and Lehman, 1972). Subsequently, the polyglycerol formed is esterified with fatty acids at about 230°C under vacuum or subjected to a transesterification process with triglycerides with soap as a catalyst (Van Heteren et al., 1983). Monoesters of diglycerol can be isolated from the reaction mixture by molecular distillation and are more surface active than corresponding monoglycerides (Stauffer, 2002).

4.5.3.6 Polyglycerol polyricinoleate (E476)

Like the fatty esters of polyglycerol, the polyricinoleate esters of polyglycerol are also made by first of all condensing glycerol. Heating ricinoleic acid (12-hydroxy oleic acid) on its own causes this acid to polycondense by forming ester bonds between the hydroxyl groups and the acid groups in the ricinoleic acid molecule. The polyricinoleic acid will have free carboxyl groups left, which can then esterify remaining hydroxyl groups in the polyglycerol (Stauffer, 2002).

4.5.3.7 Fatty acid esters of propylene glycol (E477)

The direct esterification of propylene glycol yields products that typically consist of 55 to 60% monoester with the remainder being diester. The monoester can be isolated from this product by molecular distillation, but often the extra cost is not warranted (Krog, 2001). They can also be prepared by interesterifying triglycerides with propylene glycol, but then the reaction product will also contain some monoglycerides (Stauffer, 2002).

4.5.3.8 Stearoyl lactate emulsifiers (E481)

When lactic acid is heated with stearic acid in the presence of, for instance, sodium hydroxide, the hydroxyl group in the lactic acid is esterified by the stearic acid and also by the lactic acid itself. Under the proper reaction conditions, the main product will be the stearic acid of monolactic acid, but fatty acid esters of dilactic acid and further oligomers of lactic acid will also be present. Neutralising the acid with more caustic soda yields sodium stearoyl lactate and the calcium salt results from neutralisation with the appropriate calcium compound.

References

Aalrust, E. et al. (1993), Enzymatic treatment of edible oils, US Patent 5,264,367, assigned to Röhm GmbH and Metallgesellschaft AG.

Adhikari, S. and Adhikari, J. (1986), Indian ricebran lecithin, *J. Am. Oil Chem. Soc.*, **63**, 13671369.

Adlercreutz, D. and Wehtje, E. (2004), An enzymatic method for the synthesis of mixed-acid phosphatidylcholine, *J. Am. Oil Chem. Soc.*, **81**, 553–557.

Als, G. and Krog, N. (1991), Emulsifiers as food processing aids, in *World Conference on Oleochemicals into the 21st Century*, Applewhite, T.H., Ed., AOCS Press, Champaign, IL, 67–73.

Alsop, W.G. and Krems, I.J. (1963), Process for the preparation of higher fatty acid monoglycerides, US Patent 3,080,216, assigned to Colgate-Palmolive Company.

Aneja, R. (1970), Process for separating phosphatides, British Patent 1 217 846, assigned to Unilever.

Aneja, R. et al. (1971), A process for the separation of phosphatide mixtures: the preparation of phosphatidylethano-

lamine-free phosphatides from soya lecithin, *Fette Seifen Anstrichm.*, **73**, 643–651.

Aneja, R. and Chadha, J.S. (1974), Phosphatide separation, British Patent 1 350 390, assigned to Unilever.

Babayan, V.K. and Lehman, H. (1972), Process for preparation and purification of polyglycerols and esters thereof, US Patent 3,637,774.

Baur, F.J. (1952), Mixed triglycerides, US Patent 2,615,160, assigned to The Procter & Gamble Company.

Baur, F.J. (1954), Acetin fats. II. Preparation and properties of diacetin fats from some common vegetable oils, *J. Am. Oil Chem. Soc.*, **31**, 196–199.

Betzing, H. (1980), Process to obtain oily, highly purified phosphatidylcholines, US Patent 4,235,793, assigned to A. Nattermann & Cie. GmbH.

Betzing, H. and Eikermann, H. (1970), Process for obtaining highly purified phosphatidylcholine and the product of this process, US Patent 3,555,605, assigned to A. Nattermann & Cie, GmbH.

Bognolo, G. (1997), Nonionic surfactants, in *Lipid Technologies and Applications*, Gunstone, F.D. and Padley, F.B., Eds., Marcel Dekker, New York, 633–694.

Bollmann, H. (1923), Verfahren zur Gewinnung des durch Auslaugen von Ölsaaten oder deren Preßkuchen mit einem Gemisch aus Alkohol und Benzol oder Benzin erhaltenen Lecithins, German Patent 382912.

Bollmann, H. (1930), Process for the purification of phosphatides, US Patent 1,776,720.

Bollmann, H. (1931), Improvements in and relating to the production and purification of phosphatides, British Patent 356,384.

Bornscheuer, U.T. (1995), Lipase-katalysierte Synthese von Monoglyceriden, *Fat Sci. Technol.*, **97**, 241–249.

Brandner, J.D. and Birkmeier, R.L. (1960), Relative esterifiability of the primary and secondary hydroxyl groups of glycerol, *J. Am. Oil Chem. Soc.*, **37**, 390–396.

Brandner, J.D. and Birkmeier, R.L. (1964), The equilibrium distribution of acyl groups between primary and secondary hydroxyl positions in partial esters, *J. Am. Oil Chem. Soc.*, **41**, 367–370.

Brian, R. (1976), Soybean lecithin processing unit operations, *J. Am. Oil Chem. Soc.*, **53**, 27–29.

Brown, K.R. (1943), Monoesters of inner ethers of hexahydric alcohols, US Patent 2,322,820, assigned to Atlas Powder Company.

Buer, C.H. (1936), Process for the manufacture of stable preparations having a high content of commercial lecithin, US Patent 2,064,727.

Burkhardt, H.J. (1971), Phosphatides isolated from seeds of commercial and experimental safflower varieties, *J. Am. Oil Chem. Soc.*, **48**, 697–699.

Capik, R.J. and Wright, L.W. (1972), Preparation of polyhydric alcohols from carbohydrates, US Patent 3,670,035, assigned to Atlas Chemical Industries, Inc.

Cheng, S.F. et al. (2005), Rapid synthesis of palm-based monoacylglycerols, *J. Am. Oil Chem. Soc.*, **82**, 791–795.

Cherry, J.P. et al. (1981a), A review of lecithin chemistry and glandless cottonseed as a potential commercial source, *J. Am. Oil Chem. Soc.*, **58**, 903–913.

Choo, Y.M. et al. (2004), Phospholipids from palm-pressed fiber, *J. Am. Oil Chem. Soc.*, **81**, 471–475.

Clausen, K. (2001), Enzymatic oil-degumming by a novel microbial phospholipase, *Eur. J. Lipid Sci. Technol.*, **103**, 333-340.

Cole, R.D. (1959), Hydrogenated lecithin and process for preparing same, US Patent 2,907,777, assigned to The Glidden Company.

Copeland, R. and Belcher, W.M. (2001), Methods for refining vegetable oils and byproducts thereof, US Patent 6,172,247 B1, assigned to IP Holdings, L. L. C.

Dailey, J.P. et al. (1959), Improvements in and relating to soybean phosphatide emulsifiers, British Patent 825,936, assigned to Armour and Company.

Dalby, G. (1966), Method of forming monoglycerides of carboxylic organic acids, US Patent 3,251,870, assigned to Benj. T. Rauber.

Davis, P.F. (1962), Process for the hydrogenation of phosphatides, US Patent 3,026,341, assigned to Central Soya Company, Inc.

Davis, P.F. (1967a), Fluidized lecithin composition and method, US Patent 3,357,918, assigned to Central Soya Company, Inc.

Davis, P.F. (1967b), Process of phosphatide preparation, US Patent 3,301,881, assigned to Central Soya Company, Inc.

Davis, P.F. (1970), Alkaline hydrolyzed phosphatides, US Patent 3,499,017, assigned to The Central Soya Company, Inc.

Davis, P.F. (1971), Acid hydrolyzed phosphatides, US Patent 3,576,831, assigned to The Central Soya Company, Inc.

Davis, P.F. and Fellow, M.A. (1961), Method for preparing a granular oil-free phosphatide product, US Patent 3,012,888, assigned to Central Soya Company, Inc.

De Kock, J. (1991), Process for the supercritical solvent extraction of a mixture of phosphatides, European Patent 431 709, assigned to N.V. Vandemoortele International.

Demarcq, M. (1956), Les esters d'acides gras, *Rev. Franç. Corps Gras*, **3**, 746–763.

Dijkstra, A.J. and De Kock, J. (1990), Process for fractionating phosphatide mixtures, European Patent 372 327, assigned to N.V. Vandemoortele International.

Dijkstra, A.J. and Van Opstal, M. (1986), Process for producing degummed vegetable oils and gums of high phosphatidic acid content, European Patent 0 195 991, assigned to N.V. Vandemoortele International.

Dijkstra, A.J. and Van Opstal, M. (1989), The Total Degumming Process, *J. Am. Oil Chem. Soc.*, **66**, 1002–1009.

Dijkstra, A.J. et al. (2006), Process for preparing an acetoglyceride composition comprising high amounts of monoacetyl monoacyl glycerides, PCT Patent Application WO 2006/091096 A2, assigned to Paramelt B.V.

Doig, S.D. and Diks, R.M.M. (2003a), Toolbox for exchanging constituent fatty acids in lecithins, *Eur. J. Lipid Sci. Technol.*, **105**, 359–367.

Doig, S.D. and Diks, R.M.M. (2003b), Toolbox for modification of the lecithin headgroup, *Eur. J. Lipid Sci. Technol.*, **105**, 368–376.

Drozdowski, B. and Zajac, M. (1977), Effect of concentration of some nickel catalyst poisons in oils on the course of hydrogenation, *J. Am. Oil Chem. Soc.*, **54**, 595–599.

Eichberg, J. (1939), Lecithin — its manufacture and use in the fat and oil industry, *Oil & Soap*, **16**, 51–54.

Eichberg, J. (1961), Process of modifying phosphatides, US Patent 2,983,612, assigned to American Lecithin Company Inc.

Eichberg, J. (1967), Lecithin product and method, US Patent 3,359,201, assigned to American Lecithin Company Inc.

Embree, N.D. and Brokaw, G.Y. (1956), Method of making mixed diglyceride, US Patent 2,764,605, assigned to Eastman Kodak Company.

Emtir, M.M. et al. (1995), Kinetics of esterification reaction between glycerol and oleic acid in the presence of pyridine, *Fat Sci. Technol.*, **97**, 347–351.

Feuge, R.O. and Bailey, A.E. (1946), Modification of vegetable oils VI. The practical preparation of mono- and diglycerides, *Oil & Soap*, **23**, 259–264.

Feuge, R.O. and Gros, A.T. (1950), Modification of vegetable oils. IX. Purification of technical monoglycerides, *J. Am. Oil Chem. Soc.*, **27**, 117–122.

Feuge, R.O. and Lovegren, N.V. (1956), Dilatometric properties of some butyropalmitins, butyrostearins and acetopalmitins, *J. Am. Oil Chem. Soc.*, **33**, 367–371.

Flider, F.J. (1985), The manufacture of soybean lecithins, in *Lecithins*, Szuhaj, B.F. and List, G.R., Eds., AOCS, Champaign, IL, 21–37.

Franzke, C. and Kretzschmann, F. (1963), Zur Darstellung von Monoglyceriden aus natürlichen Fetten, *Fette Seifen Anstrichm.*, **65**, 275–277.

Freund, E.H. (1966), Manufacture of succinyl monoglyceride, US Patent 3,293,272, assigned to National Dairy Product Company.

Gembicki, S.A. et al. (1986), Sorbex separations: A key to new product opportunities, in *World Conference on Emerging Technologies in the Fats and Oils Industry*, Baldwin, A.R., Ed., American Oil Chemists' Society, 380–382.

Goh, S.H. et al. (1982), Phospholipids of palm oil *(Elaeis guineensis)*, *J. Am. Oil Chem. Soc.*, **59**, 296–299.

Goldsmith, H.A. (1953), Liquid-liquid extraction process, US Patent 2,651,646, assigned to Colgate-Palmolive Company.

Grechke, Y.M. et al. (1992), Process for the synthesis of polyol fatty acid polyesters, US Patent 5,079,355, assigned to Unilever.

Griffin, W.C. (1945), Emulsions, US Patent 2,380,166, assigned to Atlas Powder Company.

Groot, W.T. de (1974), Isomerization of 1,2-diglycerides to 1,3-diglycerides, US Patent 3,845,087, assigned to Unilever.

Günther, B.R. (1984), Process for the separation of acylated phospholipids from phosphatidylcholine products containing the same, US Patent 4,443,378, assigned to A. Nattermann & Cie, GmbH.

Günther, B.-R. (1985), Method for the preparation of phosphatidylcholine of low oil content, US Patent 4,496.486, assigned to A. Nattermann & Cie. GmbH.

Haas, M.J. et al. (1994), Enzymatic phosphatidylcholine hydrolysis in organic solvents: an examination of selected commercially available lipases, *J. Am. Oil Chem. Soc.*, **71**, 483–490.

Halden, W. (1940), Über den heutigen Stand der Phosphatidforschung I., *Fette u. Seifen*, **47**, 6–9.

Hara, F. and Nakashima, T. (1996), Transesterification of phospholipids by acetone-dried cells of a *Rhizopus* species immobilized on biomass support particles, *J. Am. Oil Chem. Soc.*, **73**, 657–659.

Harris, T.L. and Bradford, L. (1966), Phosphorylated glyceride derivatives for use as emulsifying agents, British Patent 1,032,465, assigned to Cadbury Brothers Limited.

Harwood, J. (1972), Process for manufacture of symmetrical glycerides, US Patent 3,634,473, assigned to SCM Corporation.

Hasenhuettl, G.L. (2000), Design and application of fat-based surfactants, in *Introduction to Fats and Oils Technology*, O'Brien, R.D., Farr, W.E., and Wan, P.J., Eds., AOCS Press, Champaign, IL, 515–541.

Heigel, W. and Hüschens, R. (1981), Process for obtaining pure lecithin which can be used directly for physiological purposes, British Patent 2 072 189, assigned to Kali-Chemie Pharma GmbH.

Hennessy, D.J. and Moshy, R.J. (1957), Processes of enhancing phosphatides and other organic materials, US Patent 2,791,594, assigned to American Lecithin Company Inc.

Heteren, J. van et al. (1983), Polyglycerol esters, EP Patent 0 070 080, assigned to Unilever.

Hilditch, T.P. and Rigg, J.G. (1937), Manufacture of glycerides of fatty acids, US Patent 2,073,797, assigned to ICI Ltd.

Holló, J. et al. (1993), Sunflower lecithin and possibilities for utilization, *J. Am. Oil Chem. Soc.*, **70**, 997–1001.

Holmberg, K. and Osterberg, E. (1988), Enzymatic preparation of monoglycerides in microemulsion, *J. Am. Oil Chem. Soc.*, **65**, 1544–1548.

Houben, G.M.M. and Jonker, E.W. (1961a), Improved margarine, British Patent 881,714, assigned to N.V. Koninklijke Stearine Kaarsenfabrieken "Gouda-Apollo."

Houben, G.M.M. and Jonker, E.W. (1961b), Margarine antispattering substance and method of manufacturing the same, US Patent 3,006,772, assigned to N.V. Koninklijke Stearine Kaarsenfabrieken "Gouda-Apollo."

Hutton, K.J. and Guymon, J.S. (2000), Process for producing deoiled phosphatides, US Patent 6,140,519, assigned to Archer-Daniels-Midland Company.

Iwasaki, Y. et al. (2003), An aqueous suspension system for phospholipase D-mediated synthesis of PS without toxic organic solvent, *J. Am. Oil Chem. Soc.*, **80**, 653–657.

J.D. Riedel AG (1913), Verfahren zur Darstellung von Hydrolecithin, German Patent 256998.

J.D. Riedel AG (1914a), Verfahren zur Darstellung von Hydrolecithin, German Patent 279200 and addition to German patent 256998.

J.D. Riedel AG (1914b), Verfahren zur Darstellung von Hydrolecithin, German Patent 280695 and addition to German patent 256998.

J.D. Riedel AG (1924a), Verfahren zur Darstellung von Hydrolecithin, German Patent 389299 and addition to German patent 389298.

J.D. Riedel AG (1924b), Verfahren zur Darstellung von Hydrolecithin, German Patent 389298.

Jacini, G. (1959), Hydrogenation of phosphatides, US Patent 2,870,179, assigned to American Lecithin Company Inc.

Julian, P.L. et al. (1953), Process of treating phosphatides and product, US Patent 2,629,662, assigned to The Glidden Company.

Juneja, L.R. et al. (1989), Enzymatic method of increasing phosphatidyl content of lecithin, *J. Am. Oil Chem. Soc.*, **66**, 714–717.

Kleemann, A. et al. (1978), Process for the production of high percentage carboxylic acid-1-monoglycerides, British Patent 1 517 349, assigned to Degussa AG and Henkel KGaA.

Klenk, E. et al. (1962), Process for the production of natural phospholipids and substances produced thereby, US Patent 3,031,478, assigned to A. Nattermann & Cie, GmbH.

Kochhar, R.K. and Bhatnagar, R.K. (1962), Indian Patent 71,979.

Kock, M. (1978), Procédé de préparation d'huile alimentaire à partir d'huile de soja brute, French Patent 2 391 272, assigned to Akzo N.V.

Kock, M. (1983), Oilseed pretreatment in connection with physical refining, *J. Am. Oil Chem. Soc.*, **60**, 150A-154A.

Köhler, M. et al. (1993), Acylwanderung bei 2-Glycerin-Monofettsäureestern, *Fat Sci. Technol.*, **95**, 18–19.

Kolstad, J.J. et al. (1999), Method for preparation of purified monoglycerides; and products, US Patent 5,859,270, assigned to Cargill Incorporated.

Krog, N. (1994), Surfactants and emulsifiers, in *The Lipid Handbook*, Gunstone, F.D., Harwood, J.L., and Padley, F.B., Eds., Chapman & Hall, 296304.

Krog, N. (1997), Food emulsifiers, in *Lipid Technologies and Applications*, Gunstone, F.D. and Padley, F.B., Eds., Marcel Dekker, New York, 521–534.

Krog, N. (2001), Food emulsifiers: Their production and application, in *Animal Carcass Fats*, Rossell, J.B., Ed., LFRA Limited, Leatherhead, U.K., 197–218.

Kuhrt, N.H. (1953), Process for substantially complete conversion of fatty material to partial ester, US Patent 2,634,278, assigned to Eastman Kodak Company.

Kuhrt, N.H. and Welch, E.A. (1955), Method of obtaining concentrated monoglycerides, US Patent 2,727,913, assigned to Eastman Kodak Company.

Lange, R. et al. (1994), Zusammensetzung, Präparation und Eigenschaften von Rapsphospholipiden, *Fat Sci. Technol.*, **96**, 169–174.

Lascaray, L. (1939), Der Reaktionsverlauf der Autoklavenspaltung der Fette, *Fette u. Seifen*, **46**, 628–632.

Lauridsen, J.B. (1976), Food emulsifiers: Surface activity, edibility, manufacture, composition, and application, *J. Am. Oil Chem. Soc.*, **53**, 400–407.

Lauridsen, J.B. and Kristensen, F. (1971), Emulsifier for water-in-oil emulsions and process for its preparation, British Patent 1 220 488, assigned to AS Grindstedvaerket.

Liebing, H.A. and Lau, J. (1976), Über ein Verfahren zur Gewinnung von Lecithin-Fraktionen, *Fette Seifen Anstrichm.*, **78**, 123–127.

Marmor, R.A. and Moyer, W.W. (1949), Preparation of phosphatides, US Patent 2,461,751, assigned to A.E. Staley Manufacturing Company.

Mattikow, M. (1953), Antispattering margarin, US Patent 2,640,780, assigned to Benjamin Clayton, doing business as Refining Unincorporated.

Mattil, K.F. and Sims, R.J. (1952), The glycerolysis of fat in tertiary aromatic nitrogeneous bases to increase monoglyceride yield, *J. Am. Oil Chem. Soc.*, **29**, 59–61.

Mattson, F.H. and Volpenhein, R.A. (1976), Pharmaceutical composition for inhibiting absorption of cholesterol, US Patent 3,954,976, assigned to The Procter & Gamble Company.

Meyer, C.E. et al. (1960), Phosphatide emulsifying agent and process of preparing same, US Patent 2,045,869, assigned to The Upjohn Company.

Mikkelsen, B. et al. (1995), Cheese coating composition and method for producing protective coatings on cheese, European Patent 0 679 337 A2, assigned to Danisco A/S.

Miller Jr., E.J. and Mais, A. (1970), Esterification, US Patent 3,551,464, assigned to Armour Industrial Chemical Company.

Miller, R. (1956), Refining crude fatty acid monoglyceride, US Patent 2,759,954, assigned to The Chemical Foundation, Incorporated.

Morgado, M.A.P. et al. (1996), Phospholipase A_2-catalyzed hydrolysis of lecithin in a continuous reversed-micellar membrane reactor, *J. Am. Oil Chem. Soc.*, **73**, 337–346.

Mulder, T.J. (1969), Method of coating fruits and vegetables with a thin film of lecithin and method of making lecithin emulsion for said purpose, US Patent 3,451,826, assigned to Lecifruit N.V.

Mustranta, A. et al. (1994), Transesterification of phospholipids in different reaction conditions, *J. Am. Oil Chem. Soc.*, **71**, 1413–1419.

Neiman, E.R. (1955), Lecithin composition and method of producing the same, US Patent 2,708,631, assigned to Sadie Neiman.

Nieuwenhuyzen, W. van (1976), Lecithin production and properties, *J. Am. Oil Chem. Soc.*, **53**, 425–427.

Normann, W. (1903), Process for converting unsaturated fatty acids or their glycerides into saturated compounds, British Patent 1 515, assigned to Herforder Maschinenfett-und Ölfabrik Leprince und Siveke.

Normann, W. (1925), Verfahren zur Umesterung von Fettsäureestern, German Patent 417 215, assigned to Oelwerke Germania G.m.b.H.

Noureddini, H. and Medikonduru, V. (1997), Glycerolysis of fats and methyl esters, *J. Am. Oil Chem. Soc.*, **74**, 419–425.

Pardun, H. (1962a), Isolierung und Reinigung von Sojalecithin, *Fette Seifen Anstrichm.*, **64**, 536–541.

Pardun, H. (1962b), Purification of phosphatides, US Patent 3,047,597, assigned to Unilever.

Pardun, H. (1964), Analytische Methoden zur Qualitätsbeurteilung von Sojalecithin, *Fette Seifen Anstrichm.*, **66**, 467–476.

Pardun, H. (1969), Process for the treatment of phosphatides, British Patent 1,174,399, assigned to Unilever.

Pardun, H. (1971a), Process for preparing modified plant lecithins, British Patent 1 229 430, assigned to Unilever.

Pardun, H. (1971b), Verfahren zur Extraktion von Pflanzenphosphatiden, German Patent application 1 692 568, assigned to Unilever.

Pardun, H. (1971c), Verfahren zur Herstellung von Phsophatidemulgatoren für Öl-in-Wasser- und Wasser-in-Öl-Emulsionen durch partielle Hydrolyse von natürlichen und modifizierten Pflanzenphosphatiden, German Patent 2 063 128, assigned to Unilever.

Pardun, H. (1972a), Phosphatide emulsifiers, US Patent 3,661,795, assigned to Unilever.

Pardun, H. (1972b), Preparation of phosphatides, US Patent 3,652,397, assigned to Unilever.

Pardun, H. (1988), *Die Pflanzenlecithine*, Verlag für chemische Industrie H. Ziolkowsky KG, Augsburg.

Persmark, U. (1968), Main constituents of rapeseed lecithin, *J. Am. Oil Chem. Soc.*, **45**, 742–743.

Peter, S.K.F., Brunner, G., and Riha, R. (1982), Process for separating mixtures of substances of low volatility, US Patent 4,345,976, assigned to S.K.F. Peter.

Peter, S.K.F., Czech, B.A., Ender, U.M., and Weidner, E. (1995), Process for preparing pure monoglycerides, pure diglycerides and/or pure triglycerides, US Patent 5,434,280, assigned to S.K.F. Peter.

Peter, S.K.F. et al. (1987), The separation of lecithin and soya oil in a countercurrent column by near critical fluid extraction, *Chem. Eng. Technol.*, **10**, 37–42.

Peter, S.K.F. and Weidner, E. (1985), Verfahren zur Gewinnung von Lecithin, vorzugsweise zur kontinuierlichen Gewinnung von Rein-Lecithin (entölt), European Patent 0 156 374, assigned to Lucas Meyer GmbH & Co.

Peter, S.K.F. et al. (1992), Process for the recovery of monoglycerides and diglycerides from a mixture containing monoglycerides, diglycerides and triglycerides, US Patent 5,110,509, assigned to S.K.F. Peter.

Piepen, R. van der (1962), Neuzeitliche Dünnschicht-Verdampfer und deren Anwendung in der Öl — und Fett-Industrie, *Fette Seifen Anstrichm.*, **64**, 145–150.

Przybylski, R. and Eskin, N.A.M. (1991), Phospholipid composition of canola oils during the early stages of processing as measured by TLC with flame ionization detector, *J. Am. Oil Chem. Soc.*, **68**, 241–245.

Reichling, P. (1969), Verfahren zur Gewinnung vont reinen natürlichen Cholinphosphorsäurediglyceriden, German Patent 1 543 722, assigned to Knoll AG, Chemische Fabriken.

Reiser, R. and Isbell, A.F. (1971), Production of glyceryl monoalkanoates, US Patent 3,595,888, assigned to Research Corporation.

Rewald, B. (1933), Light coloured mixture of vegetable phosphatides and fatty oil, US Patent 1,895,424.

Richardson, A.S. (1941), Preparation of monoglycerides, US Patent 2,251,692, assigned to The Procter & Gamble Company.

Richardson, A.S. and Eckey, E.W. (1941), Preparation of monoglycerides, US Patent 2,251,693, assigned to The Procter & Gamble Company.

Ringers, H.J. and Segers, J.C. (1977), Degumming process for triglyceride oils, US Patent 4,049,686, assigned to Unilever.

Rizzi, G.P. and Taylor, H.M. (1976), Synthesis of higher polyol fatty acid polyesters, US Patent 3,963,699, assigned to The Procter & Gamble Company.

Sarney, D.B. et al. (1994), Lipase-catalyzed synthesis of lysophospholipids in a continuous bioreactor, *J. Am. Oil Chem. Soc.*, **71**, 93–96.

Schneider, M. (1997a), Industrial production of phospholipids — lecithin processing, *Lipid Technol.*, **9**, 109–116.

Schneider, M. (1997b), Phospholipids, in *Lipid Technologies and Applications*, Gunstone, F.D. and Padley, F.B., Eds., Marcel Dekker, New York, 51–78.

Scholfield, C.R. and Dutton, H.J. (1955), Purification of phosphatides, US Patent 2,727,046, assigned to USA, Secretary of Agriculture.

Scholfield, C.R. et al. (1952), Carbohydrate constituents of soybean "lecithin," *J. Am. Oil Chem. Soc.*, **29**, 293–298.

Schwartz, G.L. (1925), Mixed esters of lower and higher fatty acids and process of making same, US Patent 1,558,299, assigned to E.I. du Pont de Nemours and Company.

Schwieger, A. (1935), Process for the production of phosphatide preparation, US Patent 2,020,662, assigned to American Lecithin Company Inc.

Seaberg, P.A. and Hayes, L.P. (1974), Phosphatides, US Patent 3,823,170, assigned to A.E. Staley Manufacturing Company.

Seguin, J.L. (1984), Procédé de fabrication de saucisson sec et similaire, French Patent 2 541 085, assigned to Ets ABC SA.

Sen Gupta, A.K. (1975), A convenient laboratory method for preparative defatting of lecithin, *Fette Seifen Anstrichm.*, **77**, 487–488.

Shimazaki, H. et al. (1974), Process for preparing soybean phosphatides, US Patent 3,798,246, assigned to Ajinomoto Company Inc.

Solomon, B. (1973), Nature des phospholipides d'huile de colza brute, *Rev. Franç. Corps Gras*, **20**, 567–569.

Sonntag, N.O.V. (1982), Glycerolysis of fats and methyl esters — status, review and critique, *J. Am. Oil Chem. Soc.*, **59**, 795A–802A.

Sosada, M. et al. (1992), The composition and properties of purified rapeseed lecithin, *Fat Sci. Technol.*, **94**, 233–236.

Stahl, E. and Quirin, K.W. (1985), Entölung von Rohlecithin durch Hochdruck-Düsenextraktion mit Kohlendioxid, *Fette Seifen Anstrichm.*, **87**, 219–224.

Stauffer, C.E. (2002), Emulsifiers and stabilizers, in *Fats in Food Technology*, Rajah, K.K., Ed., Sheffield Academic Press, Sheffield, U.K., 228–274.

Stipp, G.K. and Kluesener, B.W. (1992), Selective esterification of long chain fatty acid monoglycerides with medium chain fatty acid anhydrides, US Patent 5,142,072, assigned to The Procter & Gamble Company.

Stockburger, G.J. (1981), Process for preparing sorbitan esters, US Patent 4,297,290, assigned to ICI Americas Inc.

Svensson, I. et al. (1992), Lipase-catalyzed transesterification of phosphatidylcholine at controlled water activity, *J. Am. Oil Chem. Soc.*, **69**, 986–991.

Szuhaj, B.F. (1975), Pan release product and process, US Patent 3,928,056.

Szuhaj, B.F. and Yaste, J.R. (1976), Phosphatide preparation process, US Patent 3,962,292, assigned to Central Soya Company, Inc.

Temelli, F. et al. (1996), Conversion of oils to monoglycerides by glycerolysis in supercritical carbon dioxide media, *J. Am. Oil Chem. Soc.*, **73**, 699–706.

Totani, Y. and Hara, S. (1991), Preparation of polyunsaturated phospholipids by lipase-catalyzed transesterification, *J. Am. Oil Chem. Soc.*, **68**, 848–851.

Ulbrich-Hofmann, R. (2003), Enzyme-catalysed transphosphatidylation, *Eur. J. Lipid Sci. Technol.*, **105**, 305–307.

Vijayalakshmi, B. et al. (1969), The nature of cottonseed phospholipids, *Fette Seifen Anstrichm.*, **71**, 757–761.

Volpenhein, R.A. (1980), Diglyceride manufacture and use in making confectioner's butter or the like, European Patent 0 010 333, assigned to The Procter & Gamble Company.

Wang, X. et al. (1997), Synthesis of phosphatidylglycerol from soybean lecithin with immobilized phospholipase-D, *J. Am. Oil Chem. Soc.*, **74**, 93–96.
Weidner, E. et al. (1993), Entölung von Rohlecithin mit Propan, *Fat Sci. Technol.*, **95**, 347–351.
Weiss, A. (1990), Enzymatische Herstellung von festen Fettsäuremonoglyceriden, *Fat Sci. Technol.*, **92** , 392–396.
Wendel, A. (2000a), Lecithin: the first 150 years, Part I: From discovery to early commercialization, *inform*, **11**, 885–892.
Wendel, A. (2000b), Lecithin: the first 150 years, Part II: Evolution to a global pharmaceutical industry, *inform*, **11**, 992–997.
Werly, E.F. (1957), Fluidizing lecithin, US Patent 2,777,817, assigned to Pillsbury Mills, Inc.
Wiesehahn, G.A. (1940), Soft lecithin preparation, US Patent 2,194,842, assigned to American Lecithin Company Inc.
Wieske, T. et al. (1968), Phosphatide anti-spattering agents for margarine, British Patent 1,113,241, assigned to Unilever.
Wittcoff, H. (1949), Fluid phosphatide composition, US Patent 2,483,748, assigned to General Mills, Inc.
Wocasek, J.J. and Koch, J.R. (1948), Fluoride catalysis at 175°C of the reaction: Glycerol plus fatty acid, *J. Am. Oil Chem. Soc.*, **25**, 335–337.
Woldhuis, J. et al. (1990), Method for the preparation of a coating preparation for cheese, a coating preparation obtainable this way and a method for coating cheese using a preparation of this type, EP Patent 0 403 030, assigned to Paramelt Syntac B.V.
Yamaguchi, S. et al. (1993), Preparation of glycerides having a high content of monoglycerides with a lipase from *Penicillium cyclopium* ATCC 34613, US Patent 5,270,188, assigned to Amano Pharmaceutical Co., Ltd.
Yang, T. et al. (2004), Diacylglycerols from butterfat: Production by glycerolysis and short-path distillation and analysis of physical properties, *J. Am. Oil Chem. Soc.*, **81**, 979–987.

4.6 Food uses of oils and fats

Because linoleic acid and linolenic acid are essential fatty acids, fat has always been an important constituent of food. When man was still a hunter-gatherer, game, fish, and nuts were probably the main sources of these fatty acids in his diet. With the advent of agriculture, fresh milk and seeds containing oil became available. Milk is highly perishable, but concentrating the fat and especially inverting the emulsion to form a continuous fat phase greatly increased the "shelf life" of this fatty food; cheese production is another way of preserving milk constituents. In hot climates, butter was further preserved by total elimination of the water phase and conversion into ghee.

In the second half of the 19th century, demand for butter exceeded supply and Emperor Napoléon III of France offered a prize for the development of a butter substitute. After first of all developing a dry fractionation process for beef tallow (1869), Mège invented margarine using the olein fraction, which he called "oléomargarine" (Van Alphen, 1964). However, the production of margarine led to a shortage of solid fats until this was alleviated by the invention of the hydrogenation process (Normann, 1903). This process not only allowed solid fats to be produced from liquid oil, but it also made fish oil and whale oil suitable for human consumption. It was also instrumental in the development of products serving different purposes, such as tub margarines that are spreadable straight from the refrigerator, cocoa butter replacers and puff pastry margarines

Since the fat content of butter could be standardised at 80%, national legislation soon prescribed that margarine should also contain 80% by weight of fat. In the U.K., the maximum water content was set at 18% and since salt is cheaper than fat, British margarine (and butter) tended to be much saltier than in the rest of Europe. As recounted by Robinson and Rajah (2002), national legislation also aimed at protecting butter against competition from margarine by imposing a variety of regulations. For example:

- Margarine had to be colourless and was sold with colour capsules so that the consumer could add the colour at home.
- In France, the use of β-carotene was prohibited and a reasonably attractive colour could only be attained by the inclusion of some palm oil that had been briefly deodorised at a lower than normal temperature. It had hardly been heat bleached and could impart an off-flavour to the fat blend.
- In some states in the U.S., margarine had to be coloured pink, brown, or even blue.
- In Belgium and France, margarine had to be sold in cube-shaped packs.
- The name "margarine" had to be printed on the wrapper in clearly legible letters of prescribed size.
- Elsewhere, the pack had to bear a red band to warn customers that this product was a substitute product.
- In Italy, sesame oil had to be added as a tracer and, in other countries, starch was also prescribed for this purpose, etc.

In Belgium, producing tub margarines meant producing cubic tubs onsite as well, since empty tubs cannot be stacked and, therefore, would be very expensive to transport. However, harmonisation within the EU and especially the landmark ruling of the European Court of Justice in the "Cassis de Dijon" case made countries withdraw such protective legislation.

This allowed butter-like products with improved spreadability through the use of milkfat fractions and/or blends of such fractions with vegetable oil, to be produced throughout the EU. Similarly, spreads with a reduced fat content were now also permitted, in addition to margarine. And mayonnaise, a strictly defined product containing 80% oil and a minimum amount of egg yolk, was joined by fancy products like dressings and sauces, which could now be developed and marketed.

Products used by pastry cooks did not suffer that much from legal constraints and in this domain, special cream margarines, shortenings, and margarines for cakes, and puff pastry margarines could be developed that offered improved technical performance. Similarly, toppings incorporating vegetable fat not only offer a saving in comparison with dairy cream, but they can also offer technical advantages through a judicious choice of fat blend and emulsifiers.

Fats and oils are also used in feed production where they primarily serve as a source of energy, so the composition is not critical. They, therefore, can be supplied from waste or by-product streams, such as gums, spent bleaching earth (Herstad, 1979), filter cakes from the dewaxing process, spent deep frying oils, etc. Feed used in aquaculture is more critical and fish oil is an essential feed ingredient (Barlow, 2004) providing highly unsaturated fatty acids, such as EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) for incorporation into the farmed fish. Fat used in calf milk replacers also has to meet some specific nutritional requirements.

4.6.1 Cream and aerated products

Creams are o/w (oil-in-water) emulsions with a fat content that is at least 12% by weight and may be as high as 55% in clotted cream; in coffee whiteners, the fat content is usually some 10 to 12% (Premlal Ranjith, 2002). In dairy cream, milkfat is the only fat present and in vegetable cream the fat phase is a blend of various vegetable oils and fats. The use of blends of vegetable fat and milkfat has not been reported.

Dairy cream can be supplied as fresh cream, but some kind of heat treatment, such as pasteurisation, sterilization, and ultra high temperature (UHT) treatment is more common. Vegetable creams are supplied as packaged products but can also be prepared by the pastry cook as an emulsion to be used for toppings after overnight storage in a refrigerator. Sour cream is the result of a lactic acid fermentation that is only applied to dairy cream.

4.6.1.1 Dairy cream

Dairy cream is produced from raw, filtered, or clarified milk by means of a stacked disk centrifugal separator like those used for degumming, chemical refining, and water washing of oil, and which were, in fact, developed for the dairy industry. The milk is heated to a constant temperature in the range 40 to 45°C by a heat exchanger before being fed to the separator. Settings are selected so as to produce skimmed milk with less than 0.05% fat. Continuous density measurement of the cream stream allows its fat content to be adjusted to the desired value by diluting it with skimmed milk. Cream categories in use in the U.K. have been listed below in Table 4.30.

TABLE 4.30 Cream categories in the United Kingdom with their minimum fat content in g per 100 g

Type of Cream	Minimum Fat
Half cream	12
Single cream	18
Sterilised cream	23
Whipping cream	35
Double cream	48
Clotted cream	55

Source: Premlal Ranjith, H.M. (2002), in *Fats in Food Technology*, Rajah, K.K., Ed., Sheffield Academic Press, Sheffield, U.K., 69–122.

To ensure food safety, the cream must be given a heat treatment, such as pasteurisation or UHT. Pasteurisation may involve a treatment of at least 15 seconds at 72°C or for a shorter period at a higher temperature. Pasteurised cream must be stored at refrigerator temperatures and even then, the shelf life is limited to 2 to 3 weeks. A longer shelf life results from UHT treatment followed by aseptic packaging, even when the packages are stored at ambient temperature.

The UHT treatment prescribes heating the product to 140°C and a holding time of not less than 2 seconds. Heating can be indirect in plate heat exchangers that can be designed to operate at >90% energy efficiency. Direct heating can be by steam injection followed by flashing off at reduced pressure as a result of which the cream cools down again. This flashing step also reduces the concentration of volatile flavouring compounds in the cream.

The UHT treatment is likely to affect the molten fat globules in the cream causing the physical properties of the cream to change. Consequently, a homogenisation step is always included to ensure that no fat ring is formed on top of the cream and that its behaviour on whipping and the properties of the whipped cream are fully predictable. During homogenisation, the cream is fed by a positive displacement pump to the high-pressure head and the homogenising valve housing; the pressure at this stage may be as high as 20 MPa. The fat globules will vary considerably in size and may be as large as 20 μm. After having been forced through the slit of the homogenisation valve where velocities of up to 250 m/s are attained (Kessler, 1981), their average particle size will have been much reduced to about 0.7 μm and the particle size distribution will have become narrower. The first, high-pressure homogenisation is commonly followed by a second, low-pressure homogenisation to break up the clusters or strings of globules formed in the first one; the pressure during this second homogenisation ranges from 0.1 to 8 MPa.

The reduction in droplet size increases the droplet surface area, and because of the limited availability of protein to cover the newly created surface in comparison with milk, it takes longer to cover the droplet surface in homogenised cream than in milk homogenised under the same conditions.

Since droplets are also closer together in cream than in milk, there will be more recoalescence in cream than in milk, resulting in a wider globule distribution in cream than in milk (Walstra et al., 1999). The surface layer of the newly formed globules consists of fragments of the original membrane, serum proteins, and micellar and submicellar casein. The composition of the original membrane is given in Table 4.31 below.

During whipping, air is beaten into the cream and air bubbles are formed. Proteins move towards the air–water interface where they denature and bind fat globules. The fat in these globules is partially crystallised because the cream has been refrigerated beforehand and some fat crystals penetrate the membrane and, thus, cause fat globules to coalesce (Walstra, 2006). In whipped cream, the liquid phase is truly continuous, whereas the solid, fatty phase is disperse. The gas phase is both continuous and disperse, in that the air is present both in cells that have been opened by the breaking of the intercellular lamellae, and in closed cells (air bubbles).

Liquid dairy cream is used in cooking for the rich taste it adds to food and sauces, and in many other applications, such as bavarois. It is also added to coffee and this may lead to a problem that is known as "feathering:" the appearance of coagulated curd-like flocs and an oily layer floating on the coffee surface. The acidity of the coffee and its calcium content are the primary cause of this feathering since they interact with the casein micelles associated with the fat globules. Lowering the calcium content of the cream reduces its tendency to feather (Anderson et al., 1977a; 1977b). The problem of feathering can also be minimised by immobilising the calcium present in cream by means of chemical additives, such as phosphates, citrates, and carbonates (Premlal Ranjith, 2002).

4.6.1.2 Vegetable cream and toppings

A vegetable cream with excellent whipping properties can be made by a pastry cook onsite by mixing equal amounts of a butter cream margarine and hydrogenated coconut oil in milk with some egg yolks at a temperature at which the fat is molten. Homogenising this mixture with a powerful hand blender before storing it in a refrigerator until the next day ultimately yields an emulsion with partially crystallised fat globules that can be used for toppings with a rich and creamy taste (G. Laridon, personal communication). The emulsion meets the following requirements:

- A fairly short and reproducible whipping time.
- No tendency to churn and produce fat granules.
- A reproducible overrun of 100 to 150%.
- A stable foam that ensures that cream piped onto a cake holds its shape.
- Does not leak serum onto the cake.
- A somewhat irregular foam structure with both open and closed cells; a topping should not look like shaving cream, however.
- On exposure to air, the rosettes should not form a crust on the outside.

It has the disadvantage that it has to be produced the night before, which means that the baker may run out when demand exceeds expectation. Accordingly, a long-life UHT product would meet a need, and such products have indeed been developed. For taste reasons, some contain milk proteins (Kubota et al., 1974; Dell et al., 1981), but others specify their absence (Darling and Dicks, 1982). The fat or fat blend used must melt sharply at body temperature. Accordingly, hydrogenated palm kernel oil, a partially hydrogenated soybean oil, or a mixture of a palm midfraction and a fully hydrogenated coconut oil are suitable. In fact, fats used in confectionery applications can often be used in vegetable creams as well.

These creams tend to use a mixed emulsifier system of, for example, lecithin and polyglycerol mono-, di- or tristearate or Tween 60 (Kubota et al., 1974). Another combination is lecithin, diacetyl tartaric acid ester of monoglycerides and some sorbitan fatty acid esters (Hidaka et al., 2002) or sodium stearoyl lactate, lecithin, and glycerol monostearate (Darling and Dicks, 1982). In these combinations, the lecithin may cause the vegetable cream to incorporate air, but prevent a sizeable overrun, whereas the other emulsifiers prevent whipping when used on their own, but ensure an overrun when used in combination with lecithin. Their amounts and ratio are obviously critical, but their use ensures reproducible whipping behaviour and topping properties. The use of stabilisers and thickeners has also been mentioned in the literature to improve the physical properties of the whipped cream, but their use is not recommended for organoleptic reasons. Another emulsifier combination comprises a lactic acid ester of monoglycerides and, for instance, an acetylated monoglyceride (Willock, 1974).

The cream can be whipped in a bowl with a whisk either manually or mechanically at atmospheric pressure. Another process (Zobel et al., 1984) aerates the emulsion under pressure and then releases the pressure, causing the dissolved gas to escape and create a foam. This is claimed to be an effective way to control the overrun.

TABLE 4.31 Gross composition of the milkfat globule membrane

Constituent	Concentration (mg/100 g fat)
Protein	1 800
Phospholipids	650
Cerebrosides	80
Cholesterol	40
Monoglycerides	+
Water	+
Carotenoids + vitamin A	0.04
Other constituents	Traces
Total	>2570

4.6.1.3 Ice cream

Ice cream is an intricate mixture of fat globules, ice crystals, and liquid water containing dissolved sugars and protein, possibly stabilisers and emulsifiers, and certainly air. A typical composition of the dry matter in ice cream is given in Table 4.32.

As illustrated in Figure 4.25, ice cream manufacture entails mixing the ingredients, homogenising the mixture, and pasteurising the resulting ice cream mix to kill pathogenic bacteria and reduce the number of spoilage organisms. Homogenisation reduces the fat globule size to an average of some 0.45 μm (Adleman and Hartel, 2001). Immediately after pasteurisation, the fat is cooled to just above freezing temperature in a scraped surface heat exchanger. Such heat exchangers have been specifically developed for ice cream manufacture, and are now also used in margarine production.

The chilled mass may then be allowed to rest for a period of 2 to 18 hours (Vaghela et al., 2005), during which period the dispersed fat globules will have time to solidify. Subsequently, the mix is pumped into the freezer where a controlled amount of air is whipped into the mix creating an overrun of some 80 to 120%. Its temperature decreases and its viscosity increases and thereby provides the shear required to create air cells with an average diameter of some 50 μm. Because of the whipping, the partially crystallised fat globules will also agglomerate, which is also referred to as destabilising (Adleman and Hartel, 2001).

TABLE 4.32 Composition of ice cream mix

Component	% Weight
Water	60–64
Sugar	13–18
Fat	10–18
Nonfat milk solids	7.5–11.5
Stabilisers/emulsifiers	0.3–0.5

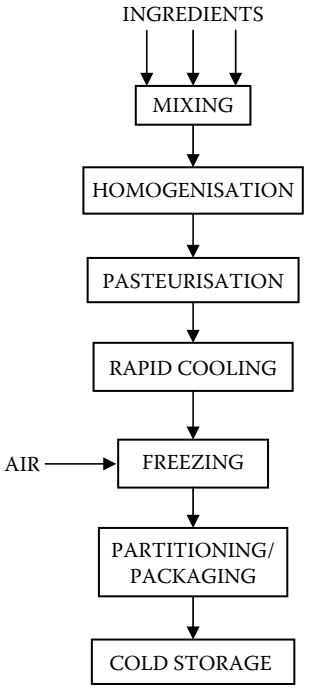


FIGURE 4.25 Flow sheet ice cream manufacture.

If particulate matter is to be included in the ice cream, it has to be added at this stage. Examples of particulate matter are pieces of fruit, chocolate chips, or raisins soaked in rum (Meziane, 2001; Binley et al., 2005). Next, the aerated ice cream mass is pumped to the packaging line and filled into containers or tubs, or divided into individual size portions. These portions may then be coated with chocolate or a compound coating. The fat used in this kind of compound coating may be a cocoa butter substitute, such as hydrogenated palm kernel stearin, because with deep frozen products there is only a low risk of fat hydrolysis and the resulting soapy flavour. Finally, the product is stored at freezing temperatures of about −25°C to about −35°C.

The role of emulsifiers in ice cream is somewhat similar to their role in whipped cream. They affect overrun by destabilising the fat globule surface and when used in small quantities, they provide significant quality enhancement. Used at high concentrations, they can promote creaming of the emulsion as butter granules form during shearing in the freezer (Adleman and Hartel, 2001).

Ice cream is not only sold as a ready-to-eat product, but also as a mix. This may be as a complete mix that is aseptically packaged after an UHT treatment (Donald and Lewis, 1987) or as a dry mix (Mishkin et al., 1970) that has to be reconstituted with water before the mixture can be processed in a conventional ice cream freezer. These mixes are, for example, used by pastry cooks, caterers, and ice cream vendors.

For dairy-based products, milkfat is used, but equivalent vegetable products may use fats with a preferably sharp melting point at body temperature, such as a partially hydrogenated vegetable oil, palm kernel oil, or coconut oil. The mixes will also contain milk solids, such as skimmed milk or skimmed milk powder, lactose, sodium caseinate, and sugar and/or corn syrup. The use of carrageenan as a stabiliser and the emulsifiers Tween® 65 and Tween® 80 is also mentioned.

4.6.2 Butter, margarine, and spreads

To distinguish them from liquid oil and shortenings, the term "yellow fats" is used to describe the group of products comprising butter, margarine, and fat-reduced spreads. These products are all primarily w/o emulsions. They are mainly used in conjunction with bread-like products, so, in most Asian countries, the market for yellow fats is quite small. Yellow fats are also used in the kitchen for shallow frying, preparation of sauces, to coat cooked vegetables prior to serving, and for making cakes and pastry.

Shortenings can also be used for several of these yellow fat applications and preferences for the one or the other are predominantly cultural. Countries that traditionally used butter switched more easily to margarine than countries traditionally using lard for these applications (Dijkstra, 2000). In Mediterranean countries, where the climate prevents the use of butter and olive oil is traditionally used instead, cooking oils are preferably used as a cheaper substitute. Fat-reduced spreads are in theory also cheaper substitutes since water is cheaper than fat, but commercial practice hardly reflects this saving.

4.6.2.1 Butter

Butter is produced by the churning of cream. Various processes, such as those of (Farrall, 1946; Horneman et al., 1949) using 80% cream and inverting the phases, have also been patented, but are no longer used for final product quality reasons (Poot and Biernoth, 1994). The cream is first of all pasteurised (82°C/20s to 100°C/1s), which also inactivates enzymes. After pasteurisation, the cream is cooled by heat exchange with ingoing cream.

The next stage in butter making is the ripening of the cream. This is a physical process that results in the partial crystallisation of the milkfat contained in the fat globules. It may be combined with a bacteriological process involving the partial conversion of lactose into lactic acid by the addition of a starter culture comprising mesophilic strains, such as *Lactococcus lactis* ssp. *lactis, L. lactis* ssp. *cremoris, Lactococcus lactis* ssp. *lactis* biovar. *diacetylis, Leuconostoc cremoris,* and *Leuconostoc lactis*. The pH reduction to some 4.6 provides the butter with a fresh taste and flavour substances, such as diacetyl are also formed during the bacteriological ripening process.

Because the triglyceride composition of milkfat varies with the time of the year, what the cow has eaten, her breed and lactation stage, and dairies assume that the consumer insists on constant butter properties, and, above all, because adjusting milkfat properties by blending has long been regarded as adulteration, the physical ripening process has been used as a means to adjust the physical properties of butter. Extending cooling increases the average solid fat content (SFC) of the fat globules and, thus, reduces the subsequent solidification in the post-churning stage. It, therefore, is used for low IV (iodine value) winter sweet cream (Robinson and Rajah, 2002). Two examples of temperature regimes are given in Table 4.33.

Churning started as a batch process with farm churns that could be turned by a dog. Nowadays, industrial churning is continuous and comprises three stages. In the first stage, the cream is beaten until pea-sized butter granules and buttermilk is formed. In the second stage the butter granules are separated from the buttermilk and washed with water and finally, in the third cylinder, brine or serum may be added to control the milkfat content of the butter and worked into the butter by worm conveyors; perforated plates and knives rotating at a increased speed ensure proper dispersion and a homogenous final product. Small serum droplets also make butter more resistant to microbial attack since a bacterium cannot multiply in a small droplet and soon dies.

TABLE 4.33 Temperature profiles for physical ripening

Stage	Duration (h)	Temperature (°C)	
		Hard/Winter	Soft/Summer
1	2	8	19
2	3	19	16
3	14–20	16	8

During churning, air is whipped into the cream and forms a foam with fat globules trapped between the lamellae of the bubbles and several fat globules adhering to an air bubble. Part of the membrane and some liquid fat is also spread over the air–water interface. Continued beating causes air bubbles to collide and coalesce so that the surface area of the air bubble diminishes. The fat globules adhering to air bubbles stick together due to the liquid surface fat and form small clumps. Further agitation causes these clumps to agglomerate and form granules (Walstra, 2006).

However, this agglomeration does not create a fully continuous fatty phase. Even after the butter has been worked and kneaded, it still contains residual fat globules and globule membrane fragments interspersed with moisture droplets (Mulder and Walstra, 1974). Consequently, a fat blend that has the same SFC profile as milkfat produces a margarine that is more solid than butter because of the fully continuous fat phase in the margarine (Podmore, 2002a).

Butter is generally packaged in a wrapper. It is stored and transported under refrigeration and has a shelf life of up to 4 months if refrigerated and over a year when deep frozen. The 25 kg blocks stored frozen in EU intervention stores have been found to be of satisfactory quality after 2 years (Robinson and Rajah, 2002).

4.6.2.2 Margarine

Margarine started as a butter substitute and was regarded as a threat by the dairy industry. Consequently, a rift developed between two sectors that were both dealing with fatty w/o (water-in-oil) emulsions with similar properties, applications, and technological challenges: Local dairy companies that might even be farmers' cooperatives vs. often multinational companies handling a wide range of consumer products besides margarine, an approach that regards fat modification and even blending as "adulteration" vs. an approach that is based on interchangeability and modification processes to increase this interchangeability; an environment that is strongly influenced by interventions and subsidies (and their lobbies) vs. commodity trading, separate research institutes, separate ministries (agriculture vs. economic affairs), separate trade fairs, scientific conferences, universities, journals (dairy vs. oil chemists) etc.

Fortunately, the rift is closing somewhat. Milkfat is being modified by fractionation, and it is even blended with vegetable oil, but the margarine industry does not yet regard milkfat as one of the many components it could select when formulating blends. It promoted vegetable fats and the absence of cholesterol, so conceding that it has not been able to match the taste of butter by incorporating milkfat would amount to admitting defeat. Besides, including this animal fat would increase the price of the blend.

Margarine has evolved enormously. Table 4.34 shows how various margarines differ in their application and, thus, in SFC. There are the health margarines in tubs with a low content of saturated fatty acids, butter cream margarines with a cool taste, and puff pastry margarines that can be used industrially and obviate the need to interrupt production to cool the pastry again.

Instead of being stuck with milkfat, the margarine industry has been free to choose and develop its fat blends in accordance with the requirements of the application. Nevertheless, this has led to different approaches: In the soybean oil dominated U.S., the presumed necessity to lower the linolenic acid content of fat blends (Dijkstra, 2005) has for a long time caused the entire fat blend used in margarine, or shortenings for that matter, to undergo a hydrogenation treatment. In Europe, where traditionally a wide variety of oils and fats are used, margarine fat blends tend to consist of a blend of a hardstock and liquid oil, even if this liquid oil contained linolenic acid.

The margarine industry has also competed with butter by developing fat blends in accordance with prevailing nutritional tenets. So at one stage, the industry was promoting the benefits of a low saturated fatty acid content and a high content of linoleic acid. Later, it launched a "more balanced" polyunsaturated fatty acid profile by replacing some expensive sunflower seed oil with cheaper soya bean oil or canola and currently, it is promoting a low *trans* isomer content. The industry has also provided its chemophobic customers with margarines and spreads made with a fat blend that, just like butter, has neither been hydrogenated nor interesterified.

Perceived nutritional benefits have also found their way onto margarine labels that now list not just the fat content but also the amounts of saturated, monounsaturated, and polyunsaturated fatty acids and *trans* isomers. However, despite their well-known nutritional differences, no distinction is made between the various saturated fatty acids or between the various *trans* isomers; only their totals are listed on the labels.

We shall first discuss label-friendly margarine fat blends that have no *trans* isomers and a relatively low saturated fatty acid content. To facilitate this discussion, a nomenclature that is similar to what is used in the relevant patent literature (Elliott et al., 1999) will be introduced:

M = medium chain fatty acid (up to C_{14})
H = saturated long chain fatty acid (from C_{16} upwards)
P = palmitic acid
S = stearic acid
U = unsaturated fatty acid
O = oleic acid
L = linoleic acid and/or linolenic acid
HUH = symmetrical triglyceride
H_2U = di-saturated, monounsaturated triglyceride, etc.

Accordingly, palm oil will consist mainly of H_2U and HU_2 triglycerides with some U_3 and H_3, whereby the latter triglycerides give the oil a sticky feel to the mouth. In randomised palm oil, the ratio of HUH triglycerides to HHU triglycerides is 0.5, but before randomisation, this ratio is much higher. H_2M, HM_2, M_3, and, to a lesser extent, H_2U triglycerides have melting points that contribute to the consistency of the margarine. On the other hand, HU_2, HMU, M_2U, MU_2, and U_3 do not contribute to the consistency of the fat except at very low temperature.

Consequently, a co-randomised 40/60 blend of coconut oil (H = 11%, M = 80%, U = 9%) and palm oil (H = 50%, U = 50%) contains only a small proportion of triglycerides that provide consistency. Accordingly, a margarine made with this interesterification product and a small amount of partially hydrogenated (IV = 95) soya bean oil exhibited an unacceptable oil-off and slump (Seiden, 1967). Adding 11% of a co-randomised 50/50 blend of coconut oil and almost fully hydrogenated high erucic acid rapeseed oil solved the problem because of the stiffening power of the H_2M and HM_2 triglycerides in this addition product.

TABLE 4.34 Solid fat content (SFC) profiles of various margarine types

Product Type	N_{10}	N_{20}	N_{25}	N_{30}	N_{35}
Health margarine	13–16	8–12	5–10	0–4	<1
Soft table margarine	16–22	10–16	8–14	4–8	<1
Table margarine	28–34	20–28	10–16	6–10	0–3
Wrapper margarine	24–30	16–24	8–12	3–6	<1
Butter cream margarine	46–52	20–26	10–14	4–8	<3
Cake margarine	46–54	22–30	14–20	8–12	4–8
Croissant margarine	50–58	32–38	20–26	14–20	8–12
Puff pastry margarine	58–66	38–46	24–30	16–22	10–16
Hard puff pastry margarine	62–70	42–50	26–32	18–24	14–20

Such triglycerides can also be provided by increasing H at the expense of U in the blend to be co-randomised and this is exactly what Fondu (1972) did by adding palm stearin (H >80%) to the blend. Ullanoormadam (2004) pursued this line of approach by blending a hard palm oil fraction produced in a two-step dry fractionation process (P >70%) with palm kernel stearin. However, the hard stocks resulting from the co-randomisation processes still contain various triglycerides, such as HMU and M_2U, that do not contribute any consistency at the temperature of use. Their presence can be avoided by fractionating the co-randomisation product and just using the stearin in the margarine fat blend (Huizinga et al., 1999; 2000).

Their presence can also be avoided by full hydrogenation of the blend components. Accordingly, Graffelman (1971) blends palm kernel oil with an oil containing H and U fatty acids, interesterifies and fully hydrogenates the blend or hydrogenates the blend components before or after blending, but before interesterification. A few months later, Delfosse (1971) does the same but uses coconut oil instead of palm kernel oil and quite some time later, Ward (1982) interesterifies saturated babassu nut oil with saturated palm oil. When the genetically modified high lauric rapeseed oil became available (Voelker and Davies, 1992; Del Vecchio, 1996), this was also interesterified with fully hydrogenated soya bean oil (Sassen and Wesdorp, 2001).

But again, these interesterification products are not ideal since they contain M_3 triglycerides that do not contribute to the consistency and reducing their content by using relatively less lauric component increases the H_3 content of the randomisation product and leads to a poor feeling in the mouth. Another way to reduce the M_3 content of a randomised fully hydrogenated lauric oil and, thus, produce a hard stock with increased HM_2 and H_2M content is by vacuum distillation (Seiden, 1970) at a temperature of 280 to 290°C; it is doubtful whether this method has ever been used industrially.

Accordingly, Kattenberg et al. (1976) fractionate the randomised product to reduce its H_3 content. Similarly, Schijf et al. (1984) use solvent fractionation to upgrade their interesterification product and produce a midfraction, just as Holemans et al. (1988) subject their randomisation product to a two-stage solvent fractionation process. By removing a first stearin fraction they reduce the H_3 content and by retaining the stearin fraction of the first olein (the midfraction) they isolate a fraction that is enriched in H_2M and HM_2 triglycerides. By blending this midfraction with a sunflower oil that had been subjected to a directed interesterification process, they arrive at a fat blend with a low saturated fatty acid content (<14%) and high linoleic acid content (>66%) that was designed to compete with a blend of sunflower seed oil enriched with a small amount of fully saturated sunflower seed oil and then subjected to directed interesterification (De Lathauwer et al., 1980).

The above *trans* isomer free products from Seiden (1967) to Ullanoormadam (2004) obtain their consistency from triglycerides with M and H fatty acids. However, consistency can also be provided by HUH triglycerides, which are also *trans* isomer free. Such triglycerides are formed during directed interesterification of sunflower seed oil (Haighton and Kattenberg, 1974; Kattenberg, 1974; Sreenivasan, 1975; De Lathauwer et al., 1980; Pronk et al., 1986), but they can also originate as such from the oil source. Accordingly, Kattenberg and Verburg (1977) provide a fat blend with H_2U triglycerides that is mainly derived from palm oil and also contains a randomised product so that the HUH:HHU ratio is lower than in palm oil itself.

Margarines without M fatty acids and with low *trans* isomer content have also been developed by the interesterification of a fully hardened oil and a liquid oil (Melnick and Gooding, 1960; List et al., 1995).

The blends discussed above are primarily for table margarines sold to the consumer in tubs with a label that lists fat ingredients. In the EU, mentioning the *trans* isomer content is not yet obligatory except when it is linked to a more general claim. In the U.S., the *trans* isomer content must be mentioned when it is more than 0.5 g per portion of 14 g (List, 2004). The ingredients of margarine sold to commercial pastry cooks do not need to be listed on the wrapper, but may be mentioned on a technical data sheet. After using the margarine in his/her products, the baker does not have to mention this, since the products are in sealed packaging.

It is not surprising that bakery margarines have continued to contain *trans* isomers, since they provide the triglycerides with the melting properties that lead to good aeration and plasticity. In fact, the consequences of reducing the *trans* isomer content in margarine has been described by Sassen and Wesdorp (2001) as follows: "The recent change-over to low or zero *trans* spreads has caused various problems, such as increased brittleness, a poorer melt down behaviour in the mouth, less favorable flavour release, and serious structural defects, particularly graininess. … Further problems occurred in the manufacturing plants. The low or zero *trans* margarine fats tend to crystallize more slowly, which not only made the fats less plastic and more brittle, but also reduced the throughput of the spread production lines. This was necessary for allowing the spread to get a firm enough consistency. Obvious consequences were capacity losses, extra investments, and increased costs for labour and energy." Accordingly, the fat blend in puff pastry margarines may still contain palm stearin and partially hydrogenated palm oil, which provide the margarine with plasticity and a long plastic range. On the other hand, fat blends for puff pastry margarines with less than 10% *trans* isomers have also been described (Van der Schee, 1997).

Margarines also comprise an aqueous phase (Opfer, 1978). This can be just water, but it can also contain dairy products. Milk solids in margarine brown on shallow frying and form the basis of meat gravy. The aqueous phase may be subjected to a fermentation process for flavour development. This fermentation process lowers the pH and gives the margarine a fresh taste. In the absence of fermentation, the pH can be lowered by dissolving some citric acid in the aqueous phase. Other ingredients that may be added to the aqueous phase are salt, water-soluble flavours, and potassium sorbate (E202).

Oil-soluble flavourings are added to the fat blend together with colouring agents, such as β-carotene (E160a), fat-soluble vitamins, and emulsifiers. Traditionally, the emulsifiers used in margarine production are small amounts (<0.5%) of lecithin (E322) (Fresenius, 1902) and monoglycerides (E471). These emulsifiers should only provide the w/o emulsion with sufficient stability to survive processing. However, when the fat melts in the mouth, the emulsion should break to release the water-soluble flavours and allow the tongue to taste the salt and acid.

Margarine used in shallow frying may cause spattering. In countries where oil or lard is used for shallow frying, this spattering is not considered a great disadvantage, since oil and lard are even worse in this respect. However, in countries where salted butter is used and which causes less spattering in shallow frying, margarine should not spatter either. Salted margarine causes less spattering than when little or no salt is present, and incorporating air, as in margarines that were produced with a chilling drum and a complector (Robinson and Rajah, 2002), also reduces the tendency to spatter (Pardun, 1988).

Using the methanol-soluble (Mattikow, 1953) fraction of lecithin or the extract obtained with aqueous ethanol (Wieske et al., 1968) also reduces spattering, presumably by reducing the PE content of the lecithin. This can also be achieved by acetylating the lecithin and removing the acetylated PE by extraction with acetone or methyl acetate (Aneja, 1972). A mixture of partially hydrolysed lecithin and saturated monodiglycerides is also effective in reducing spattering (Pardun, 1972).

Today, margarine is almost exclusively produced by using scraped-surface heat exchangers (Opfer, 1978). These pieces of equipment comprise two concentric tubes with a refrigerant, such as liquid ammonia, circulating in their annular space. Inside the inner tube, a heated shaft with floating scraper blades rotates. They operate continuously and allow continuous packaging, but the emulsions they are fed with are produced batchwise because of the many ingredients and different recipes that may be involved. Both the fatty phase and the aqueous phase batches are produced by a combination of weighing and volumetric measurement using metering pumps or measuring cylinders. Molten monodiglycerides and lecithin are commonly stored in heated tanks and administered in dosed amounts by pumps. Vitamins, β-carotene, and oil-soluble flavourings are measured and added manually to the batch.

Similarly, the aqueous phase is prepared batchwise, but batch sizes vary. A batch reconstituted milk, for instance, will serve a number of emulsion batches. Salt can be added as saturated brine by a metering pump, but water-soluble flavourings are added manually. The aqueous phase must be pasteurised and this can either be before or preferably after it has been mixed with the fatty phase. Mixing of the two phases can be by metering pumps located just before the scraped-surface heat exchangers or in a slowly agitated buffer vessel into which the melted rework from the packaging line is also returned. Of course, there are a number of variants on the layout described above (see Podmore, 2000, Figure 7.1).

Variable speed triplex piston pumps (Robinson and Rajah, 2002) forward the emulsion to scraped-surface heat exchangers where it is cooled and the fat starts to crystallise. Because the heat exchanger is cooled by liquid ammonia, its wall temperature is <0°C so that fat is likely to crystallise in its α-polymorph. Under the prevailing conditions, this polymorph is not stable and will convert to the β′-polymorph. This takes more or less time and liberates latent heat of crystallisation. Accordingly, the equipment downstream of the scraped-surface heat exchanger or two such exchangers in a series, is a working unit that provides the stream of partially crystallised emulsion with some residence time. This working unit is not a heat exchanger. It has a central rotor shaft that may be an extension of the axis in the scraped-surface heat exchanger and that carries a number of pins that intermesh with stationary pins fitted inside the housing of the unit. (See also www.gerstenbergschroeder.com.)

In this unit, the emulsion temperature will rise, so that a further cooling treatment in a scraped-surface heat exchanger is required. This can be complemented with another treatment in a second working unit, but table margarines can also be fed directly to the packaging machine. If undercooled, they will stiffen in the package, which may make them somewhat brittle. Like wrapper margarines, puff pastry margarines having a high solid fat content benefit from a second working unit, and before being partitioned they are also passed through a resting tube equipped with sieves to homogenise the margarine and break up the crystal network.

In addition, puff pastry margarines may be given a tempering treatment to improve their plasticity. This treatment involves storing the margarine for up to 2 days at a temperature that is slightly higher than the product temperature on leaving the production line before transporting the product to refrigerated storage. During this tempering, some Ostwald ripening may occur and mixed crystals may give rise to differently mixed crystals as shown by the change in DSC profile during the tempering treatment.

Margarine production equipment comprising scraped-surface heat exchangers and working units has a modular design. Accordingly, it is possible to change the place of the working unit in the chain of events, change its volume, by-pass a cooling unit, etc. Consequently, the equipment can be adapted to the crystallisation behaviour of the fat blend being processed.

4.6.2.3 Spreads

Spreads are margarine with a reduced fat content. According to the Council Regulation 2991/94 of the European Community (EU, 1994), which was subsequently adapted by the Council Directives No 577/1997, No 1278.1997, No 2181/1997, No 623/1998, and No 568/1999, margarine is still defined as a w/o emulsion with a fat content of 80 to 90%. The next category is called three-quarter-fat margarine and contains 60 to 62% fat. Organoleptically, it is difficult to distinguish this spread from margarine and, like margarine, it can also be used for cooking. Then there is half-fat margarine with a fat content of 39 to 41% and finally, there are spreads that have to define their own fat content.

In the U.S., the term "spread" is the commonly used term for products containing less fat than margarine, but there is no Federal standard. The term "diet margarine" is used for the "halvarine" or "minarine" containing 40% fat, but again, there is no Federal standard for this type of product.

The equipment used to produce spreads is the same as for margarine, including the equipment for the constitution of the emulsion, the chilling/crystallisation equipment and the packaging equipment. However, the formulation of the aqueous phase may well include gelatine (E485) in an attempt to make the product taste somewhat less watery (Norton and Underdown, 1992). Preparing the emulsion requires care and possibly more time to prevent it from inverting.

4.6.3 Anhydrous fat products

For water-continuous emulsions like cream and aerated products and even for oil-continuous emulsions like butter, margarine, and spreads, microbial spoilage is the most serious quality threat. For anhydrous fat products, such as shortenings and salad oils, oxidative deterioration is the main threat to guard against. With the exception of anhydrous milkfat (AMF), ghee, olive oil, and possible some gourmet salad oils, all other fatty products will have undergone a deodorisation treatment before leaving the plant. This treatment will have stripped all air out of the product.

Consequently, air will dissolve into freshly deodorised oil when this oil is exposed to air. According to Battino et al. (1968), the solubility of oxygen in olive oil at ambient temperature is some 115 (ml/l.atm). The solubility of nitrogen is somewhat less at 70 (ml/l.atm) and it varies only slightly according to the type of oil (Schaffer and Haller, 1943). In industrial practice, it is common to saturate oil that is destined to be bottled as salad oil with nitrogen and/or to store the oil in tanks under a nitrogen blanket. This does not affect the solubility of the oxygen, but it has an effect upon the rate of its dissolution in the oil.

If the oil were to be devoid of both oxygen and nitrogen, contact with air would cause both gases to be drawn into the oil. However, when the oil is already saturated with nitrogen, no more nitrogen will dissolve in the oil and the oxygen in the air, therefore, has to diffuse through the nitrogen in the air towards the oil surface. Since diffusion is a relatively slow process, prior saturation of oil with nitrogen slows down the uptake of oxygen. Moreover, dissolved oxygen has to diffuse from the oil surface towards the bulk of the oil, unless of course convection (agitation) continuously brings fresh oil to this surface.

Oil in a closed bottle will absorb oxygen from the headspace unless the filling machine incorporates a nitrogen flushing system. This absorption will decrease the headspace pressure but this decrease will immediately be compensated by some nitrogen escaping from the saturated oil into the headspace.

Fats, such as shortening and deep-frying fats, that are solidified in a scraped-surface cooler before being wrapped can also be dosed with nitrogen. This will hardly slow down the rate of oxygen uptake since in the solid matrix, this is entirely diffusion controlled. The small gas bubbles present in the solidified fat will make it look much whiter, make cutting the bar somewhat easier and by increasing the volume rather than the weight, make it look more than it is.

4.6.3.1 Anhydrous milkfat (AMF)

The production of anhydrous milkfat has been described in Section 3.2.3 and its fractionation has been discussed in Section 4.4.4.1. This section, therefore, will concentrate on the food uses of AMF and its fractions. When the Commission faced a "butter mountain," one of the measures utilized to eliminate it was to allow limited sales of AMF at a reduced/subsidised price to the consumer at certain times of the year. It was processed/packaged like a deep-frying fat and sold in 250 g wrappers.

The Commission also allows sales of AMF and its fractions at reduced price to industrial customers like bakeries and ice cream or chocolate (Hartel, 1996) manufacturers. On the other hand, the use of AMF fractions in spreadable butter never qualified for any subsidy. Cold spreadable butter has a much reduced SFC at low temperatures, as shown by Table 4.35.

This table refers to fats that only contain milkfat. By including other products, such as liquid vegetable oil, spreadable butter blends can be obtained that also exhibit a more balanced fatty acid profile. A comparison between the data in Table 4.34 and in Table 4.35 shows that AMF has an SFC profile that is quite close to that of a butter cream margarine, which is not surprising, since that

TABLE 4.35 Solid fat content (SFC) profiles for anhydrous milkfat (AMF) and experimental butters

Product	N_0	N_{10}	N_{20}	N_{25}	N_{30}	N_{35}	N_{40}
AMF	57.2	49.1	20.7	13.2	7.0	2.0	0
Pastry butter	64.7	59.6	36.1	26.8	18.6	10.4	2.9
Spreadable butter	32.4	25.9	10.3	6.4	3.4	0	0

Source: Kaylegian, K.E. (1999), *Lipid Techn.*, **11**, 132–136.

TABLE 4.36 Main uses of milkfat and its fractions in bakery products

Bakery Product Type	Milkfat Product
Biscuits	AMF + 20% olein
Ice cream cones, waffles	Various oleins
Viennoiserie	AMF + stearin
Puff pastry products	AMF + stearin
Cakes	AMF
Butter sponges	Various oleins
Couverture chocolate for ice cream bars	Various oleins
Butter cream	AMF + oleins

Source: De Greyt, W.F.J. and Huyghebaert, A.D. (1993), *Lipid Techn.*, **5**, 138–140.

margarine was developed as the starting material for butter cream substitutes. Similarly, pastry butter closely resembles puff pastry margarine and spreadable butter is like soft table margarines but still has a steeper SFC. It is less spreadable than margarine when taken from the refrigerator and soon loses its consistency when kept outside the refrigerator.

A number of bakery applications of AMF and its fractions have been listed in Table 4.36. Mixing stearin into AMF increases its plastic range, which facilitates rolling and obviates the need for intermediate refrigeration. Oleins are used or mixed into AMF for low temperature applications and those demanding sharp melting.

4.6.3.2 Ghee, vanaspati, and shortenings

Like AMF, ghee is also dehydrated butter, but whereas the temperature in AMF production is raised only to 70 to 80°C, a much higher temperature of between 110 and 140°C is used for ghee production (Podmore, 2002b). This high temperature causes milk solids to react with each other and the reaction products give ghee its distinctive flavour. Temperatures at the lower end of the range give the ghee a nutty, lightly cooked aroma that is highly appreciated. However, water takes longer to evaporate at these low temperatures. High temperatures may give the ghee more of a cooked or even burnt flavour.

After production, the ghee is poured into metal cans that are set aside to cool down. This cooling process is quite slow and causes fractional crystallisation. Since the walls of the container act as a heat exchanger surface, crystals will deposit on those walls and also sink to the bottom of the can. In Asia and the Middle East, ghee is mainly used for cooking and some recipes call for the olein fraction of the ghee, whereas for others, the ghee is stirred with a spoon before a mixture of olein and stearin is scooped from the can (Achaya, 1997).

Because ghee is anhydrous, it is not prone to microbiological spoilage. The development of rancidity is the main reason why its shelf life is limited to up to 8 months in tropical temperatures. Because ghee is more expensive than its substitute vanaspati, the use of ghee is becoming more and more limited to high-quality cooking. Liquid vegetable oil is also making inroads on ghee usage.

According to Podmore (2002b), vanaspati was first imported into India from The Netherlands after World War I as a substitute for ghee for bulk users, such as restaurants and sweetmeat manufacturers. Local production started in 1930 and soon a number of rules and regulations were issued:

- Moisture content, maximum 0.25%
- Slip melting point, 31 to 41°C
- Free fatty acids (as oleic acid), maximum 0.25%
- Unsaponifiable matter, maximum 2.0%
- Nickel content, maximum 1.5 ppm

In addition, the agricultural origin of permitted ingredients is specified and sesame oil is prescribed as a marker. Groundnut oil was originally permitted as a blend component, but can now only be used as such. Interesterification products were not permitted for quite some time, but the ruling has changed; they are being produced and used in vanaspati (S. Ghosh, personal communication).

Like ghee, vanaspati is packaged in tinned cans, but containers like glass jars and plastic buckets are also used (P. Vasudevan, personal communication). Like ghee, vanaspati should deposit rather coarse crystals that can be spooned out of the container. Flavours can be added to make it resemble ghee, but as a rule, no emulsifiers are added.

In this latter respect, shortenings are quite different from vanaspati. "Superglycerinerated" shortenings were developed in the U.S. by mixing about 3% of monoglycerides into the shortening (Stauffer, 2002). As reported by Lauridsen (1976), the mesomorphic state of the monoglyceride has a great effect on the specific volume of the cake batter and the finished cake. Only the lamellar dispersion and the α-crystalline gel phase can provide satisfactory batter and cake volumes.

This superglycerinated shortening was originally lard-based, but it could also include a hard fat obtained by almost full hydrogenation to extend its plastic range

(Gudheim, 1939).[11] Subsequently, the physical properties were further improved and made to resemble a vegetable shortening by randomisation (Vander Wal and Van Akkeren, 1951; Mattil and Nelson, 1953). Directed interesterification was then found to be an even better method of extending the plastic range (Hawley and Holman, 1956).

The SFC profile of the shortening is a function of its intended use and is comparable with the margarine blend SFC profiles listed in the lower part of Table 4.34 (Hawley and Dobson, 1956; Holman and Going, 1959). Accordingly, a shortening to be used as the fat base in a biscuit filling cream will melt sharply and be fully molten at body temperature, just as with a butter cream margarine. Shortenings used for cake manufacture will have an SFC profile that falls within the limits indicated for cake margarine, etc.

For industrial use, bakery margarines and shortenings have the disadvantage that they need to be wrapped for transport and intermediate storage, and also require unwrapping. Accordingly, money has been saved by supplying shortenings in bulk. These can be fully liquid shortenings comprising liquid oil, emulsifiers, and optionally a small amount of a solid stearin (a vegetable oil hydrogenated to a low iodine value) that tends to crystallise in the β-polymorph (Norris, 1976).

The particular shortening described in this patent is produced by rapidly chilling a liquid blend of all ingredients to a temperature in the range of 28 to 31°C and working it at that temperature to ensure that the β-crystals are formed. This results in a very stable suspension that can be transported and stored as such. The emulsifiers used (soft mono- and diglycerides and, for example, ethoxylated mono- and diglycerides) make the liquid shortening suitable for bread dough. A liquid shortening that provides cakes with enhanced moistness has been described by Busken (1993). It also comprises mostly liquid oil, emulsifiers, and an optional amount of <5% solid stearin. The emulsifers used in this instance are: propylene glycol monoesters and diacetyl tartaric acid esters of monoglycerides. Another combination of emulsifiers used in a liquid shortening comprises an ester of polyglycerol and an ester of propylene glycol (Rossen, 1970). Liquid shortenings comprising soft or hard mono- and diglycerides and fatty acid esters of polyglycerol have also been described (Varvil, 1982). Such liquid shortenings can also be mixed with water and provide stable emulsions to be used as such (Gawrilow, 1980).

A different type of shortening that also obviates the need for wrapping and unwrapping, is the pourable or pumpable shortening. It is well-known that fat products with an SFC of 15% can be solid and that with a higher SFC of, for example, 30%, they can still be pumpable. Table margarines illustrate the former and the fractionation process provides several examples of the latter. Solidified blocks can also be made pumpable by crushing (Yoneda et al., 2000). As pointed out by Price (1989), a common problem with pumpable shortenings is the tendency of the solid component to separate from the liquid component over a relatively short period, particularly when the shortening is exposed to temperature extremes. She also mentions that it may become more viscous after exposure to temperatures above ambient.

A crystal network will prevent the shortening from being poured so its formation must be prevented. This can be achieved by agitating the shortening until it is fully crystallised. The effect of temperature changes can be minimised by providing the shortening with a flat SFC profile. In that case, raising the temperature will not cause much solid fat to melt and subsequent cooling will hardly induce any crystallisation, thereby ensuring that a crystal network is not formed. Accordingly, the use of low IV-hardened fat that crystallises in the β-polymorph is recommended (Mitchell Jr., 1950; Bell et al., 1969; Reid and Morgan Jr., 1974). Sedimentation of the crystal slurry is avoided by grinding the crystals (Haighton and Mijnders, 1968; Price, 1989).

4.6.3.3 Salad and cooking oils

The formal distinction between salad oils and cooking oils is that the former are not heated and are only used for making, for instance, mayonnaise or French dressing, whereas the latter can be used for shallow frying and cooking and baking in general. Oils for deep-frying are also heated, but will be discussed in the next section. In practice, the distinction between salad oil and cooking oil is marginal in that oils sold as "salad oil" can be used for cooking and often are. However, not all oils can be used to make mayonnaise, since oils that partially solidify at refrigeration temperatures, such as groundnut oil or olive oil, would break its emulsion (O'Brien, 2000). Nondewaxed oils would also break this emulsion when the mayonnaise is stored in the refrigerator, but the housewife need not worry about this possibility since the oils she buys will have been dewaxed.

In the U.S., the higher melting triglyceride content of cottonseed oil sold as salad oil will have been reduced by a fractionation process (winterization) and soya bean oil-based salad oil will have been brush hydrogenated to an IV of around 100 and subsequently fractionated (Melnick et al., 1953). Consequently, both of these oils can be used for making mayonnaise.

In Europe, what salad oil is used (if any) and for what purpose depends very much on the country and even the region concerned. In Greece, olive oil is predominant (Dijkstra, 2000), but in France, groundnut oil was the most important salad oil when France still had colonies providing this commodity; nowadays, sunflower seed oil,

[11] The patent indicates the extent of the hydrogenation by the titer of the product. See Section 6.3.5 for a discussion of this parameter.

being domestic, is a popular oil, and is also considered to offer health benefits.

In general, salad oils in Europe are sold on the basis of their agricultural origin: rapeseed oil (canola), soya bean oil, sunflower seed oil, corn germ oil, etc. Oils are also sold as mixtures like the "Iso-4" in France, which has a health connotation. Oils can also be sold as oil for a specific purpose, but the agricultural origin is often also mentioned. At one stage, grape seed oil was promoted as the ideal oil for fondue bourguignon and the label mentioned both the origin and the intended application. Gourmet oils are most definitely sold on the basis of their agricultural origin, if only in an attempt to justify their selling price.

If "salad oils" or rather liquid oils are sold on a business to business (B2B) basis, the customer may specify what antioxidants, etc., he wants to be dissolved into the oil. Salad oils sold as consumer items may contain some antioxidants, such as BHA (butylated hydroxyanisole, E320), BHT (butylated hydroxytoluene, E321), or propyl gallate (E310). Although allowed in the U.S. and quite effective, the antioxidant TBHQ (*t*-butylhydroquinone) is not allowed in Europe. Oils may contain some citric acid (E330) since this is commonly added to oils after they have been deodorised and cooled to below 150°C (Gimeno Villacampa, 1957a).

In addition to liquid oils produced and refined in bulk, such as soya bean oil, canola, sunflower seed oil, corn germ oil, and the oils with local importance like cottonseed oil, rice bran oil, and groundnut oil, the market is also supplied with speciality oils, also referred to as "gourmet oils" (Gimeno Villacampa, 1957b). Examples of these oils are: cold pressed sunflower seed oil, almond oil, hazelnut oil, walnut oil (Dijkstra, 2003), but there are many more and these are often local seed oils sold locally. In general, these vegetable oils are hardly refined at all with the exception of grapeseed oil. As staple oil in Greece, olive oil cannot be called gourmet oil, but in non-Mediterranean areas, it is often regarded as such.

These oils are often used to give a certain cachet to the cuisine. A "salade périgordine" comprising lettuce, warm fried duck's gizzard and bacon cubes, and walnuts may be lubricated with some local walnut oil. A restaurant proclaiming Asian cooking may use some deep red palm olein to distinguish itself from its competitors and most pizzerias will provide customers with a bottle of olive oil spiced with herbs and peppers.

Nowadays, salad oils are usually sold in PET (polyethylene terephthalate) bottles but in some European countries, opaque PVC (polyvinyl chloride) bottles are still used for salad oil. The bottles are blown upstream of the filling line, often to a registered design. Parisons constitute the starting material for the PET bottles, whereas granules can be used for the PVC bottles. In Europe, by far the most common bottle size for oil is 1 litre.

Nontransparent bottles are the most sensible since they prevent light catalysed degradation reactions. However, since most people store their bottles of table oil inside a cupboard anyway, it does not matter that much if the bottle is transparent. Olive oil, being somewhat special, is still also sold in green or clear glass bottles, and the gourmet oils are mainly sold in glass bottles. Cans are also used especially for volumes of more than a litre.

In the kitchen, cooking oils are easier to dispense than butter or margarine, but the latter have the advantage that they contain emulsifiers and milk solids that are essential ingredients of meat gravy, and butter has its unique flavour compound. In addition, when used in shallow frying, emulsions show when they have reached frying temperature since that is when the water has been boiled off. To combine all the advantages listed above, liquid margarines were developed.

Melnick and Josefowicz (1969) did this by preparing and crystallising a fat phase from a mixture of liquid oil, a small amount (<4%) of a vegetable oil that had been hydrogenated to a low (<20) IV, and emulsifiers, and then mixing an aqueous phase comprising skimmed milk, salt, preservatives, and flavourings into the partially crystallised fat phase. Oil separation was only noted after 10 days storage at ambient temperature, but "a few inversions of the bottle were adequate to reestablish a uniform emulsion." A low-fat product with a continuous water phase was also developed (Bosco and Sledzieski, 1984); it contains potassium sorbate as a bactericide.

In addition to the oil separation mentioned above, the aqueous phase in the liquid margarine also may separate and collect at the bottom of the bottle. Increasing the solid fat content of the margarine will make it more viscous and retard these separation processes, but this viscosity makes it more difficult to completely empty the bottle. Accordingly, Wilton and Baurén (1972) disperse gas bubbles in the liquid margarine and note a markedly improved stability with an only moderate increase in product viscosity; they do not provide an explanation. This was subsequently provided by Benjamins et al. (2003), who disperse the gas bubbles in the dispersed aqueous phase, thereby lowering its density and, thus, decreasing the driving force for separation.

Tack and Dijkstra (1982) prevented this phase separation by omitting the aqueous phase. They made a suspension of a spray dried product comprising milk powder, salt, and flavour compounds in a mixture of liquid oil and emulsifiers and attained a stable suspension by including a small amount of fully hydrogenated fat. In a frying test, the product showed very little spattering.

4.6.3.4 Deep frying fats and oils

During deep fat frying, food containing water is brought into contact with fat that is maintained at a temperature well above 100°C. This causes the moisture on the outside of the food to evaporate and thereby dehydrate the outer layer of the food product which becomes crisp. This layer, thus, becomes less lipophobic so that some oil can

penetrate into the food. At the same time, heat transfer from the hot oil to the food product is improved, the inside of the product heats up by conduction and the dehydrated outer layer assumes a temperature that is close to the oil temperature. At this increased temperature, all kinds of reactions, such as dehydration and Maillard reactions, may then take place, causing the crisp outer layer to brown and acquire the taste the consumer is looking for.

When the oil has a high temperature of, for instance, 200°C, the crisping and browning reactions will be fast so that the inside of the food product may not yet be heated through when the outside is already so brown that the product should be taken from the fryer. Accordingly, thick pieces of food like cod fillets, for instance, should not be fried at too high a temperature. When the food product needs to be cooked rather than just reheated, the ensuing residence time also necessitates a moderate temperature to prevent the outside from burning. On the other hand, if the temperature is too low, browning may be very slow, resulting in an almost fully dehydrated product that has absorbed far more oil than intended or desirable. Raising the temperature will increase the rate of browning, but it may have to be raised so much that the food is not hot or properly cooked inside.

Accordingly, there may not be a single deep frying temperature that achieves both heating/cooking and browning, and several ways out of this dilemma have been developed. In Europe, prefried potato chips ("French fries") are produced industrially by frying sliced potatoes at a fairly low temperature; this forms a skin on the chip, cooks it, and also leads to substantial dehydration. These prefried chips are then either sold at refrigeration temperature to restaurants and fast-food eateries as "fresh prefries" or they can be deep-frozen and sold as such to the housewife. After a short, final deep frying treatment at 180°C, the chips are hot, crisp, and ready to serve. Another way out of this dilemma involves blanching and drying the potato slices before deep fat frying (Wilmot, 2005).

During prefrying, the fat will deteriorate, but only slowly. The processor, therefore, aims at a steady state situation whereby he tops up with fresh oil or fat and the prefries act as the purge for the fat degradation products. The fryer contents are only completely replaced with fresh oil or fat when something goes wrong or at plant shut down. In restaurants and in private homes, the situation is quite different. There, the fryer operates at a much higher temperature, may stand idle, and may process different products. This causes the fat to deteriorate so that it has to be replaced earlier.

This replacement can be stepwise. A fish and chip shop that also serves deep fried meat products, such as sausages, chicken, and/or croquettes, for instance, can operate three deep fryers. The first one is used for chips, the second one for the meat products, and the last one for fish. Fresh fat is fed to the first one and fishy fat is discarded from the last one. Multiple use is attained by ladling fat from the first fryer to the second and from the second to the third. Fat quality is judged by smelling the hot fat or by its tendency to foam, and this subjective method works surprisingly well. When the quality of deep frying fat in Belgian catering establishments was investigated, it turned out that they changed their deep frying fat somewhat earlier than the investigators thought to be necessary. Kitchens in hospitals and jails were the exception in that they replaced their fat far later.

Several more objective ways to assess the quality of used frying fat have been developed and some of these involve the use of kits allowing the acidity of the oil to be estimated without titration. However, the most meaningful way is the determination of polar compounds, and measuring the increase of the dielectric constant of the fat with the Food Oil Sensor® has been found to correlate quite closely with the polar compound content of the fat. This Food Oil Sensor® method has the disadvantage that the increase in the dielectric constant can only be calculated if the fresh fat has been measured. It has to be measured for each batch of frying fat because the reading depends on its partial glyceride content. Consequently, it can hardly be used by visiting food inspectors, but for inhouse quality control, it is highly recommended.

When the rate of oxidation of oils and fats is measured as the Oil Stability Index (OSI) with the Rancimat® equipment, large differences are found between the various oils and fats. At 120°C, they range from 2.4 hour for liquid sunflower seed oil, 3.7 hour for liquid soya bean oil, and 4.7 hour for liquid rapeseed oil to over 70 hour for hydrogenated products, such as hydrogenated palm oil with a melting point of 45°C and soya bean oil hardened to a melting point of 44°C (Dijkstra et al., 1995; 1996). These differences are far greater than the differences in frying life experienced in deep fat frying. Liquid oil will be changed more frequently than a partially hydrogenated soya bean oil, but not 20 times more frequently.

There are several reasons for this. During the oil stability measurement, air is blown through the oil. That is an entirely different situation from deep fat frying where air is stripped from the oil by the water vapour originating from the product being fried. The oil temperature is also widely different and the mechanism operating at 120°C may well differ from the prevalent mechanism at 180°C, just as it differs from what happens at ambient temperature, which is why the OSI does not predict shelf life (Lacoste et al., 1999).

Deep fat frying oil may well deteriorate because its foam is exposed to air, and the foam movement and its collapse ensure that fresh oil is continuously exposed to air so that it can absorb oxygen. This tentative mechanism explains why frying oil deterioration is autocatalytic: deterioration causes oil to start foaming and foam causes oxygen to be absorbed and oil to degrade. It also explains

why only 1 ppm (Podmore, 2002b) of dimethyl polysiloxane (E900) can prolong the frying life of the oil: it suppresses foam formation and, thus, reduces oxygen absorption.

The beneficial effect of the use of dimethyl polysiloxane reduces the importance of which oil or fat is used for deep fat frying. Consumers can chose what flavour they prefer, be it coconut or palm oil, provided they ascertain that the label mentions E900. However, producers should make sure that this palm oil does not contain any coconut oil since even small amounts of coconut oil cause oils and fats with longer chain fatty acids to foam (Naudet et al., 1948). This phenomenon has been linked to a heterogeneous triglyceride structure, but no explanation has been provided. Interesterification of a foaming mixture of a lauric oil like coconut oil or palm kernel oil with a long chain oil generates a product that no longer foams (Naudet and Desnuelle, 1951).

Because of the rule that deep-frying fats should not contain more than 2% linolenic acid, soya bean oil and rapeseed oil have to be hydrogenated before they can be sold for deep-frying purposes. This hydrogenation inevitably forms some *trans* isomers that provide the product with some solid fat at ambient temperature. The product can be dispensed by pouring and is opaque because of the many small crystals present that have been formed in a scraped-surface heat exchanger. After having been heated and allowed to cool down again, the fat crystals will be larger and collect at the bottom of the fryer.

4.6.4 Mayonnaise and dressings

Mayonnaise and dressings are oil-in-water (o/w) emulsions. Consequently, their water phase is continuous and, thus, prone to microbiological intrusion that might lead to spoilage. The latter can be prevented by a high salt concentration, a high acidity (pH 3.0-3.5), or a combination of both. Accordingly, vinegar is an essential ingredient of mayonnaise, salad dressings, and similar sauces. In the U.S., the vinegar is often cider based, whereas in the U.K., malt vinegar is highly appreciated. Not surprisingly, the vinegar used in France is based upon white wine; lemon juice can also be used. In addition to salt and vinegar, the mayonnaise will contain egg yolks as an emulsifier, mustard or mustard oil, and perhaps some pepper or other seasoning. Whereas cookbooks hardly ever mention sugar as an ingredient for mayonnaise, industrially produced mayonnaise invariably contains some sugar.

Mayonnaise has higher oil content than salad dressing and sauces. Originally, the minimum oil content prescribed was between 65 and 80 wt %, but this has been harmonised to at least 70 wt% vegetable oil and 5 wt% egg yolk (Dubruille, 1996). Given the density difference between the heavy aqueous phase and the much lighter oily phase, the volume fraction of the oil is more than 83%. This is more than can be attained by a close packing of spheres and, consequently, the oil droplets in mayonnaise are somewhat distorted. Macroscopically, this distortion causes the mayonnaise to behave like a Bingham plastic (Stauffer, 2002). It also means that incorporation of some additional oil into the mayonnaise will make it stiffer. Mayonnaise with a high yield value is very suitable for garnishing dishes since it prevents it from collapsing or losing its sharp edges after piping.

Such a high yield value makes it difficult to mix the mayonnaise evenly in a salad, so attaining the lubricity required necessitates extending the continuous aqueous phase, which results in a dressing. This then has to be stabilised by including a cooked starch (Premlal Ranjith, 2002), or thickeners (Podmore, 2000). Sauces and sandwich spreads are also water-continuous emulsions. They may include a whole range of ingredients like tomato purée, cheese (Roquefort), and/or all kinds of chunky vegetable derived ingredients having no relation to lipid science and technology.

With respect to the oil to be used in mayonnaise and the like, the purchasing specifications for sunflower seed oil, canola, and soya bean oil given by Dubruille (1996) list maximum contents of nickel (0.1 ppm), iron (0.1 ppm), and copper (0.01 ppm), and maximum values for microbiological parameters, but surprisingly, the cold stability is not specified. However, this is the most important quality parameter of oil to be used in mayonnaise and similar products. It must have been dewaxed or winterised (O'Brien, 2000) except for use in French dressing (vinaigrette), which is basically oil, vinegar, and seasonings (Podmore, 2002b). The only emulsifier allowed in mayonnaise is egg yolk.

4.6.5 Chocolate and confectionery products

The production of cocoa butter has been described in Section 3.3.3. In Section 4.1, it is mentioned that hydrogenation enabled the development of *trans* isomer rich intermediate products that on fractionation yield cocoa butter replacers (CBRs). With respect to interesterification, enzymatic catalysis (Section 4.3.3) mentions cocoa butter equivalents (CBEs) as the first product made by this process and, finally, Section 4.4.4.2 discussing fractionation products also mentions cocoa butter substitutes (CBSs) as an additional product used in chocolate and confectionery product manufacture. Some of their characteristics are mentioned in Table 4.37.

These various confectionery fats have been developed because of the fundamental imbalance stemming from the composition of chocolate: it contains additional cocoa butter and, thus, makes surplus cocoa powder available. The mass balance shown in Table 4.38 illustrates this for both plain chocolate and milk chocolate.

The table assumes that 100 parts cocoa beans lead to the production of 40 parts cocoa butter and 40 parts cocoa powder (Kattenberg, 1996). Table 4.38 shows that

TABLE 4.37 Characteristics of confectionery fats

Property	Cocoa Butter (CB)	CB Equivalents (CBEs)	CB Replacers (CBRs)	CB Substitutes (CBSs)
Starting material	Cocoa nibs	Fats with already high content of SMUTs	Blend of vegetable oils	Lauric oils (palm kernel oil)
Primary treatment	Grinding	Fractionation to obtain stearine or midfraction	Hydrogenation (*trans* equilibrium)	Dry fractionation
Secondary treatments	Deodorisation	Deodorisation blending	Fractionation, deodorisation	Hydrogenation, deodorisation
Compatibility with CB	Complete (by definition)	Good	Reasonable	Very poor
Crystal polymorph	β_2-3	β	β'	β'
Tempering	Yes	Yes	No	No

Note: SMUT = symmetrical monounsaturated triglyceride.

TABLE 4.38 Mass balance of chocolate production (per 100 parts of chocolate)

Ingredient	Dark Chocolate		Milk Chocolate	
Cocoa Butter Equivalents (CBE)	**Without**	**With**	**Without**	**With**
Cocoa mass	40	40	13	13
Additional cocoa butter (CB)	12	7	20	15
CBE	–	5	–	5
Whole milk powder	–	–	20	20
Sugar	48	48	47	47
Mass equivalent of added CB	24	14	40	30
Total amount cocoa mass	64	54	53	43
Surplus cocoa powder	12	7	20	15

producing 100 parts of chocolate requires between 64 parts cocoa mass (for dark chocolate without CBE) and 43 parts of cocoa mass (for milk chocolate with CBE). This production of 100 parts chocolate also leads a surplus of 7 parts cocoa powder in the case of dark chocolate containing CBE and 20 parts cocoa powder in the case of milk chocolate not containing CBE. By incorporating up to 5% CBE in chocolate, this surplus cocoa powder decreases by up to 5 parts per 100 parts chocolate.

The European Union (EU) Chocolate Directive 2000/36 also defines chocolate compositions and the vegetable fats that may be used to replace up to 5% of the cocoa butter in products that may still be called "chocolate" (Stewart and Kristott, 2004); fats obtained by enzymatic interesterification, therefore, are not allowed. As shown in Table 4.39, these vegetable fats all have a naturally high content of symmetrical monounsaturated triglycerides (SMUT) and, thus, resemble cocoa butter in chemical terms.

Almost 80% of the triglycerides in cocoa butter are SMUT, so that cocoa butter is far closer to being a reasonably pure compound than other fats and especially animal fats. Accordingly, the behaviour of the crystalline phase of cocoa butter can be described as if it were a pure compound and, like a pure compound, it exhibits melting point depression by other compounds. This explains the lack of compatibility between for example a lauric CBS and cocoa butter. Individually, both fats melt sharply at body temperature, but a mixture of the two forms a eutectic, which has a much lower melting point and also a much flatter SFC profile. Milk fat has a similar effect on cocoa butter and causes milk chocolate to melt at a lower temperature than plain chocolate, and to be softer and break with less of a snap. The phase behaviour of confectionery fats is quite different from the behaviour of fat blends for margarine or shortening. Those blends have a single crystalline phase that is in equilibrium with an oily phase and the amount of crystals left is determined by their melting point profile. CBEs crystallise like cocoa butter and, thereby, hardly affect the physical properties of the chocolate like sharp melting, gloss, shrinkage on solidifying, and snap on breaking.

This table clearly shows that only illipé butter has such a high SMUT content that it does not require fractionation before it can be included as a CBE in chocolate without lowering the SMUT content of the chocolate. Palm oil will require two or more fractionation steps to produce some kind of midfraction, whereas for the other four fats, a single fractionation step to isolate the stearin fraction will suffice (Best et al., 1960).

The various CBEs also differ in the POP/SOS ratio. Whereas illipé butter is relatively rich in POS, palm oil is rich in POP, and SOS is the main triglyceride in the other four fats that may be used for CBE production. CBEs are produced by blending a suitable palm midfraction with an SOS-rich fraction. The blend ratio affects the CBE properties and also the properties of the chocolate containing the CBE. Accordingly, a rather soft South American cocoa butter can be improved by an SOS-rich CBE, which is then referred to as a cocoa butter improver (CBI). SFC values have been listed in Table 4.40 for a number of cocoa butters from different origins and their fractions do indeed show quite large differences.

The treatment of the cocoa bean involving fermentation, drying, shipment, storage, breaking, winnowing, dutching, drying, roasting, and grinding, which transforms the beans into cocoa liquor or cocoa mass, has been described in Sections 2.2.3 and 3.3.3. Chocolate manufacture then

TABLE 4.39 Symmetrical monounsaturated triglyceride (SMUT) composition and content of fats permitted for use in chocolate

Fat	Origin	POP	POS	SOS	SMUT
Cocoa butter	Africa, So. America, Malaysia	16	38	23	77
Palm oil	Malyasia, Indonesia	25	3	trace	28
Illipé butter	Borneo (Indonesia)	9	29	42	80
Shea butter	West Africa	1	6	30	37
Kokum gurgi fat	India	trace	13	41	54
Mango kernel fat	India	1	13	41	56
Sal fat	India	2	11	48	61

Note: P = palmitic acid; O = oleic acid; S = stearic acid

TABLE 4.40 Solid fat content (SFC) values of cocoa butter

Origin	N_{20}	N_{25}	N_{30}	N_{35}
Brazil	59	50	27	0
Malaysia	82	78	59	3
Cameroon	71	64	39	0
Ivory Coast	76	70	47	1
West African (stearin)	91	85	73	16
West African (olein)	48	36	0	0

Source: Kattenberg, H.R. (1996), Functional properties of cocoa butter in relation to the origin of the cocoa beans and processing, *Paper presented at the SCI Seminar Production and Application of Confectionary Fats, 15-16 Oct., London* 1996, 1–11 and Padley, F.B. (1997), in *Lipid Technologies and Applications*, Gunstone, F.D. and Padley, F.B., Eds., Marcel Dekker, New York, 391–432.

entails the addition of cocoa butter, which in all likelihood has been deodorised (Kellens and De Greyt, 2006), to the cocoa liquor, plus sugar and minor ingredients like lecithin and vanilla, and, of course, milk solids in the case of milk chocolate. The milk solids may be added as whole milk powder or as skimmed milk powder and anhydrous milkfat or even a milkfat fraction.

Very fine sugar is used, but this still has to be ground so that it is not perceived as a particle in the mouth when the cocoa butter in the chocolate has melted. For this purpose, a set of rollers mounted one above the other is used; in the trade, this particular grinding or rolling process is also referred to as "refining." The sugar particle size aimed for is typically 20 to 30 μm (Beckett, 1999). Extra cocoa butter and/or CBEs can also be added at this stage, i.e., after rolling, and then the mixture is subjected to a prolonged period of heating while it is also slowly agitated. This stage is known as "conching" and it assists the flavour development of the chocolate (Lipscomb, 1954).

In another chocolate manufacturing process, milk crumb is used. In this process, whole milk is dehydrated under vacuum at an elevated temperature in the presence of sugar or sugar and cocoa mass to yield crumb, which is then mixed with the remaining chocolate components, such as cocoa butter, CBE, and minor ingredients before being refined and conched (Padley, 1997). After conching, the liquid chocolate is tempered and dispensed into moulds or used in an enrobing process, before being cooled to induce final crystallisation.

No tempering is required when using cocoa butter replacers or substitutes and as shown by Table 4.41 the recipes for compound chocolate using the lauric CBS minimise the amount of cocoa butter itself; all the butter in the recipe originates from the low-fat cocoa powder, which contains only 10 to 12% cocoa butter. After the ingredients have been mixed and refined in a roller mill, a short conching treatment is given for flavour development. The CBR is compatible with a certain amount of cocoa butter, but even so, the amount of cocoa liquor in the recipe is kept low.

Apart from in mouldings and coatings, confectionery fats are also used in centre filling creams, in biscuits, and chocolates. In the latter case, migration of fats from the filling cream to the enrobing chocolate can cause problems like softening and bloom formation. Using filling fats with a relatively high SMUT content makes them less incompatible with the chocolate coating and, thus, reduces the risk of fat bloom (Lonchampt and Hartel, 2004).

Another chocolate product is chocolate spread (Iseli, 1977). The viscosity of this product (its spreadability) should not vary too much with temperature. Therefore, the product should have a low cocoa butter content and be based on cocoa powder with a low (<10%) butter content. In addition the spread will contain a liquid vegetable oil, such as, for instance, groundnut oil, milk solids, finely ground sugar, flavouring, and optionally hazelnuts. Lecithin is the preferred emulsifier. Low calorie spreads have also been described (Benesh, 2002).

4.6.6 Miscellaneous

Because of the enormous and increasing variety of foods available, the food uses of oils and fats are also varied and expanding. A number of main categories have been discussed above, but even so, there remain quite a few miscellaneous applications, some of which are discussed below.

4.6.6.1 Pan-release agents

Pan-release agents are mixtures comprising oils or fats and emulsifiers and sometimes also an aqueous phase. They are used in baking to facilitate the ease of releasing food from baking containers and trays. Since they affect the

TABLE 4.41 Compound chocolate recipes

Ingredient	With Cocoa Butter Replacers				With Cocoa Butter Substitutes			
	Dark	Milk		White	Dark	Milk		White
Cocoa liquor	10	10	0	0	0	0	0	0
Cocoa powder	15	0	5	0	14	5	7	0
Whole milk powder	0	6	0	20	0	10	0	0
Skim milk powder	0	12	17	5	6	8	19	20
CBR (high *trans*)	28	28	34	30				
CBS (lauric)					32	32	29	32
Sugar	47	44	44	45	48	45	45	48
Total	100	100	100	100	100	100	100	100
Total fat content	35.0	35.2	34.7	35.4	33.5	35.2	29.9	32.2
CB as % age of total fat	20.0	15.6	1.4	0	4.2	1.4	2.3	0
Total cocoa solids	25	10	5	0	14	5	7	0

Source: Stewart, I.M. and Timms, R.E. (2002), in *Fats in Food Technology*, Rajah, K.K., Ed., Sheffield Academic Press, Sheffield, U.K., 159–191.

percentage of broken biscuits, their economic impact is considerable. Moreover, "in continuous commercial baking processes, the prevention of sticking or adhesions of the bread product to the bread pan surface is most important so as to avoid interruption of the smooth flow of automatic equipment. When sticking occurs, manual removal of the bread products is usually required, which often either damages the pan or the bread product and sometimes both." (Gawrilow, 1975). These agents should preferably be able to withstand baking temperatures and obviate deposits of charred incrustations on the baking pans. For the release property, emulsifiers are used and charring is counteracted by avoiding extreme unsaturation.

Lecithin was modified by acetylation and hydroxylation (Szuhaj, 1975) and unsaturation of the accompanying oil was avoided by using synthetic triglycerides with saturated medium chain fatty acids (Szuhaj and Yaste, 1978) or an interesterification product also containing some longer chain fatty acids (Teran et al., 2004). A mixture of monodiglycerides and ethoxylated monodiglycerides was used by Gawrilow (1975), whereas mixing the monodiglycerides with a polysorbate was advocated by Strouss (1985). A very simple and surprising formulation comprises only deoiled lecithin and oil (Paul and Stoltz, 2004).

4.6.6.2 Bread improvers

According to Stauffer (2002), the main use of monoglycerides in the U.S. is in margarine production, but half that amount is used in yeast-raised goods for the retardation of staling. Like any straight chain aliphatic compound, the monoglyceride forms a lipid–starch complex (Krog, 1971) and when in the lamellar mesophase, the monoglyceride is especially effective as a starch complexing agent (Krog and Jensen, 1975).

Diacetyl tartaric acid esters of monoglycerides (DATEM) are widely used to increase the final loaf volume. Stearoyl lactate provides bread with a very regular crumb structure and is very suitable for bread for toasting. Modern bread improvers also contain enzyme preparations with xylanolytic activity, carbohydrate or pyranose oxidase, or serine protease (Arnaut et al., 2003). However, these products fall outside the scope of the present work.

4.6.6.3 Fat flakes and powders

Fat flakes are made with a slowly rotating cooling drum onto which some fat solidifies when it passes through a bath of molten fat. Liquid fat is entrained onto the surface and allowed to crystallise fully before it is removed from the drum by a scraper blade. This blade also bends the coat of solid fat so that it breaks into flakes. Rapid and complete crystallisation is essential in this process, and it can only be applied to high melting fats like, for example, fully hydrogenated fats. Inclusion of hydrogenated coconut or palm kernel oils can assist in achieving the required percentage of solids (Podmore, 2002a).

Powdered fats are similar to flaked fats, but they are produced by chilling in a spray tower. In this process, the particle size of the droplets to be crystallised is very critical. If the droplets are large, they will fall more rapidly than smaller droplets so that they reach the bottom of the tower more quickly. Because more latent heat has to be removed from large droplets than from small ones, the large ones may still be insufficiently crystallised when they reach the bottom of the tower. Subsequent crystallisation will liberate additional latent heat and may lead to the formation of clumps. If the droplets are too small, there may be so many droplets that some of them are without a nucleus to initiate crystallisation. These droplets remain liquid when falling to the bottom of the tower where they moisten other crystallised droplets and become crystallised themselves to act as a cement for the others.

Fat powder is a different product in that it contains other constituents besides fat, such as milk powder, starch, or dextrin (Podmore, 2002a). Fat powder is made by spray drying an emulsion comprising the various ingredients. Because of the carrier present, the fat in the fat powder may have a lower SFC than is necessary for powdered or

flaked fat. Accordingly, the fat may be milk fat or preferably a stearin fraction of milk fat and the fat content of the powder can be as high as 80 wt %.

Powdered fat and fat powder are easy to handle, dose, and mix into other powdery ingredients. Powdered lauric fats are used in ice cream mixes. Powdered fats are also used in instant soups, cake mixes, coffee whiteners, etc. They are also used in bread improvers, whereby the emulsifiers in these improvers has been mixed with the fat before this was made into a powder product.

4.6.6.4 Peanut butter

Peanut butter produced shortly after World War II was similar to products presently sold in health food shops in that on storage, solids settle at the bottom of the jar and an oily layer floats on top of the sediment. Mixing the two invariably leads to oil spilling over the rim of the jar, which creates a most unwelcome mess. Consequently, the cure for this problem involved the addition of a small (2.25 to 4 wt %) amount of hydrogenated vegetable oil to warm peanut butter, which was a great improvement (Mitchell, 1951). Subsequently, it was found that 0.5 to 1.0 of 12-hydroxystearic acid or its triglyceride also works wonders (Elliger et al., 1972). Using just a small amount of palm stearin gives the fatty phase in peanut butter an SFI of 2 to 3 at 27°C and is also an effective solution (Gooding, 1975).

References

Achaya, K.T. (1997), Ghee, vanaspati and special fats in India, in *Lipid Technologies and Applications*, Gunstone, F.D. and Padley, F.B., Eds., Marcel Dekker, New York, 369–390.

Adleman, R. and Hartel, R.W. (2001), Lipid crystallisation and its effect on the physical structure of ice cream, in *Crystallization Processes in Fats and Lipid Systems*, Garti, N. and Sato, K., Eds., Marcel Dekker Inc., New York, 329–356.

Alphen, J. van (1964), De Chevreul à Mège Mouriès, *Oléagineux*, **19**, 525–528.

Anderson, M. et al. (1977a), Changes during storage instability and composition of ultra-heat-treated aseptically-packed cream of 18% fat content, *J. Dairy Res.*, **44**, 111–123.

Anderson, M. et al. (1977b), Extending shelf life of UHT creams, *J. Soc. Dairy Technol.*, **30**, 229–232.

Aneja, R. (1972), Process for separating phosphatides, US Patent 3,704,254, assigned to Unilever.

Arnaut, F. et al. (2003), Method and composition for the prevention or retarding of staling and its effect during the baking process of bakery products, PCT Patent Application WO 03/084334 A2, assigned to Puratos.

Barlow, S. (2004), Raw material supply issues within the fish oil industry, *Paper presented at the 3rd EuroFedLipid Congress,* Edinburgh, Abstracts page 5.

Battino, R. et al. (1968), The solubilities of seven gases in olive oil with reference to theories of transport through the cell membrane, *J. Am. Oil Chem. Soc.*, **45**, 830–833.

Beckett, S.T., Ed. (1999), *Industrial Chocolate Manufacture and Use*, 3rd ed., Basil Blackwell, Oxford, U.K.

Bell, R.J. et al. (1969), Liquid shortening process, US Patent 3,455,699, assigned to Anderson Clayton & Co.

Benesh, Y. (2002), Chocolate spread, PCT Patent Application WO 02/30212 A2, assigned to Olivia Gourmet (1996) Ltd.

Benjamins, J. et al. (2003), Pourable water and oil containing emulsions comprising gas bubbles, US Patent 6,589,587 B1, assigned to Unilever.

Best, R.L. et al. (1960), Improvements relating to cocoa butter substitutes, British Patent 827,172, assigned to Unilever.

Binley, G.N. et al. (2005), Frozen ice confection, US Patent 6,955,829 B2, assigned to Good Humor–Breyers Ice Cream, division of Conopco Inc.

Bosco, P.M. and Sledzieski, W.L. (1984), Low-fat, butter-flavored, liquid spread and process, US Patent 4,468,408, assigned to Nabisco Brands Inc.

Busken, D. (1993), Bakery goods with liquid shortening system, US Patent 5,254,356, assigned to Bunge Foods Corporation.

Darling, D.F. and Dicks, E. (1982), Artificial cream composition and method of preparation, US Patent 4,360,535, assigned to Unilever.

De Greyt, W.F.J. and Huyghebaert, A.D. (1993), Food and non-food applications of milk fat, *Lipid Techn.*, **5**, 138–140.

De Lathauwer, R. et al. (1980), Process for the directed interesterification of a triglyceride oil or oil mixture, European Patent 0 009 207, assigned to N.V. Vandemoortele International.

Delfosse, J.K.F. (1971), Margarine fat composition, British Patent 1 244 868, assigned to Unilever.

Del Vecchio, A.J. (1996), High-laurate canola, *inform*, **7**, 230–243.

Dell, W.J. et al. (1981), Cream-containing frozen whipped topping composition, US Patent 4,251,560, assigned to General Foods Corporation.

Dijkstra, A.J. (2000), Edible oils in Europe, *inform*, **11**, 386–394.

Dijkstra, A.J. (2003), My visit to a walnut oil mill, *inform*, **14**, 755–756.

Dijkstra, A.J. (2005), No solution yet to the linolenic acid mystery, *inform*, **16**, 283.

Dijkstra, A.J. et al. (1995), Interpreting oil stability data, in *Oils-Fats-Lipids 1995, Proceedings of the 21st ISF Congress*, Castenmiller, W.A.M., Ed., P.J. Barnes & Associates, Bridgewater, England, 629–637.

Dijkstra, A.J. et al. (1996), Interpreting the oil stability index, *Oléagineux Corps Gras Lipides/OCL*, **3**, 378–386.

Donald, A.J.S. and Lewis, M.J. (1987), Ice-cream formulation, British Patent Application 2 191 676 A, assigned to Franesk Limited.

Dubruille, D. (1996), Huiles et mayonnaises, *Oléagineux Corps Gras Lipides/OCL,* **3**, 36–38.

Elliger, C.A. et al. (1972), Thickening action of hydroxystearates in peanut butter, *J. Am. Oil Chem. Soc.*, **49**, 536–537.

Elliott, P.W. et al. (1999), Edible spread, US Patent 5,869,124, assigned to Unilever.

EU (1994), Council Directive (EU) No 2991/1994 of 5 December 1994 defining product categories of fatty spreads, *Off. J.*, L 316/2, 09/12/1994.

Farrall, A.W. (1946), Butter manufacture, US Patent 2,406,819, assigned to The Creamery Package Mfg. Company.

Fondu, M.P.V. and Willems, M.A.G. (1972), Margarine fat containing randomized fat component, US Patent 3,634,100, assigned to Unilever.

Fresenius, C. (1902), Verfahren zur Verbesserung naturbutterähnlicher Speisefette, German Patent 142 397, assigned to Reeser Margarine-Fabrik G.m.b.H.

Gawrilow, I. (1975), Hydrated pan release agents, US Patent 3,906,117, assigned to SCM Corporation.

Gawrilow, I. (1980), Hydrated stable fluent shortening containing solid phase emulsifier components, US Patent 4,226,894, assigned to SCM Corporation.

Gimeno Villacampa, M. (1957a), Estabilización de los aceites refinados de soja, oliva y algodón con ácido cítrico, *Grasas Aceites*, **8**, 162–165.

Gimeno Villacampa, M. (1957b), Stabilisation à l'acide citrique des huiles raffinées de soja, d'olive et de coton, *Rev. Franç. Corps Gras*, **4**, 559–559.

Gooding, C.M. (1975), Peanut butter, US Patent 3,882,254, assigned to CPC International Inc.

Graffelman, H.A. (1971), Margarine fat and process for preparing same, US Patent 3,617,308, assigned to Unilever.

Gudheim, A. (1939), Blending edible fats, US Patent 2,174,365, assigned to Unilever.

Haighton, A.J. and Kattenberg, H.R. (1974), Interesterification process, US Patent 3,855,254, assigned to Unilever.

Haighton, A.J. and Mijnders, A. (1968), Preparation of liquid shortening, US Patent 3,395,023, assigned to Unilever.

Hartel, R.W. (1996), Applications of milk-fat fractions in confectionary products, *J. Am. Oil Chem. Soc.*, **73**, 945–953.

Hawley, H.K. and Dobson, R.D. (1956), Molecular rearrangement process, US Patent 2,733,251, assigned to The Procter & Gamble Company.

Hawley, H.K. and Holman, G.W. (1956), Directed interesterification as a new processing tool for lard, *J. Am. Oil Chem. Soc.*, **33**, 29–35.

Herstad, O. (1979), Waste fat in fat-containing bleaching earth as feed for chickens, *Acta Agricul. Scand.*, **26**, 87–93.

Hidaka, H. et al. (2002), Oil-and-fat feedstock for production of cream and low-oil cream composition, US Patent 6,497,914 B1, assigned to Fuji Oil Company Ltd.

Holemans, P.M.J. et al. (1988), Fat and edible emulsions with a high content of *cis*-polyunsaturated fatty acids, US Patent 4,791,000, assigned to Unilever.

Holman, G.W. and Going, L.H. (1959), Rapid directed interesterification of glycerides, US Patent 2,875,066, assigned to The Procter & Gamble Company.

Horneman, H.C. et al. (1949), Dairy process, US Patent 2,466,896, assigned to Sugar Creek Creamery Company and Cherry-Burrell Corporation.

Huizinga, H. et al. (1999), Process for making a margarine hardstock, US Patent 5,858,445, assigned to Unilever.

Huizinga, H. et al. (2000), Process for making a margarine hardstock blend, US Patent 6,156,370, assigned to Unilever.

Iseli, W. (1977), Method of producing a milk chocolate flavoured spread, British Patent 1 488 305, assigned to Cadbury Typhoo Limited.

Kattenberg, H.R. (1974), Beschleunigung der gelenkten Umesterung mittels periodischer, variabler Temperaturführung, *Fette Seifen Anstrichm.*, **76**, 79–82.

Kattenberg, H.R. (1996), Functional properties of cocoa butter in relation to the origin of the cocoa beans and processing, *Paper presented at the SCI Seminar Production and Application of Confectionary Fats, 15-16 Oct., London 1996,* 1–11.

Kattenberg, H.R. and Verburg, C.C. (1977), Plastic fat product, US Patent 4,055,679, assigned to Unilever.

Kattenberg, H.R. and Verhagen, A.M. (1976), Margarine fat, US Patent 3,956,522, assigned to Unilever.

Kaylegian, K.E. (1999), Contempory issues in milk fat fractionation, *Lipid Technol.*, **11**, 132–136.

Kellens, M.J. and De Greyt, W.F.J. (2006), Vapour scrubbing process and apparatus, European Patent Appication 1 637 201 A1, assigned to De Smet Engineering N.V.

Kessler, H.G. (1981), *Food Engineering and Dairy Technology*, (translated by M. Wotzilka), Verlag A. Kessler, Freising, Germany.

Krog, N. (1971), Amylose complexing effect of food grade emulsifiers, *Starch/Stärke*, **23**, 206–210.

Krog, N. and Jensen, B.N. (1975), Interaction of monoglycerides in different physical states with amylose and their antifirming effects in bread, *J. Food Technol.*, **5**, 77–87.

Kubota, H. et al. (1974), Whippable topping composition and process for producing same, US Patent 3,840,682, assigned to Fuji Oil Company Ltd.

Lacoste, F. et al. (1999), Comparison of Rancimat stability test and ambient storage of edible oil, *Paper presented at the 23rd ISF World Congress,* Brighton, U.K.

Lauridsen, J.B. (1976), Food emulsifiers: Surface activity, edibility, manufacture, composition, and application, *J. Am. Oil Chem. Soc.*, **53**, 400–407.

Lipscomb, A.G. (1954), Ungelöste Probleme bei der Herstellung von Nahrungsmitteln: Schokolade, *Fette Seifen Anstrichm.*, **56**, 803–809.

List, G.R. (2004), Processing and reformulation for nutrition labelling of *trans* fatty acids, *Lipid Technol.*, **16**, 173–177.

List, G.R. et al. (1995), Preparation and properties of zero *trans* soybean oil margarines, *J. Am. Oil Chem. Soc.*, **72**, 383–384.

Lonchampt, P. and Hartel, R.W. (2004), Fat bloom in chocolate and compound coatings, *Eur. J. Lipid Sci. Technol.*, **106**, 241–274.

Mattikow, M. (1953), Antispattering margarine, US Patent 2,640,780, assigned to Benjamin Clayton, doing business as Refining Unincorporated.

Mattil, K.F. and Nelson, D.W. (1953), Process of producing modified lard, US Patent 2,625,483, assigned to Swift & Company.

Melnick, D. and Gooding, C.M. (1960), A highly nutritional margarine, US Patent 2,921,855, assigned to Corn Products Company.

Melnick, D. et al. (1953), Soya bean oil compositions, US Patent 2,627,469, assigned to The Best Foods Inc.

Melnick, D. and Josefowicz, E.L. (1969), Process for preparing liquid margarine, US Patent 3,472,661, assigned to Corn Products Company.

Meziane, J. (2001), Process for producing an ice confection, US Patent 6,299,923 B1, assigned to Nestec S.A.

Mège, H. (1869), Production de certains corps gras d'origine animale, French Patent 86 480.

Mishkin, A.R. et al. (1970), Powdered ice cream mix, British Patent 1 207 294, assigned to Nestlé's Products Limited.

Mitchell Jr., P.J. (1950), Permanently pumpable oleaginous suspension, US Patent 2,521,242, assigned to The Procter & Gamble Company.

Mitchell Jr., P.J. (1951), Nut butter manufacture, US Patent 2,562,630.

Moustafa, A. (1995a), Consumer and industrial margarines, in *Practical Handbook of Soybean Processing and Utilization*, Erickson, D.R., Ed., AOCS Press, Champaign, IL, 339–362.

Moustafa, A. (1995b), Salad oil, mayonnaise and dressings, in *Practical Handbook of Soybean Processing and Utilization*, Erickson, D.R., Ed., AOCS Press, Champaign, IL, 314–362.

Mulder, H. and Walstra, P. (1974), *The Milk Fat Globule, Emulsion Science as Applied to Milk Products and Comparable Foods*, Pudoc, Wageningen and CAB, Farnham Royal, U.K.

Naudet, M. and Desnuelle, P. (1951), Influence exercée par l'interesterification sur le pouvoir moussant des mélanges coprah (ou palmiste)-arachide, *Oléagineux*, **6**, 338–339.

Naudet, M. et al. (1948), Sur l'origine des mousses dans les mélanges d'huiles d'arachide et de coprah, *Bull. mens. ITERG*, 32–24.

Normann, W. (1903), Process for converting unsaturated fatty acids or their glycerides into saturated compounds, British Patent 1 515, assigned to Herforder Maschinenfett-und Ölfabrik Leprince und Siveke.

Norris, M.E. (1976), Preparing fluid shortening for use in yeast raised bakery products, US Patent 3,943,259, assigned to SCM Corporation.

Norton, I.T. and Underdown, J. (1992), Water-in-oil dispersion and process for preparing such dispersion, US Patent 5,151,290, assigned to Unilever.

O'Brien, R.D. (1995c), Soybean oil products utilization: Shortenings, in *Practical Handbook of Soybean Processing and Utilization*, Erickson, D.R., Ed., AOCS Press, Champaign, IL, 363–379.

O'Brien, R.D. (2000), Liquid oils technology, in *Introduction to Fats and Oils Technology*, O'Brien, R.D., Farr, W.E., and Wan, P.J., Eds., AOCS Press, Champaign, IL, 463–485.

Opfer, W.B. (1978), Margarine production — a review of modern manufacturing processes, *Chem. Ind.*, 681–687.

Padley, F.B. (1997), Chocolate and confectionary fats, in *Lipid Technologies and Applications*, Gunstone, F.D. and Padley, F.B., Eds., Marcel Dekker, New York, 391–432.

Pardun, H. (1972), Phosphatide emulsifiers, US Patent 3,661,795, assigned to Unilever.

Pardun, H. (1988), *Die Pflanzenlecithine*, Verlag für chemische Industrie H. Ziolkowsky KG, Augsburg.

Paul, L. and Stoltz, E.I. (2004), Pan spray formulation and delivery system, US Patent Application 2004/0191373.

Podmore, J. (2000), Applications of edible oils, in *Edible Oil Processing*, Hamm, W. and Hamilton, R.J., Eds., Sheffield Academic Press, Sheffield, U.K., 190–218.

Podmore, J. (2002a), Bakery fats, in *Fats in Food Technology*, Rajah, K.K., Ed., Sheffield Academic Press, Sheffield, U.K., 30–68.

Podmore, J. (2002b), Culinary fats: solid and liquid frying oils and speciality oils, in *Fats in Food Technology*, Rajah, K.K., Ed., Sheffield Academic Press, Sheffield, U.K., 333–359.

Poot, C. and Biernoth, G. (1994), Margarine and butter production, in *The Lipid Handbook*, 2nd ed., Gunstone, F.D., Harwood, J.L., and Padley, F.B., Eds., Chapman & Hall, London, 288–295.

Premlal Ranjith, H.M. (2002), Water continuous emulsions, in *Fats in Food Technology*, Rajah, K.K., Ed., Sheffield Academic Press, Sheffield, U.K., 69–122.

Price, J.E. (1989), Pourable shortening and process for its preparation, US Patent 4,889,740, assigned to Beatrice/Hunt-Wesson, Inc.

Pronk, J.N., Soltau, M., and Wieske, T. (1986), Fat having butter-like properties and a reduced tendency to develop graininess and process for producing such, US Patent 4,590,087, assigned to Unilever.

Reid, E.J. and Morgan Jr., P.W. (1974), Pourable liquid shortening, US Patent 3,857,985, assigned to Hunt-Wesson Foods Inc.

Robinson, D.J. and Rajah, K.K. (2002), Spreadable products, in *Fats in Food Technology*, Rajah, K.K., Ed., Sheffield Academic Press, Sheffield, U.K., 192-227.

Rossen, J.L. (1970), Fluid shortening, US Patent 3,528,823, assigned to Unilever.

Sassen, C.L. and Wesdorp, L.H. (2001), Edible fat spread, US Patent 6,238,723, assigned to Unilever.

Schaffer, P.S. and Haller, H.S. (1943), The solubility of gases in butter oil, cottonseed oil, and lard, *Oil & Soap*, **20**, 161–162.

Schee, G.L. van der (1997), Lamination fat product, European Patent Application 0 786 209 A1, assigned to Unilever.

Schijf, R. et al. (1984), Margarine fat blend, and a process for producing said fat blend, US Patent 4,486,457, assigned to Unilever.

Seiden, P. (1967), Corandomzed margarine oils, US Patent 3,353,964, assigned to The Procter & Gamble Company.

Seiden, P. (1970), Process for vacuum distilling randomly interesterified triglycerides to produce novel triglyceride fractions, US Patent 3,494,944, assigned to The Procter & Gamble Company.

Sreenivasan, B. (1975), Margarine, US Patent 3,859,447, assigned to Unilever.

Stauffer, C.E. (2002), Emulsifiers and stabilizers, in *Fats in Food Technology*, Rajah, K.K., Ed., Sheffield Academic Press, Sheffield, U.K., 228–274.

Stewart, I.M. and Kristott, J. (2004), European Union Chocolate Directive defines vegetable fats for chocolate, *Lipid Technol.*, **16**, 11–14.

Stewart, I.M. and Timms, R.E. (2002), Fats for chocolate and sugar confectionary, in *Fats in Food Technology*, Rajah, K.K., Ed., Sheffield Academic Press, Sheffield, U.K., 159–191.

Strouss, O.L. (1985), Pan release agent and its preparation, US Patent 4,547,388.

Szuhaj, B.F. (1975), Pan release product and process, US Patent 3,928,056, assigned to Central Soya Company.

Szuhaj, B.F. and Yaste, J.R. (1978), Pan release agent, US Patent 4,127,419, assigned to Central Soya Company.

Tack, A. and Dijkstra, A.J. (1982), Frying oil composition and process of production, European Patent 0 045 504, assigned to N.V. Vandemoortele International.

Teran, P.L. et al. (2004), Low-residue, easy-cleaning and low-viscosity structured lipid pan release compositions and methods, US Patent Application 2004/0115332 A1.

Ullanoormadam, S.R. (2004), Trans free hard structural fat for margarine blend and spreads, US Patent 6,808,737, assigned to Premium Vegetable Oils Berhad.

Vaghela, M. et al. (2005), Aerated frozen product, US Patent 6,890,577 B2, assigned to Nestec S.A.

Vander Wal, R.J. and Van Akkeren, L.A. (1951), Modified lard and process of producing same, US Patent 2,571,315, assigned to Armour and Company.

Varvil, R.D. (1982), Fluid shortening, US Patent 4,335,157, assigned to SCM Corporation.

Voelker, T.A. and Davies, H.M. (1992), Plant medium-chain thioesterases, PCT Patent Application WO 92/20236 A1, assigned to Calgene, Inc.

Walstra, P. (2006), Fat crystallization, in *Food Structure and Behaviour*, Blanshard, V.M.V. and Lillford, P., Eds., Academic Press, London, 67.

Walstra, P. et al. (1999), *Dairy Technology*, Marcel Dekker, New York.

Ward, J. (1982), Edible fat product, US Patent 4,341,812, assigned to Nabisco Brands Inc.

Wieske, T. et al. (1968), Phosphatide anti-spattering agents for margarine, British Patent 1,113,241, assigned to Unilever.

Willock, J.T. (1974), Whippable composition, US Patent 3,785,831, assigned to General Foods Limited.

Wilmot, K. (2005), Reduced fat chip and manufacturing process, US Patent Application 2005/0220973, assigned to McCain Foods Limited.

Wilton, I.E.M. and Baurén, K.L.E. (1972), Pourable margarine, US Patent 3,682,656, assigned to Margarinbolaget AB.

Yoneda, S. et al. (2000), Method of dry fractionating fat and stationary crystallization apparatus, EP Patent 1 028 159, assigned to Fuji Oil Company Ltd.

Zobel, F.A. et al. (1984), Process for whipping an emulsion, US Patent 4,478,867, assigned to General Foods Corporation.

5

SYNTHESIS

M. S. F. Lie Ken Jie, J. L. Harwood and F. D. Gunstone (with W. H. Cheung and C. N. W. Lam)

5.1 Unsaturated fatty acid synthesis via acetylene

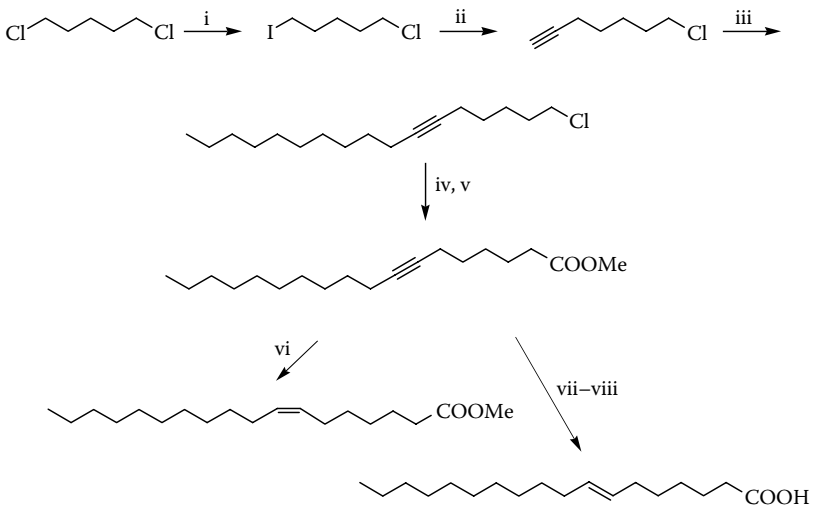
The need for the laboratory synthesis of unsaturated fatty acids arises when these acids do not occur naturally, are found only at low level in obscure sources, or when an isotopically labelled sample is required. Many synthetic procedures have been employed and those based on the reactivity of acetylene (ethyne) and its derivatives have been widely exploited. The first synthesis of an unsaturated C_{18} fatty acid, oleic [18:1(9*c*)], was recorded in 1934 (Noller and Bannerot, 1934). With the emergence of the methylene-interrupted (skipped) polyenoic fatty acids as a physiologically important group of fatty acids, extensive use of the acetylenic route and later via the Wittig reaction (first applied to fatty acid synthesis by Bergelson and Shemyakin (1964)), led to the production of numerous polyunsaturated fatty acid isomers for physical, chemical, and biological studies. Linoleic acid [18:2 (9*c*,12*c*)] (an essential fatty acid) was first synthesised in 1950 (Raphael and Sondheimer, 1950).

Synthesis strategies for unsaturated fatty acids during the 1960s and 1970s were aimed at the development of efficient and stereoselective methodologies. The first total synthesis of arachidonic acid (AA) [20:4(5*c*,8*c*,11*c*,14*c*)] was reported by Osbond's group (1961) and of eicosapentaenoic acid (EPA) [20:5(5*c*,8*c*,11*c*,14*c*,17*c*)] by Pabon et al. (1965). With the application of the Wittig reaction for *cis*-olefination, the most popular strategy today for the synthesis of different geometric isomeric polyunsaturated fatty acids is a mixed approach involving *cis*-reduction of an acetylenic intermediate followed by chain extension (*cis*-olefination) by the Wittig reaction (see Section 5.2). The synthesis of unsaturated fatty acids has been reviewed by Kunau (1973), Sprecher (1979), and Vatèle (1999).

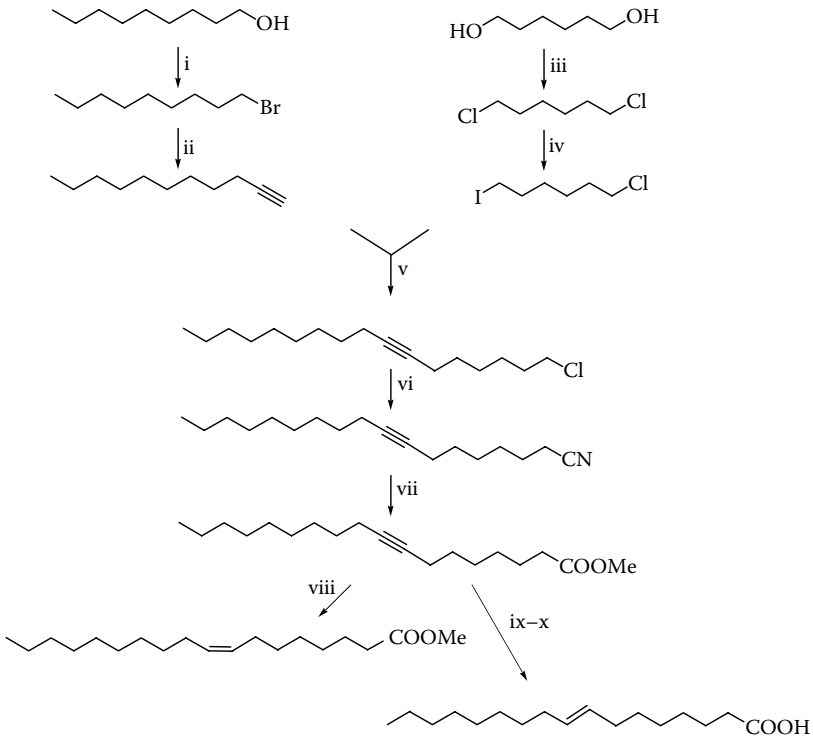
The usefulness of acetylene (ethyne) and its derivatives depends on the ease with which such compounds can be alkylated (Normant and Alexakis, 1981) and subsequently reduced stereospecifically to *cis*-alkenes (Marvell and Li, 1973) with Lindlar catalyst (Lindlar, 1952) or to the *trans*-alkene by sodium or lithium reduction in liquid ammonia.

Acetylene (ethyne) can be converted to its mono-sodium or mono-lithium salt by reaction with sodium or lithium amide, which is readily produced by dissolving the metal in anhydrous liquid ammonia containing a trace of Fe(III) nitrate. The liquid ammonia acts as reagent and solvent. Bubbling anhydrous acetylene through the solution of metal amide in liquid ammonia gives the mono-sodium or mono-lithium acetylide exclusively. The acetylide is next condensed with an alkyl bromide or iodide to yield the corresponding 1-alkyne. The latter is then treated with a 1.2 molar quantity of sodium or lithium amide in liquid ammonia and the resulting sodium or lithium acetylide from the 1-alkyne is further condensed with an ω-bromo-1-alkanol or an α,ω-chloro-bromo/iodo-alkane to yield 1-hydroxy-alkyne or 1-chloro-alkyne, respectively. 1-Hydroxy-alkyne is then oxidized (chromic acid) to the requisite alkynoic acid. 1-Chloro-alkyne is treated with sodium cyanide and then with anhydrous HCl in methanol to give the corresponding one-carbon extended methyl ester product. Most of the positional isomers of octadecynoic

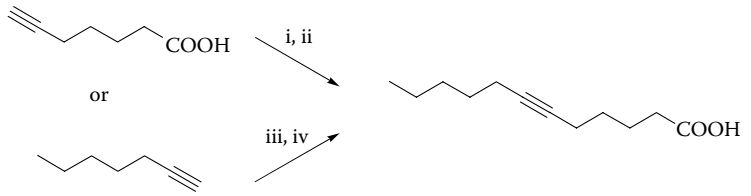
acid (C_{18} acetylenic fatty acids and the corresponding *cis*- and *trans*-olefinic acids) were prepared by this method as illustrated by the synthesis of methyl octadec-7-ynoate, methyl octadec-8-ynoate, (Gunstone and Ismail, 1967) (Schemes 5.1(i) and (ii)) and dodec-6-ynoic acid (Barve and Gunstone, 1971) (Scheme 5.1(iii)). Modification is



SCHEME 5.1 (i) *Synthesis of methyl octadec-7-ynoate, methyl octadec-7-cis-enoate and octadec-7-trans-enoic acid.* (Adapted from Gunstone, F.D. and Ismail, I.A. (1967) *Chem. Phys. Lipids,* **1**, 209–224.)
Reagents: i, NaI/acetone; ii, NaC≡CH, NH_3; iii, $NaNH_2$, NH_3, $CH_3(CH_2)_9Br$; iv, KCN/DMSO; v, HCl(g), MeOH; vi, H_2, Lindlar catalyst/quinoline; vii, KOH, H_3O^+; viii, Li, distilled NH_3



SCHEME 5.1 (ii) *Synthesis of methyl octadec-8-ynoate, methyl octadec-8-cis-enoate and octadec-8-trans-enoic acid.* (Adapted from Gunstone, F.D. and Ismail, I.A. (1967) *Chem. Phys. Lipids*, **1**, 209–224.)
Reagents: i, HBr, H_2SO_4; ii, NaC≡CH, NH_3; iii, $SOCl_2$; iv, NaI/acetone; v, $NaNH_2$, NH_3; vi, KCN/DMSO; vii, HCl(g), MeOH; viii, H_2, Lindlar catalyst/quinoline; ix, KOH, H_3O+; x, Li, distilled NH_3



SCHEME 5.1 (iii) *Synthesis of dodec-6-ynoic acids.* (Adapted from Barve, J.A. and Gunstone, F.D. (1971) *Chem. Phys. Lipids,* **7**, 311–323.)
Reagents: i, BuLi, HMPA; ii, $CH_3(CH_2)_4Br$; iii, BuLi, HMPA; iv, $Br(CH_2)_4COOH$

necessary when the unsaturated centre is close to either end of the molecules.

Semihydrogenation of chromatographically purified acetylenic fatty ester to the *cis*-olefinic fatty ester is best achieved with hydrogen over Lindlar's catalyst (Lindlar, 1952) in ethyl acetate. In all cases, the presence of a little quinoline enhances the selectively to yield >98% of the *cis*-isomer. Another effective hydrogenation catalyst is the borohydride-reduced nickel (P-2 Ni) developed by Brown and Ahuja (1973), which reduces alkynes to *cis*-alkenes selectively. This catalyst was used by Nunn et al. (1992) in the synthesis of leukotrienes (a class of conjugated polyunsaturated fatty acids). Conversion of an acetylenic fatty acid to the *trans* olefinic fatty acid can be readily achieved with sodium or lithium dissolved in pure liquid ammonia (by condensing gaseous ammonia with solid CO_2 in a cold finger). *Cis*-olefinic fatty esters can also be transformed (stereomutation) to the corresponding *trans*-olefinic fatty ester by refluxing the *cis*-olefinic ester in dioxan with a catalytic amount of freshly prepared sodium *p*-toluenesulphinic acid (Snyder and Scholfield, 1982).

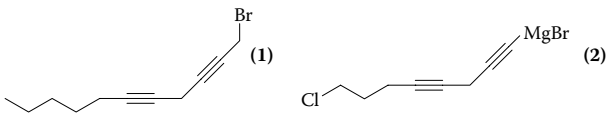
Another method is to expose the *cis*-olefinic fatty acid or ester to 2-mercaptoethanol in *iso*-propanol under ultraviolet light as was used to obtain the all-*trans*-arachidonic acid from arachidonic acid or its methyl ester (Anagnostopoulos et al., 2005). Impurities present after reduction may include saturated acids, traces of the undesired geometric isomers (*cis* or *trans*), which are best removed by silver-ion silica chromatography. *Trans* fatty acids can be purified by crystallization when available in sufficiently large quantities.

Gunstone and Ismail (1967) and Barve and Gunstone (1971) prepared all the octadecynoic acids and from them all the *cis*- and *trans*-octadecenoic acids. Lie Ken Jie and Lam (1974) synthesised all positional isomers of methyl undecynoate and *cis*-undecenoate. Gilman and Holland (1974) synthesised all the C_{10}, C_{11}, C_{12}, C_{13}, and C_{14} monoynoic acids, while Pomonis and Hakk (1990) prepared long-chain monoene acids (C_{24} and C_{28}). Monoynoic acids can also be produced from olefinic acids by partial synthesis *via* bromination followed by dehydrobromination (KOH/EtOH) (Gunstone and Hornby, 1969; Lie Ken Jie and Kalluri, 1998). However, this procedure is not suitable for the preparation of poly-ynoic fatty acids from the corresponding poly-enoic acids.

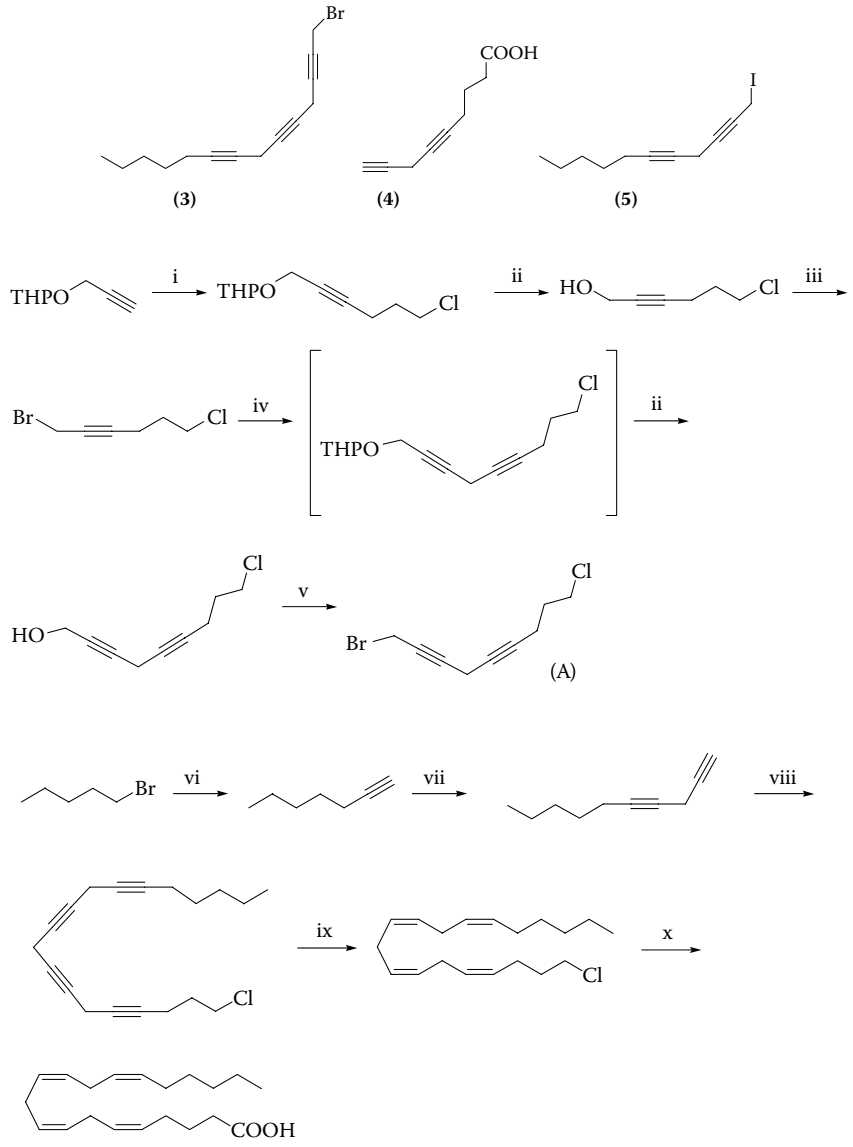
Linoleic acid was first synthesised via acetylenic intermediates in the early 1950s, but it was not until 1961 that a general procedure for obtaining the natural methylene-interrupted polyenoic fatty acids was described. Propargyl alcohol (3-hydroxy-1-propyne) is the ideal three-carbon starting block. This provides an acetylenic bond and a hydroxyl function for the synthesis of methylene-interrupted polyunsaturated fatty acids. One of the first syntheses reported for arachidonic acid [20:4(5*c*,8*c*,11*c*,14*c*)] involved the coupling (via the sodium salt of an acetylenic function) of two methylene-interrupted diacetylenic intermediates to yield a C_{19} 1-chloro-tetrayne. The latter compound was reduced to the all *cis*-C_{19} tetraene and chain extended by one carbon (carbonation via Grignard reaction with CO_2) to give the requisite arachidonic acid (Rachlin et al., 1961) (Scheme 5.1(iv)).

Osbond et al. (1961) prepared arachidonic acid in nine steps from 1-bromo-pentane. Three of the acetylenic bonds were derived through the successive coupling of propargyl alcohol or its tetrahydropyranyl ether derivative. Extension of the C_{14} tri-yne intermediate with hex-5-ynoic acid furnished arachidonic acid (Scheme 5.1(v)).

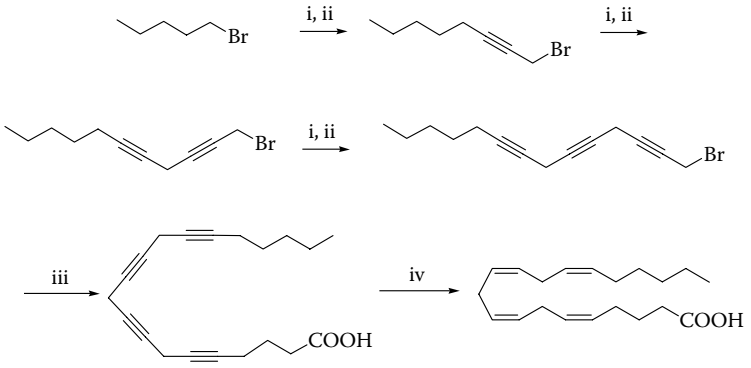
Ege et al. (1961) coupled two methylene interrupted diacetylenic intermediates (1 and 2) to give the C_{19} tetrayne chloride, which on carbonation yielded arachidonic acid in low yield.



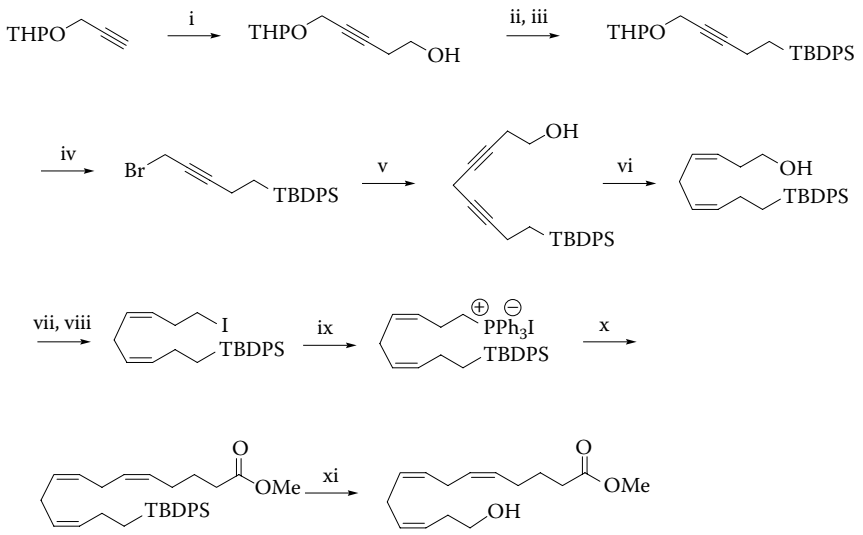
To improve the yield of these multistep reaction sequences to arachidonic acid, Van der Steen et al. (1963) proposed the use of 2,5-hexadiyne-1-ol as a C_6 fragment, which allowed the introduction of two acetylenic bonds in a single step. This approach was exploited by Beerthuis et al. (1971) and by Belosludtsev et al. (1986). Kunau (1971a, 1971b) developed a useful two-step route to 1-bromo-2,5,8-tetradecatriyne (3) for the synthesis of arachidonic acid. A further development led to the production of arachidonic acid from the coupling of a C_9 fragment (4) and a C_{11} fragment (5). This approach was also applied to the synthesis of fatty acids containing five and six olefinic bonds.



SCHEME 5.1 (iv) *Synthesis of arachidonic acid*. (Adapted from Rachlin, A.I., Wasyliw, N., and Goldberg, M.W. (1961) *J. Org. Chem., 26*, 2688–2693.)
Reagents: i, $NaNH_2$, $Br(CH_2)_3Cl$; ii, H_3O^+; iii, PBr_3; iv, THP-$OCH_2C{\equiv}CH$, $NaNH_2$ (THP = tetrahydropyranyl); v, PBr_3; vi, $NaC{\equiv}CH$; vii, $NaNH_2$, $BrCH_2C{\equiv}CH$; viii, $NaNH_2$, A, ix, H_2, Lindlar catalyst/quinoline; x, Mg, CO_2.



SCHEME 5.1 (v) *Synthesis of arachidonic acid*. (Adapted from Osbond, J.M., Philpott, P.G., and Wickens, J.C (1961) *J. Chem. Soc*., 2779–2787.)
Reagents: i, $BrMgC{\equiv}CCH_2OMgBr$, CuCl; ii, PBr_3; iii, $NaC{\equiv}CH(CH_2)_3COONa$, NH_3; iv, H_2, Lindlar catalyst/quinoline



SCHEME 5.1 (vi) *Synthesis of 14-hydroxy-(all-cis-)5,8,11-tetradecatrienoate.* (Adapted from Han, L. and Razdan, R.K. (1998) *Tetrahedron Lett*., **39**, 771–774.)
Reagents: i, *n*-BuLi, THF, ethylene oxide; ii, TBDPSCl, imidazole (TBDPSCl = *tert*-butyldiphenylsilylchloride); iii, Dowex-MeOH; iv, CBr_4, Ph_3P, CH_2Cl_2; v, CuI, *n*-Bu_4NI, Na_2CO_3, DMF, 3-butyn-1-ol; vi, $2Ni(OAc)_2$-$NaBH_4$, H_2; vii, MsCl, Et_3N, CH_2Cl_2; viii, NaI, acetone; ix, Ph_3P, MeCN; x, *n*-BuLi, HMPA, THF, $CH_3OOC(CH_2)_3CHO$; xi, *n*-Bu_4NF-THF, AcOH.

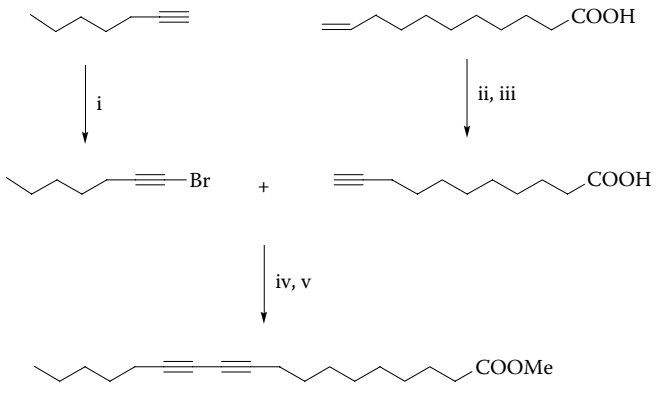
An elegant procedure for preparing 14,15-dihydro-arachidonic acid was based on the coupling of propargylic halides or tosylates with 1-alkynes catalyzed by Cu(I) iodide (Jeffery et al., 1992). Adopting a similar approach 14-hydroxy-(all-*cis*)-5,8,11-tetradecatrienoate, a useful intermediate for the synthesis of arachidonic acid analogues, was prepared by Cu(I) catalyzed propargylic substitution (Han and Razdan, 1998) (Scheme 5.1(vi)).

Several sets of C_{18} *cis,cis*- and *trans,trans*-diene esters were prepared by the acetylenic route where the number of methylene groups between the two unsaturated centres in the isomer varied from 0 to 4 (Gunstone and Lie Ken Jie, 1970; Gunstone, et al., 1971). The complete series of C_{18} dimethylene-interrupted diacetylenic fatty ester isomers was prepared by Lam and Lie Ken Jie (1975) from which the corresponding *cis,cis*- and *trans,trans*-olefinic C_{18} isomers were derived by reduction (Lam and Lie Ken Jie, 1976). Examples in Schemes 5.1(vii-ix) illustrate the methods used in obtaining the diunsaturated fatty esters with varying number of methylene groups between the unsaturated centres.

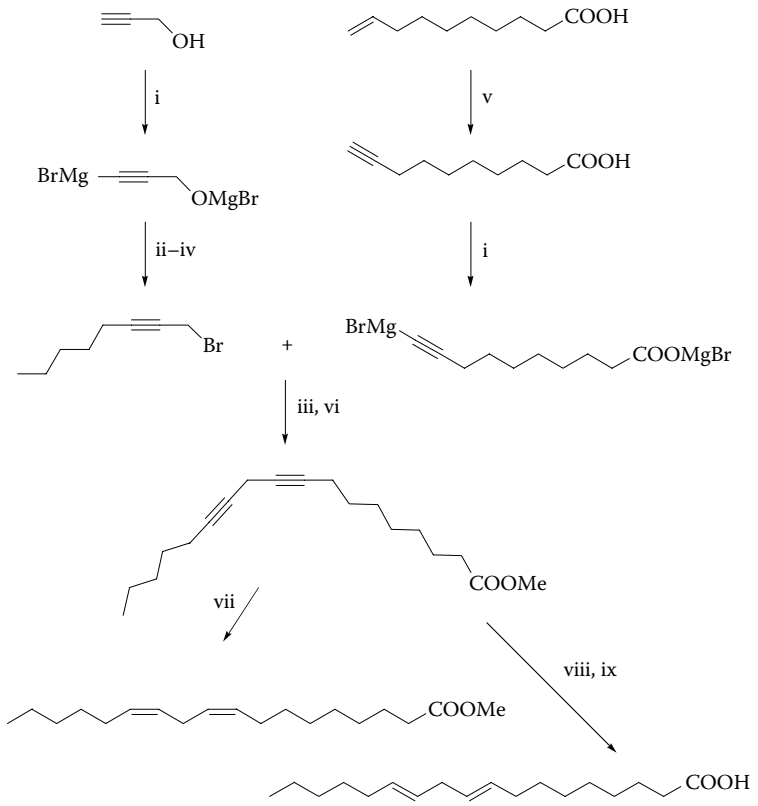
(See References for Section 5.1 at the end of Section 5.5.)

5.2 Fatty acid synthesis by the Wittig reaction

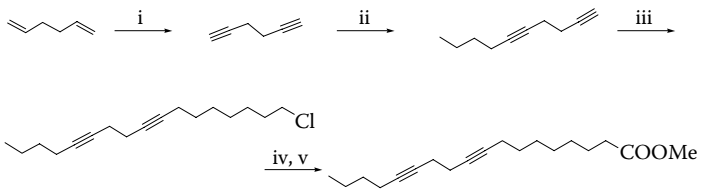
The Wittig reaction is comparable in importance to the acetylenic route as a means of synthesizing olefinic fatty acids and has also been used extensively to prepare the long-chain alcohols and their esters that are important as



SCHEME 5.1 (vii) *Synthesis of methyl octadeca-10,12-diynoate.* (Adapted from Gunstone, F.D. and Lie Ken Jie, M. (1970) *Chem. Phys. Lipids*, **4**, 1–14.)
Reagents: i, NaOBr; ii, Br_2; iii, KOH/EtOH; iv, NH_2NH_2, CuCl, Et_2NH; v, $MeOH/H_3O^+$

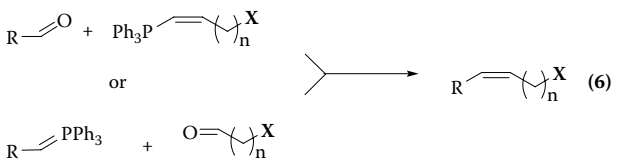


SCHEME 5.1 (viii) *Synthesis of methyl octadeca-9,12-diynoate, octadeca-9-cis,12-cis-dienoate and octadeca-9-trans,12-trans-dienoic acid.* (Adapted from Gunstone, F.D. and Lie Ken Jie, M. (1970) *Chem. Phys. Lipids,* **4**, 1–14.)
Reagents: i, EtMgBr; ii, $CH_3(CH_2)4Br$; iii, CuCl; iv, PBr_3/Et_2O; v, Br_2, KOH/EtOH; vi, BF_3/MeOH; vii, H_2, Lindlar catalysis/quinoline; viii, KOH, EtOH; ix, Li, distilled NH_3



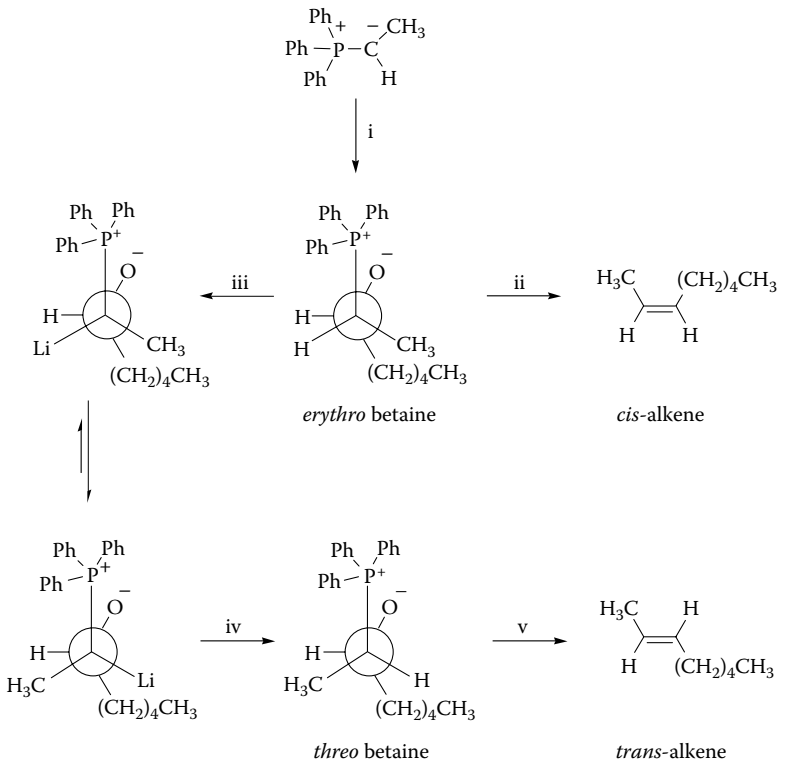
SCHEME 5.1 (ix) *Synthesis of methyl octadeca-9,13-diynoate.* (Adapted from Lam, C.H. and Lie Ken Jie, M.S.F. (1975) *J. Chromatogr.,* **115**, 559–570.)
Reagents: i, Br_2, $NaNH_2$; ii, $NaNH_2$, $CH_3(CH_2)_3Br$; iii, $LiNH_2$, $I(CH_2)_7Cl$; iv, KCN, DMSO; v, HCl(g)/MeOH

insect pheromones. The Witting reaction leads to the alkene (6) by reaction of an aldehyde (or ketone) and an alkyl triphenylphosphonium salt or its ylid (derived from an alkyl bromide). The group X is selected to give the necessary functionality at the end of the long chain.



For effective use of this reaction, it is important to appreciate the factors that influence the configuration of the double bond, of which the most important is the structure of the ylid. Stabilized ylids with an α-double bond or an α-electron-withdrawing group (such as an ester) give the *trans* alkene in >95% purity, while nonstabilized ylids with electron-donating groups (such as alkyl) give *cis*-alkenes in >98% purity. An example of the use of the Wittig reaction in organic synthesis is described in Scheme 5.2(i), which describes the synthesis of *2cis*- and 2-*trans*-octene (Schlosser and Christmann, 1966).

When the reaction is conducted in a polar aprotic solvent (such as dimethylformamide, DMF) the production of *cis*-alkene is favoured. The stereochemistry and mechanism of the Wittig reaction have been reviewed (Vedejs and Peterson, 1994; Murphy and Lee, 1999). Vatèle (1999) has described the various synthetic methods for the preparation of β,γ-unsaturated fatty acids for use in the Wittig reaction.

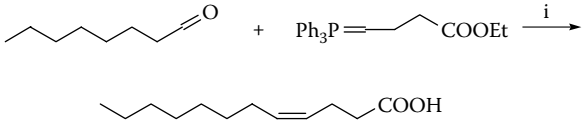


SCHEME 5.2 (i) *Wittig reaction — synthesis of 2-octene.* (Adapted from Schlosser, M. and Christmann, K.F. (1966) *Angew. Chem. Int. Ed. Engl.* **5**, 126.)
Reagents: i, $CH_3(CH_2)_4CHO$, THF; ii, warm; iii, PhLi, THF; iv, HCl, Et_2O; v, *tert*. BuOH.

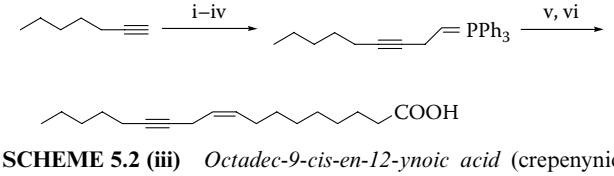
Formation of the ylid requires a suitable base and, while several have been used (see examples in Scheme 5.2(ii) to Scheme 5.2(x)), particularly good results are claimed for sodium *bis*-trimethylsilylamide at –78°C. To obtain highly steroselective *cis*-olefins in Wittig reactions, salt-free ylid solutions are prepared with sodium amide (Bestmann, 1965) or sodium or potassium hexamethyldisilazide (Bestmann et al., 1976; Viala and Santelli, 1988; Labelle et al*.,* 1990) in THF at low temperature, or by generating the ylid with *t*-butyllithium (Bestmann and Stransky, 1974), $LiN(SiMe_3)_2$ or LDA (lithium dimethylamide) (Dussault and Lee, 1995) in HMPT (hexamethylphosphoric triamide) or HMPA (hexamethylphosphoric amide) (Corey et al., 1980).

Aldehydes required for the Wittig reaction are prepared from unsaturated fatty acids or cycloalkenes by ozonolysis, from α,ω-diols by partial oxidation and from α,ω-alkadienes by partial hydroboration followed by oxidation (Bergelson and Shemyakin 1964; Bestmann and Vostrowsky 1979; and Vatèle 1999). Gravier-Pelletier et al. (1990) have reviewed synthetic routes to long-chain hydroxy acids, many of which involve the Wittig reaction.

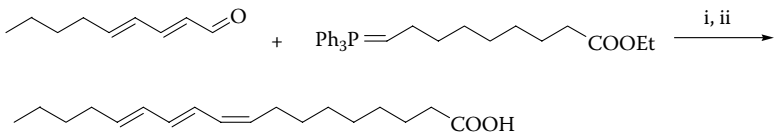
Viala and Santelli (1988) described the preparation of the phosphonium salt ($Ph_3P^+[CH_2]_2CH[O^iPr]_2Br^-$) from acrolein (propenal) and found it to be very effective as a three-carbon homologating agent under Wittig conditions. They converted hexanal to methyl arachidonate in four steps with an overall yield of 58% (Scheme 5.2(viii)). Heitz et al. (1989) followed a similar approach in the synthesis of all-*cis*-1-bromo-4,7,10,13-nonadecatetraene (a precursor to C-1 labelled arachidonic acid), using instead the more readily available diethyl acetal phosphonium salt ($Ph_3P^+[CH_2]_2CH[OEt]_2Br^-$) as the C_3 building block. The β,γ-unsaturated diethyl acetals intermediate from the Wittig coupling were hydrolyzed under very mild conditions (trifluoroacetic acid in chloroform at 0°C) to



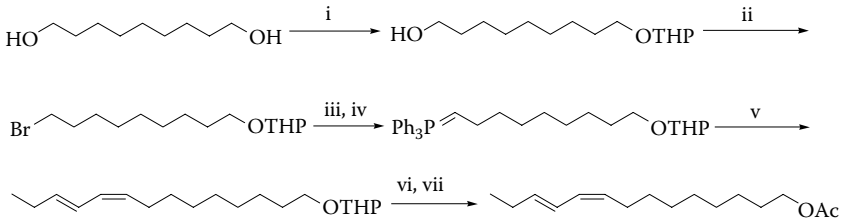
SCHEME 5.2 (ii) *Dodec-4-cis-enoic acid*. (Adapted from Bestmann, H.J. and Vostrowsky, O. (1979) *Chem. Phys. Lipids,* **24**, 335–389.)
Reagent: i, Wittig reaction, ester hydrolysis by KOH, H_3O^+.



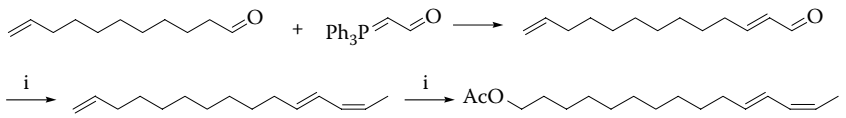
SCHEME 5.2 (iii) *Octadec-9-cis-en-12-ynoic acid* (crepenynic acid). (Adapted from Bradshaw, R.W. et al. (1971) *J. Chem. Soc., C*, 1156–1158.)
Reagents: i, $LiNH_2$, ethylene oxide; ii, PBr_3; iii, PPh_3, C_6H_6; iv, BuLi, Et_2O; v, $MeOOC(CH_2)_7CHO$; vi, KOH, H_3O^+.



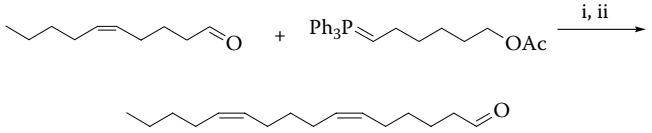
SCHEME 5.2 (iv) *Octadeca-9-cis,11-trans,13-trans-trienoic acid* (catalpic acid). (Adapted from Bergelson, L.D. and Shemyakin, M.M. (1964) *Angew. Chem. Int. Ed.,* **3**, 250–260.)
Reagents: i, DMF, I; ii, KOH, H_3O^+.



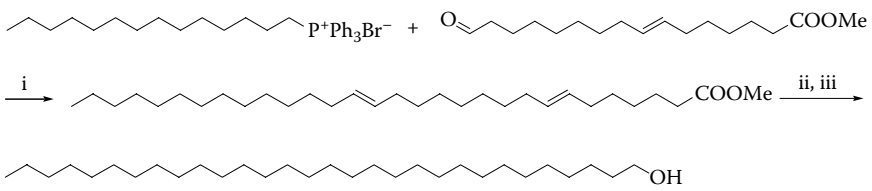
SCHEME 5.2 (v) *Tetradeca-9-cis,11-trans-dienyl acetate.* (Adapted from Hall, D.R., Beevor, P.S., Lester, R., Poppi, R.G. and Nesbitt, B.F. (1975) *Chem. Ind*., 216–217.)
Reagents: i, 2,3-dihydro-*2H* pyran; ii, PBr_3, iii, PPh_3, CH_3CN, K_2CO_3; iv, dimsyl sodium, DMSO; v, $CH_3CH_2CH{=}CHCHO$; vi, MeOH, H_3O^+; vii, Ac_2O, pyridine.



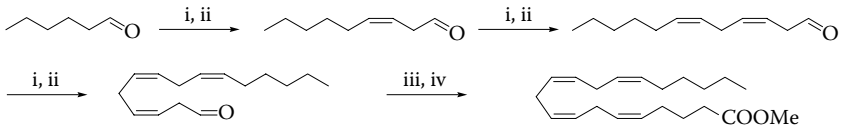
SCHEME5.2 (vi) *Pentadeca-11-trans,13-cis-dienyl acetate.* (Adapted from Bestmann, H.J. and Vostrowsky, O. (1979) *Chem. Phys. Lipids,* **24**, 335–389.)
Reagents: i, $CH_3CH{=}PPh_3$; ii, 9-BBN (9-borabicyclo[3.3.1]nonane); Ac_2O, pyridine.



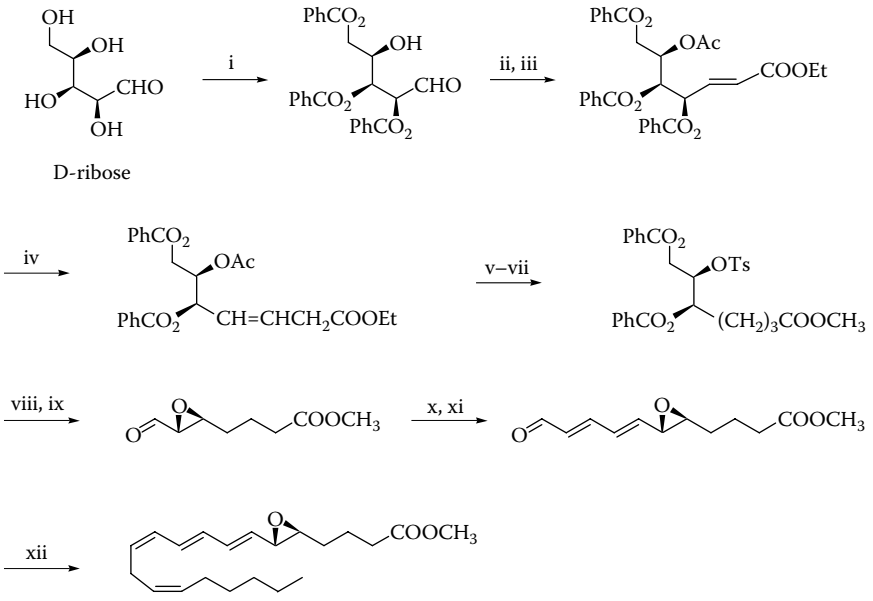
SCHEME5.2 (vii) *Hexadeca-6-cis,11-cis-dienal.* (Adapted from Bestmann, H.J. and Vostrowsky, O. (1979) *Chem. Phys. Lipids,* **24**, 335–389.)
Reagents: i, KOH, H_3O^+; ii, $C_5H_5NHCrO_3Cl$.



SCHEME 5.2 (viii) *Triacontanol.* (Adapted from Subramanian, G.B.V. and Rastogi, A. (1991) *Chem. Ind*., 436–440.)
Reagents: i, K_2CO_3, 1,4-dioxan, formamide (trace); ii, H_2, Pd/C; iii, $LiAlH_4$.



SCHEME 5.2 (ix) *Methyl arachidonate.* (Adapted from Viala, J. and Santelli, M. (1988) *J. Org. Chem.,* **53**, 6121–6123.)
Reagents: i, $Ph_3P^+(CH_2)_2CH(OPri)_2Br^-$, $NaN(SiMe_3)_2$, THF, HMPA; ii, H_2O, TsOH; iii, $Ph_3P^+(CH_2)_4COOH\ Br^-$, $NaN(SiMe_3)_2$, THF, HMPA; iv, CH_2N_2.



SCHEME 5.2 (x) *Synthesis of methyl ester of Leukotriene A*. (Adapted from Corey, E.J., Clark, D.A., Goto, G., Marfat, A., Mioskowski, C, Samuelsson, B. and Hammarström, S., (1980) *J. Am. Chem. Soc.,* **102**, 1436–1439.)
Reagents: i, pyridine, benzoyl chloride, HBr, acetic acid, Na_2CO_3, Ag_2CO_3; ii, Ph_3P=CHCOOEt, benzoic acid, dimethoxyethane; iii, Ac_2O, trace H_2SO_4; iv, Zn amalgam, Et_2O, HCl; v, Pd/C, H_2, MeOH; vi, HCl, MeOH; vii, tosyl chloride, pyridine; viii, K_2CO_3, MeOH; ix, Collin's reagent, CH_2Cl_2; x, LiCH=CHCH=CHOEt, Na_2CO_3; xi, CH_3SO_2Cl, Et_3N, CH_2Cl_2; xii, $I^-Ph_3P^+CHCH_2CH=CH(CH_2)_4CH_3$, *n*-BuLi, HMPA (hexamethylphosphoric acid), THF.

yield the requisite unsaturated aldehyde intermediate for use in the next Wittig coupling reaction.

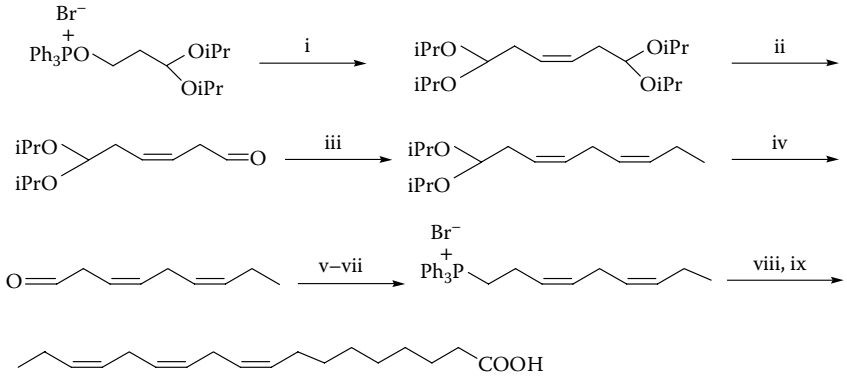
Sandri and Viala (1995) developed two new C_6 *cis,cis*-1,4-diene homologating units, which can react in turn either as ylid or aldehyde in a subsequent Wittig reaction to yield methylene-skipped polyunsaturated fatty acids, such as α-linolenic acid (Scheme 5.2(xi)) and EPA (all-*cis*-5,8,11,14,17-eicosapentaenoic acid) (Viala and Sandri, 1992).

Eynard et al. (1998) developed two new C_6 homologating units ($Bu^tPh_2SiO(CH_2)_2CH$=$CHCH_2CHO$ and $Ph_3P^+(CH_2)_2CH$=$CHCH_2CH(OEt)_2I^-$ from a common intermediate, *cis*-$HO(CH_2)_2CH$=$CHCH_2CH(OEt)_2$, for the synthesis of EPA (Scheme 5.2(xii)). A C_9 building block was employed by Taber and You (1995) in the synthesis of DHA (all-*cis*-4,7,10,13,16,19-docosahexaenoic acid) (Scheme 5.2(xiii)).

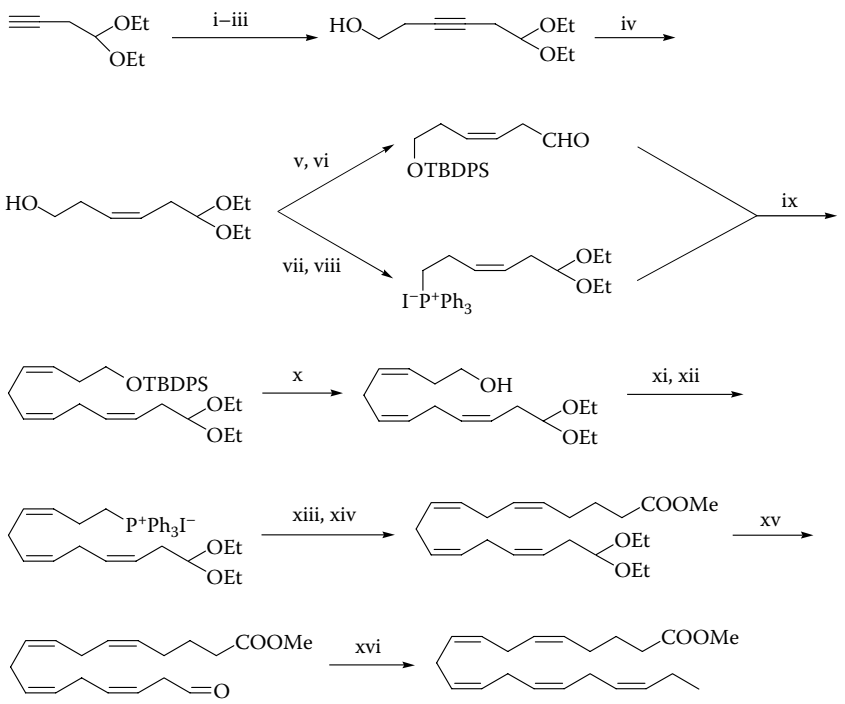
(See References for Section 5.2 at the end of Section 5.5.)

5.3 Isotopically labelled fatty acids

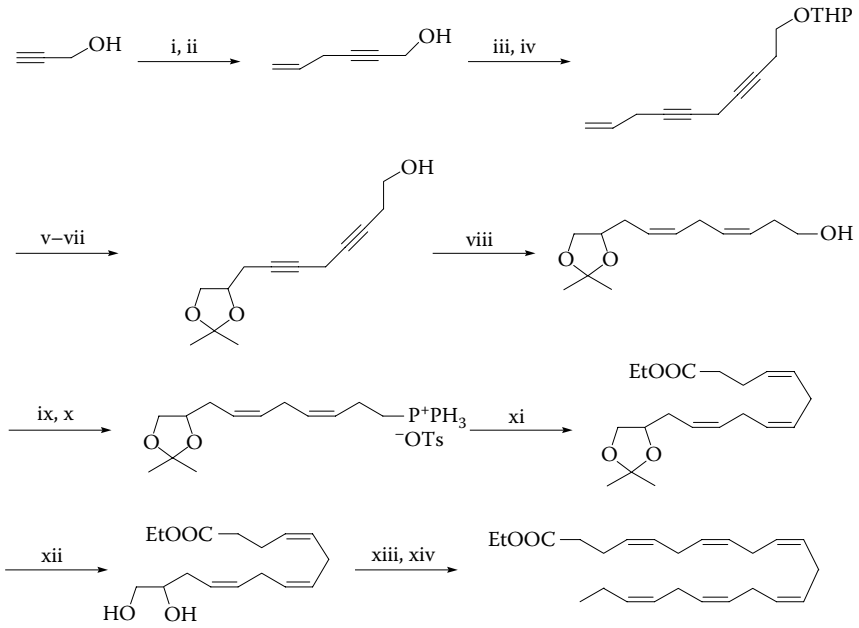
Fatty acids labelled with stable isotopes, such as deuterium (^2H) or carbon-13 (^{13}C) or with radioactive isotopes, such as tritium (^3H) and carbon (^{11}C, ^{14}C), are used in studies of reaction mechanism (e.g., hydrogenation, oxidation), nuclear magnetic spectroscopy, mass spectrometry, lipid biosynthesis, and metabolism. Compounds containing radioactive isotopes (^3H, ^{14}C) are examined by liquid



SCHEME 5.2 (xi) *Synthesis of α-linolenic acid.* (Adapted from Sandri, J. and Viala, J. (1995 *Synthesis*, 271–275.)
Reagents: i, NaN(SiMe$_3$)2, O_2; ii, HCOOH, CH_3COCH_3/H_2O; iii, NaN(SiMe$_3$)$_2$, Ph_3P^+ $^-CHCH_2CH_3$; iv, HCOOH, THF/H_2O; v, $LiALH_4$; vi, Ph_3PBr, pyridine; vii, Ph_3P; viii, NaN(SiMe$_3$)$_2$, $CHO(CH_2)_7COOMe$; ix, LiOH, THF/H_2O.



SCHEME 5.2 (xii) *Synthesis of all-cis-5,8,11,14,17-eicosapentaenoic acid.* (Adapted from Eynard, T. et al. (1998) *J. Labelled Cpd. Radiopharm*, **XLI**, 411–421.)
Reagents: i, *n*-BuLi; ii, $BF_3{\cdot}Et_2O$; iii, ethylene oxide; iv, H_2, Lindlar catalyst/ quinoline; v, TBDPSCl, imidazole (TBDPSCl = *tert*-butyldiphenylsilylchloride); vi, 50% CF_3COOH/CH_2Cl_2; vii, PPh_3, I_2, imidazole; viii, PPh_3; ix, *n*-BuLi, HMPA/THF; x, *n*-Bu_4NF; xi, PPh_3, I_2, imidazole; xii, PPh_3, $CaCO_3/CH_3CN$; xiii, *n*-BuLi, HMPA; xiv, $CHO(CH_2)_3COOMe$; xv, 50% CF_3COOH/CH_2Cl_2; xvi, *n*-$Pr^+PPh_3I^-$, HMPA/THF.



SCHEME 5.2 (xiii) *Synthesis of ethyl all-cis-4,7,10,13,16,19-docosahexaenoic acid.* (Adapted from Taber, D.F. and You, K (1995) *J. Org. Chem.,* **60**, 139–142.)
Reagents: i, EtMgBr, CuBr; ii, $CH_2{=}CHCH_2Br$; iii, TsCl (4-$CH_3C_6H_4SO_2Cl$)/KOH; iv, $BrMgC{\equiv}C(CH_2)_2OTHP$; v, OsO_4, $K_3Fe(CN)_6$; vi, Dowex/MeOH; vii, H_2SO_4/ CH_3COCH_3; viii, P-2 Ni, H_2; ix, TsCl, pyridine; x, PPh_3; xi, $NaN(SiMe_3)_2$/ THF, $CHO(CH_2)_2COOEt$; xii, HCl/THF; xiii, $NaIO_4/H_2O$-CH_2Cl_2/SiO_2; xiv, $PPh_3{}^+OTs^-$-$(CH)_2CH{=}CHCH_2CH{=}CHCH_2CH_3$.

scintillation counting or radio gas chromatography. Non-radioactive labels are studied by mass spectrometry or by nuclear magnetic resonance spectroscopy. Though some labelled acids are commercially available, it is frequently necessary to synthesise these compounds as required, sometimes by suitable adaptation of the synthetic procedures

described in Section 5.1 and Section 5.2. This account is confined to fatty acids labelled with one or more of the hydrogen (^{2}H, ^{3}H) or carbon (^{11}C, ^{13}C, ^{14}C) isotopes.

The subject has been reviewed by Crombie (1996), Kunau (1973), Sprecher (1977), Emken (1978), Tulloch (1979), Rakoff (1982), Westerman and Ghrayeb (1982), and Lie Ken Jie et al. (1997). Adlof (1999) has written a very comprehensive review with lists of over 170 stable isotopically labelled fatty acids and a number of suppliers of isotopically labelled fatty acids. Descomps (1995), Sauerwald et al. (1996), and Demmelmair et al. (1997) have reviewed the use of stable isotopes in the study of human lipid metabolism.

5.3.1 Deuterium-labelled fatty acid

5.3.1.1 Synthesis of deuterium-labelled saturated fatty acids

Depending on the position on the alkyl chain and the number of isotopic labels to be incorporated into the saturated fatty acid, several methods are available. Starting with commercially available deuterated acetic acid, total replacement of the hydrogen atoms of the terminal carbon atom (ω-carbon) of the alkyl chain by deuterium is achieved by the Kolbe reaction with diacids (Klok et al., 1974) (Scheme 5.3.1(i)).

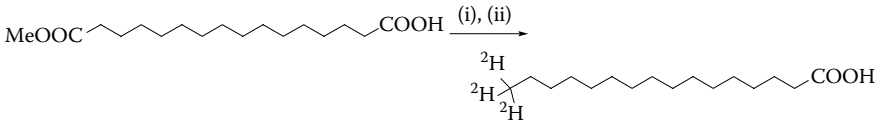
A versatile procedure has been developed by treating the tosyl derivative of ω-hydroxy-fatty acids with sodium borodeuteride or labelled lithium dimethyl cuprate to substitute one of the three hydrogens of the resulting methyl group by a deuterium atom. This method is also used to label the terminal methyl group with other isotopes (^{3}H, ^{13}C or ^{14}C) (Tamvakopoulos and Anderson, 1990) (Scheme 5.3.1(ii)).

Wilkinson's catalyst [$(Ph_3)_3RhCl(I)$] and deuterium gas have been used to reduce olefinic or acetylenic fatty acid methyl esters to saturated, deuterium-labelled fatty esters. Reduction of methyl linolenate by this method furnished methyl octadecanoate-[9,10,12,13,15,16-$^{2}H_6$] (Rakoff, 1982). Lie Ken Jie and Choi used deuterated picolinyl esters of polyunsaturated fatty esters obtained by a similar procedure to determine the positions of double bonds by mass spectrometric analysis (Lie Ken Jie and Choi, 1992). When other metal catalysts are used instead of Wilkinson's catalyst extensive isotopic scrambling occurs. Acidic hydrogens *alpha* to carbonyl or keto groups are readily exchanged with deuterium by using Na and $CH_3O^{2}H$ (Aasen et al., 1970) or $^{2}H_2O$ and pyridine (Tucker et al., 1971).

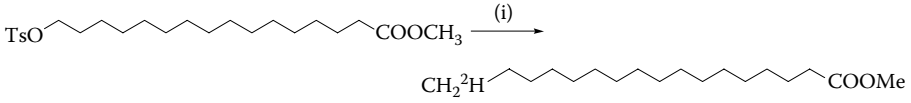
To incorporate deuterium atoms at a specific position in the alkyl chain, reduction of the methyl ester function of a fatty ester with lithium aluminum deuteride gives the corresponding 1,1-$^{2}H_2$-alkanol, which is converted to the bromide and chain extended to the required deuterated fatty acid (DasGupta et al., 1982) (Scheme 5.3.1(iii)).

5.3.1.2 Synthesis of deuterium-labelled unsaturated fatty acids

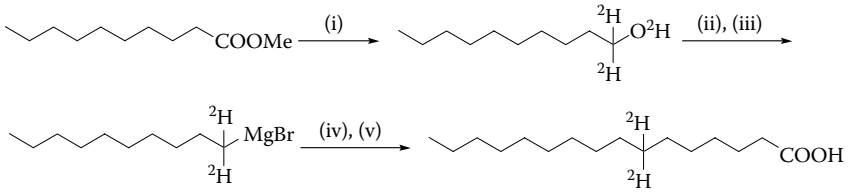
The most successful way of preparing deuterium-labelled unsaturated fatty acids of the *cis*-configuration with ^{2}H atoms on the olefinic carbons is to reduce the corresponding acetylenic ester with deuterium gas in the



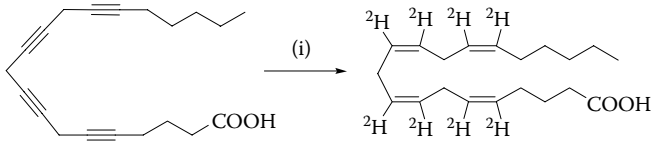
SCHEME 5.3.1 (i) *Synthesis of hexadecanoic acid-16-$^{2}H_3$.* (Adapted from Klok, R. et al. (1974) *Recl. Trav. Chim. Pays-Bas,* **93**, 222–224.)
Reagents: i, $C^{2}H_3COO^{2}H$; ii, 2Na (-2e).



SCHEME 5.3.1 (ii) *Synthesis of hexadecanoic acid-16-$^{2}H_1$.* (Adapted from Tamvakopoulos, C.S. and Anderson, V.E. (1990) *J. Labelled Cpd. Radiopharm.*, **28**, 187–191.)
Reagent: i, NaB_2H_4.



SCHEME 5.3.1 (iii) *Synthesis of palmitate-7-$^{2}H_2$.* (Adapted from DasGupta, S.K. et al. (1982) *J. Lipid Res.,* **23**, 197–200.)
Reagents: i, $LiAl^{2}H_4$; ii, HBr; iii, Mg; iv, $Br(CH_2)_5COOMgCl$; v, H_3O+.



SCHEME 5.3.1 (iv) *Synthesis of deuterated arachidonic acid.* (Adapted from Taber, D.F. et al. (1982) in Methods in Enzymology, Academic Press, New York, pp. 366–369.)
Reagent: i, $^2H_2$, Lindlar catalyst.

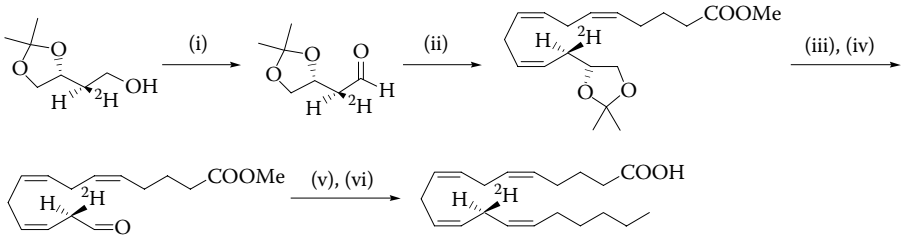
presence of Lindlar catalyst. Taber et al. (1982) prepared labelled arachidonic acid (all-*cis*-5,8,11,14-eicosatetraenoic acid-5,6,8,9,11,12,14,15-$^2H_8$) from the corresponding acetylenic precursor (Scheme 5.3.1(iv)).

To probe the reaction mechanism of prostaglandin H synthase, Peng et al. (2002) synthesised both 13(R)- and 13(*S*)-deuterium-labelled arachidonic acids in high enantiomeric purity (Scheme 5.3.1(v)).

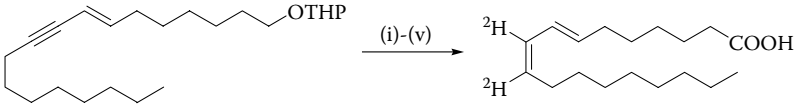
Other useful catalysts reported in the reduction of acetylenic intermediates to the corresponding *cis*-olefinic fatty acid are *bis*-(2-deuteriocyclohexyl)borane-B-^{2}H (Svatos, et al.,1994; Tamvokopoulos and Anderson, 1990), deuterated disiamylborane/acetic acid-d_4 for the synthesis of a conjugated linoleic acid (CLA) isomer, *viz.* 7-*trans*,9-*cis*-octadecadienoic acid–9,10-$^2H_2$ (Broustal and Loreau, 2004) (Scheme 5.3.1(vi)) and P-2 Ni (Taber et al., 1982).

In the synthesis of methyl 9-*cis*,11-*trans*- and 9-*trans*,11-*trans*-octadecadienoate-17,17,18,18-$^2H_2$ (CLA isomers), two key intermediates were prepared: *viz. trans*-2-nonenyl-8,8,9,9-$^2H_2$ bromide and MeOOC$(CH_2)_7$CHO (from the reductive ozonization of methyl oleate). Wittig coupling of the two intermediates furnished a mixture of deuterated CLA isomers, which were separated by reversed-phase and silver resin chromatography. Once a ^{2}H-labelled compound or an intermediate has been made, it can be incorporated into a long-chain acid by chain extension (via the nitrile, by malonation, or by enamine synthesis) or by involvement in a Wittig coupling synthesis as illustrated in the synthesis of CLA isomers (Scheme 5.3.1(vii)) (Adlof, 1997).

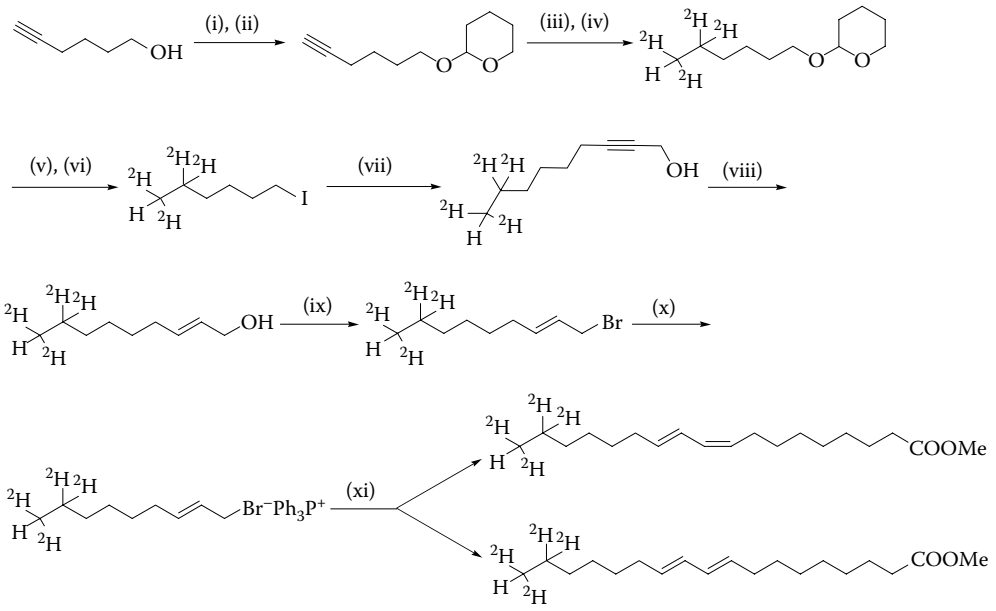
Reduction of acetylenic acids with lithium aluminium deuteride in ^2H_2O gives the corresponding *trans*-olefinic-$^2H_2$ fatty acid. This method was successfully applied to the synthesis of β-parinaric acid, (all-*trans*)-octadeca-9,11,13,15-tetraenoic acid-9,10,11,12,13,14,15,16-$^2H_8$ (Goerger and Hudson, 1988). Replacement of all hydrogen atoms of a long chain fatty acid by deuterium yields the corresponding perdeuterated fatty acid. This reaction can be accomplished by reaction of the fatty acid with either PtO_2 in ^2H_2O (Hsiao et al., 1974), ^2H_2O, PtO_2 and Na_2O_2 (Dinh-Nguyen et al., 1972) or the potassium salt of the fatty acid with ^2H_2O, PtO_2 and Na_2O_2 (Reeves et al., 1979).



SCHEME 5.3.1 (v) *Synthesis of 13(R)- and 13(S)-deuterium-labelled arachidonic acids.* (Adapted from Peng, S. et al. (2002) *J. Am. Chem. Soc*., **124**, 10786–10796.)
Reagents: i, PCC; ii, NaHMDS; iii, BF_3-HOAc; iv, $Pb(OAc)_4$; v, $BrPh_3P(CH_2)_5CH_3$; vi, LiOH, CH_3OH, THF.



SCHEME 5.3.1 (vi) *Synthesis of a deuterium labelled conjugated linoleic acid* (*7-trans,9-cis-octadecadienoic-9,10-2H_2 acid*). (Adapted from Broustal, G. and Loreau, O. (2004) *J. Labelled Cpd. Radiopharm*., **47**, 875–880.)
Reagents: i, NaB^2H_4, 2-methyl-but-2-ene, $BF_3.Et_2O$; ii, acetic acid-d4; iii, $PPh_3.Br_2$; iv, Mg; v, CO_2.



SCHEME 5.3.1 (vii) *Synthesis of deuterated conjugated linoleic acid (CLA) isomers.* (Adapted from Adlof, R. (1997) *Chem. Phys. Lipids*, **88**, 107–112.)
Reagents: i, dihydropyran; ii, *p*-toluenesulfonic acid; iii, $^2H_2$, benzene; iv, $(Ph_3P)RhCl$; v, H_3PO_4/P_2O_5; vi, KI; vii, $HC{\equiv}CCH_2OH$; viii, Li/NH_3; ix, Li/NH_3; x, Ph_3PBr_2; xi, Ph_3P; xii, *n*-BuLi, $MeOOC(CH_2)_7CHO$.

5.3.2 ^{13}C-labelled fatty acids

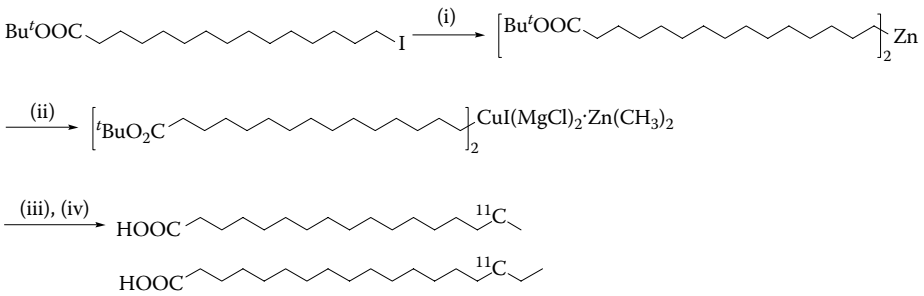
Reagents used for the incorporation of ^{13}C atoms are $^{13}CO_2$, $K^{13}CN$, or $^{13}CH_3I$, all of which are very expensive and therefore limited to small scale synthesis. Syntheses of ^{13}C labelled fatty acids follow similar procedures to those described in Sections 5.1 (acetylenic intermediates) and Section 5.2 (Wittig reaction). A large-scale preparation of octadeca-9-*cis*,12-*trans*-dienoic acid-1-^{13}C, octadeca-9-*cis*,12-*cis*,15-*trans*-trienoic acid-1-^{13}C and the (all-*cis*)-octadeca-9,12,15-trienoic acid-1-^{13}C was reported (Loreau et al., 2000).

5.3.3 ^{11}C-labelled fatty acids

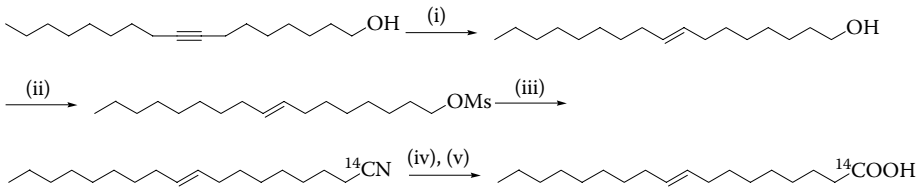
The application of ^{11}C-labelled fatty acids is limited by the short half-life (22 min) of this isotope. However, the short half-life allows ^{11}C-labelled fatty acids to be used in following the kinetics of biological processes, such as blood flow, membrane transport, and as an imaging agent for cardiac fatty acid metabolism (Buckman et al., 1994). Wüst et al. (2000) have developed a new approach to the synthesis of ^{11}C-labelled fatty acids (Scheme 5.3.3(i)).

5.3.4 ^{14}C-labelled fatty acids

Reagents used to incorporate a ^{14}C atom are $K^{14}CN$, $^{14}CO_2$, $^{14}CH_3I$. Fatty esters with the label at C-1 position are produced by the standard 1-carbon chain extension procedure from the alkyl chloride or bromide (RCl or RBr) with $K^{14}CN$ followed by treatment of the resulting nitrile with HCl (g) in anhydrous methanol to yield the requisite $R^{14}COOCH_3$. Carbonation reaction of the Grignard reagent (from alkyl bromide and magnesium) with $^{14}CO_2$ (generated from $Ba^{14}CO_3$) furnishes $R^{14}COOH$. A comprehensive list of synthetic ^{14}C-labelled fatty acids is available (Adlof, 1999).



SCHEME 5.3.3 (i) *Synthesis of [^{11}C]-labelled fatty acids.* (Adapted from Wüst, F. et al. (2000) *J. Labelled Cpd. Radiopharm.*, **43**, 1289–1300.)
Reagents: i, $ZnEt_2$; ii, $Me_2CuI(MgCl)_2$; iii, [1-^{11}C] alkyl iodide; iv, TFA.



SCHEME 5.3.4 (i) *Synthesis of octadec-9-trans-enoic acid-1-^{14}C.* (Adapted from Valicenti, A.J. et al. (1985) *Lipids*, **20**, 234–242.
Reagents: i, Li/NH_3; ii, MsCl/pyridine; iii, K^{14}CN/DMSO; iv, hydrolysis; v, H_3O+.

2- or 3-^{14}C-Labelled fatty acids have been prepared from 1-^{14}C-labelled fatty acids by a series of standard one-carbon chain extension reactions as was the case for the preparation of tetracosa-9,12,15,18,21-pentaenoic acid-[3-^{14}C] (Voss et al., 1991). Unsaturated fatty acids with a ^{14}C-label at the C-1 position can be obtained by decarboxylation of the corresponding unlabelled analogs to shorten the chain by one carbon (to the *nor*-alken-1-ol) and "reconstitution" of the carboxyl group by a one-carbon-^{14}C chain extension procedure via the Grignard reagent of the corresponding bromide derivative of the alken-1-ol with $^{14}CO_2$ (Valicenti et al., 1985) (Scheme 5.3.4(i)).

Unsaturated fatty acids labelled at an internal carbon atom are prepared by the Wittig coupling of an alkyl or alkenyltriphenylphosphonium halide and the appropriate ^{14}C-labelled aldehydic ester, as exemplified in the synthesis of methyl oleate-9-^{14}C (Barley et al., 1973) (Scheme 5.3.4(ii)).

Incorporating a ^{14}C label at the terminal methyl end of the fatty acid chain is achieved using $^{14}CH_3I$. Methyl oleate-[18-^{14}C] was prepared by Pichat et al. (1969) as illustrated in Scheme 5.3.4(iii).

(See References for Section 5.3 at the end of Section 5.5.)

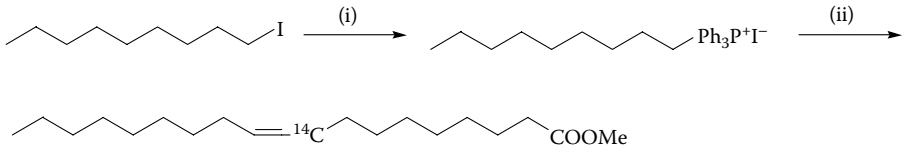
5.4 Synthesis of acylglycerols

Efforts have focused on preparing specifically structured and configurationally pure triacylglycerols. Mono- and diacylglycerols are normally obtained as intermediates in these processes. General methods for synthesizing mono-, di-, and triacylglycerols are reviewed by Quinn et al. (1967), Smith (1972), Jensen and Pitas (1976), Bhati et al. (1980), Gunstone and Norris (1983), Jensen (1995), and Sonnet (1999).

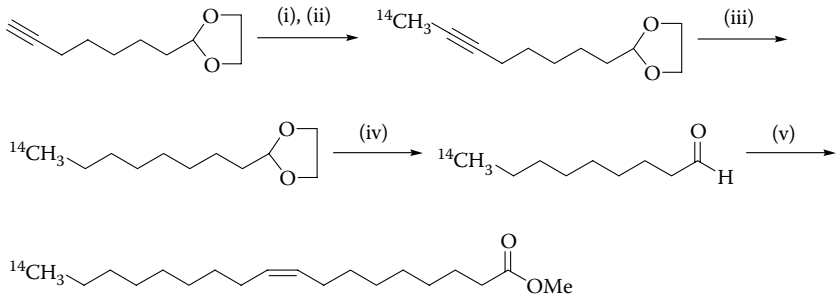
5.4.1 Acylglycerols by chemical synthesis

Acyl chlorides (RCOCl) or acid anhydrides $(RCO)_2O$) provide rapid and reliable acylation. Saturated acid chlorides are prepared by reaction of fatty acid with thionyl chloride (Jensen and Pitas, 1976), while unsaturated fatty acid chlorides are best produced with oxalyl chloride (ClOCCOCl) (Mattson and Volpenhein, 1962). Notes on precautions to be taken during the preparations are detailed by Buchnea (1978).

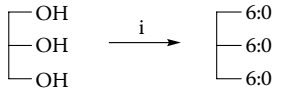
A very successful esterification method employs 1,1′-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP), which permits the carboxylic acid



SCHEME 5.3.4 (ii) *Synthesis of methyl oleate [9-^{14}C].* (Adapted from Barley, G.C. et al. (1973) *J. Chem. Soc.* (Perkin I), 151–154.)
Reagents: i, PPh_3; ii, $MeOOC(CH_2)_7$ ^{14}CHO.



SCHEME 5.3.4 (iii) *Synthesis of methyl oleate-[18-^{14}C].* (Adapted from Pichat, L. et al. (1969) *Bull. Soc. Chim Fr.*, **4**, 1198–1200.)
Reagents: i, C_6H_5Li; ii, $^{14}CH_3I$; iii, H_2, Pt; iv, H_2SO_4; v, $(Ph)_3P{=}CH(CH_2)_7COOCH_3$.



SCHEME 5.4.1 (i) *Synthesis of glycerol trihexanoate.* (Adapted from Lie Ken Jie, M.S.F. and Lam, C.C. (1995a) *Chem. Phys. Lipids*, **77**, 155–171.)
Reagents: (i) hexanoic acid, 1,1'-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP).

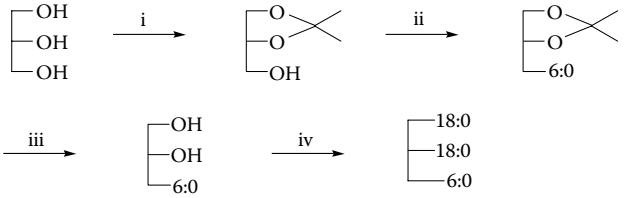
(fatty acid) to be acylated to the glycerol molecule (Neises and Steglich, 1978; Kodali, 1987; Lie Ken Jie and Lam, 1995a, 1995b, 1995c; Mazur et al*.,* 1991) (Scheme 5.4.1(i)).

Fatty acid anhydrides can be prepared via mixed-acid anhydride intermediates using either trifluoroacetic anhydride or acetyl chloride (Mattson et al., 1964). After refluxing, the excess reagent is removed by distillation and the mixed-acid anhydrides converted to fatty acid anhydrides by heating under pressure.

5.4.2 Structured triacylglycerols by chemical synthesis

Interest in relating the structure of dietary fat molecules (triacylglycerols) to human health has led to efforts to synthesise triacylglycerols of specific structure (Bell et al., 1997). Publications on the chemistry, nutrition and biotechnology of food lipids (Akoh and Min, 2002), structurally modified lipids (Gunstone, 2001), modified food fats (Christophe, 1998), functional foods and nutraceuticals (Gunstone, 2003), functional lipids (Akoh, 2005), lipids in infant nutrition (Huang and Sinclair, 1998), and healthful lipids (Akoh and Lai, 2005) have recently received considerable attention.

Commercial applications have focused on the production of triacylglycerols containing readily metabolized medium-chain acids and other potentially desirable fatty acids (such as γ-linolenic acid, arachidonic acid) at appropriate position(s) of glycerol (Akoh, 1995; Bell et al., 1997; Haumann, 1997). Low-calorie fats, such as *Salatrim* (Smith et al*.,* 1994; Softly et al., 1994; Klemann, 1994) and *Caprenin* (Haumann, 1997), contain short (C_2-C_4) or medium-chain C_8-C_{10} fatty acids that have been incorporated into the triacylglycerol molecule by chemical interesterification. Lie Ken Jie and Lam have

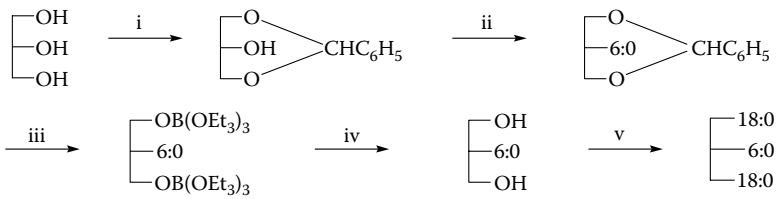


Scheme 5.4.2 (i) *Synthesis of rac-glycerol 1,2-distearate-3-hexanoate via isopropylidene glycerol*. (Adapted from Lie Ken Jie, M.S.F. and Lam, C.C. (1995a) *Chem. Phys. Lipids*, **77**, 155-171.)
Reagents: i, CH_3COCH_3, p-toluenesulfonic acid; ii, CH3(CH2)4COOH, DCC (1,1'-dicyclohexylcarbodiimide), DMAP (4-dimethylaminopyridine), CH_2Cl_2; iii, boric acid, triethyl borate, H_2O; iv, $CH_3(CH_2)_{16}COOH$, DCC, DMAP, CH_2Cl_2.

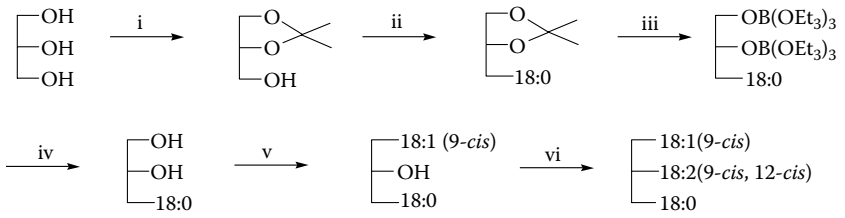
synthesised numerous mixed *rac*- triacylglycerols containing short and long chain saturated acyl chains at specific positions of the glycerol "backbone" (Lie Ken Jie and Lam, 1995a) (Schemes 5.4.2(i) and (ii)), and mixed triacylglycerols containing saturated, acetylenic and olefinic acyl moieties (Lie Ken Jie and Lam, 1995b, 1995c) (Scheme 5.4.2(iii)).

The question of the stereospecific description of the absolute configuration of glycerol derivatives has received considerable attention. Various methods are discussed by Buchnea (1978), who also emphasizes the advantages of the stereospecific numbering (*sn*) system, now recommended by the IUPAC-IUB Commission. This system recognizes that the two primary methylene groups of glycerol are prochiral and are not interchangeable in their reactions with chiral structures, such as enzymes. If the secondary hydroxyl group is shown to the left of C-2 in a Fischer projection, then the carbon atom above is called C-1 and that below is termed C-3.

The problem of 1,2-acyl migration (Mattson and Volpenhein, 1962) led to the need for protection of the selective hydroxyl groups of the glycerol substrate during the course of preparation of mono-, di- and triacylglycerols. Isomerization occurs readily under basic or acidic condition. For example, (*S*)-glycerol 1,2-dipalmitate equilibrates with the 1,3-isomer at 40 to 65°C in about 3 h (Kodali and Duclos, 1992). Migration is also observed in the solid state (Dorset and Pangborn, 1979; Dorset, 1987) and the rate of isomerization depends on chain length (Boswinkel et al. 1996). Thus, during the course of



SCHEME 5.4.2 (ii) *Synthesis of glycerol 1,3-distearate-2-hexanoate via benzylidene glycerol*. (Adapted from Lie Ken Jie, M.S.F. and Lam, C.C. (1995a) *Chem. Phys. Lipids*, **77**, 155–171.)
Reagents, i, benzaldehyde, sulfuric acid; ii, $CH_3(CH_2)_4COOH$, DCC (1,1′-dicyclohexylcarbodiimide), DMAP (4-dimethylaminopyridine), CH_2Cl_2; iii, boric acid, triethyl borate; iv, H_2O; v, $CH_3(CH_2)_{16}COOH$, DCC, DMAP.



SCHEME5.4.2 (iii) *Synthesis of rac-glycerol 1-oleate-2-linoleate-3-stearate.* (Adapted from Lie Ken Jie, M.S.F. and Lam, C.C. (1995a) *Chem. Phys. Lipids*, **77**, 155–171; and Lie Ken Jie, M.S.F. and Lam, C.C. (1995c) *Chem. Phys. Lipids*, **78**, 1–13.)
Reagents, i, CH_3COCH_3, *p*-toluenesulfonic acid; ii, $CH_3(CH_2)_{16}COOH$, DCC (1,1'-dicyclohexylcarbodiimide), DMAP (4-dimethylaminopyridine), CH_2Cl_2; iii, boric acid, triethyl borate; iv, H_2O; v, $CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$, DCC, DMAP; vi, $CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$, DCC, DMAP, CH_2Cl_2.

preparation of specific acylglycerols, it is necessary to incorporate protective groups for specific hydroxyl groups of the glycerol, which can be removed with minimal acyl migration. In addition, since racemic derivatives of *sn*-glycerol cannot be resolved, at least one protective group must be used during the initial preparation of the enantiomeric glycerol. Eibl and Woolley (1986) compared the benzyl, allyl and trityl groups.

5.4.2.1 Benzyl group ($PhCH_2$-)

This group was first used as a blocking group for the synthesis of monoacid diacyl-*sn*-glycerols when the group was added by reacting sodium or potassium isopropylidene-*sn*-glyceroxide with benzyl chloride ($PhCH_2Cl$). 1-Benzyl- and 3-benzyl-*sn*-glycerols can be further protected by trityl groups (see below) so that mixed acid diacyl- and triacyl-*sn*-glycerols can be produced. Removal of the benzyl group requires catalytic hydrogenolysis, so its use is limited to preparations with saturated fatty acids, although, of course, unsaturated fatty acids can be introduced after the benzyl group has been removed (Buchnea, 1978). 4-Methylbenzyl (Golding, 1988) is reported to be a better protecting group than benzyl. It is removed by reaction with 2,3-dichloro-5,6-dicyano-1,4-benzo-quinone (DDQ).

5.4.2.2 Isopropylidene group (Me_2C =)

This is a widely used protective group for chemical reactions involving multiple hydroxyl functions as in the acid-catalysed reaction between glycerol and acetone ($Me_2C = O$). Its first use for the preparation of intermediates in acylglycerol synthesis was by Fischer and Baer (1937), who synthesise 1,2- and 2,3-isopropylidene-*sn*-glycerols. Such substances can then be acylated or blocked (e.g., by benzyl groups) in the remaining hydroxyl moiety. Care must be taken that no racemization or loss of the protective group takes place on storage (Buchnea, 1967). The isopropylidene group is best removed by trimethyl borate in the presence of boric acid (Mattson and Volpenhein, 1962). Kodali (1987) claims improved results with dimethylboron bromide (Me_2BBr) at –50°C.

5.4.2.3 Triphenylmethyl (trityl) group (Ph_3C-)

Triphenylmethyl chloride (Ph_3CCl) reacts mainly with the primary hydroxyls of glycerol, and the trityl group has been extensively used for acyglycerol preparations (Verkade, 1953). This blocking group is removed by catalytic hydrogenolysis, with hydrogen chloride in diethyl ether or petroleum ether, or with a molar equivalent of boron trifluoride-methanol in dichloromethane at 22°C for 15 min (Iwama and Foglia, 1988). Detritylation leads to the formation of by-products, which must be removed by crystallization or by silicic acid column chromatography. Therefore, the benzylidene protective group (PhCH=) is more useful for many purposes (Buchnea, 1978).

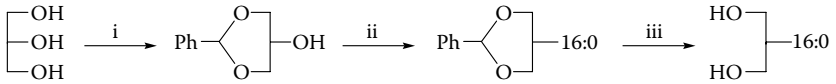
5.4.2.4 2,2,2-Trichloroethoxycarbonyl group (Cl_3CCH_2OCO-)

This protective group can be used during the preparation of unsaturated 1,2- and 2,3-diacyl-*sn*-glycerols. The 2,2,2-trichloroethoxycarbonyl group is introduced onto the free primary hydroxyl group of isopropylideneglycereol using 2,2,2-trichloroethyl chloroformate. This protecting group is retained, without migration, when the isopropylidene group is removed and is itself removed with zinc in acetic acid at room temperature (Pfeiffer et al., 1968).

5.4.2.5 Other blocking groups

Various other blocking groups are discussed by Buchnea (1978) and Gunstone and Norris (1983) with respect to their use in acylglycerol synthesis. Moss et al. (1987) recommend the use of 2-iodobenzoyl as a protecting group. This can be removed by chlorination followed by mild basic hydrolysis with methanolic sodium carbonate or bicarbonate.

Greene and Wuts (1999) have described numerous protecting groups for alcohols and 1,2-diols and procedures for deprotecting these groups. For example, in the preparation of 1,2-diacylglycerols, dimethylboron dibromide can be used to cleave the 1,2-acetonide of glycerol (Kodali, 1987). This reagent can also be used for detritylation and debenzylation (Kodali and Duclos, 1992), thus circumventing the catalytic hydrogenolysis procedure. Detritylation can be achieved by the use of methanolic borontrifluoride (Hermetter and Paltauf, 1981), while *N*-bromosuccin-imide in dimethyl sulfoxide removes readily the *t*-butyldimethylsilyl protecting group (Batten et al., 1980; Burgos et al., 1987) in the preparation of 1,2-diacylglycerols.



SCHEME 5.4.3 (i) *Synthesis of glycerol 2-palmitate.* (Adapted from Jensen, R.G. and Pitas, R.E. (1976) in *Advances in Lipid Research*, Vol. 14 (Eds., R. Paoletti and D. Kritchevsky), Academic Press, New York, pp. 213–247.)
Reagents: i, benzaldehyde, sulphuric acid; ii, palmitoyl chloride, pyridine; iii, boric acid, triethyl borate, H_2O.

5.4.3 Racemic acylglycerols

5.4.3.1 1-Acylglycerols

Glycerol is reacted with acetone in the presence of *p*-toluenesulphonic acid to yield 1,2-isopropylidene glycerol, which can be acylated with a fatty acid. Excess isopropylidene glycerol is removed with sodium acetate solution, and the extent of the acetonization and acylation reactions can be determined by quantification of the amount of water released during the reaction (Jensen and Pitas, 1976). The isopropylidene ester can then be cleaved with boric acid and the 1-acylglycerol purified by crystallization. Purity can be checked by thin layer chromatography using a solvent system where 1- and 2-monoacylglycerols are separated (Thomas et al., 1965) or by NMR spectroscopy. An alternative method of cleavage is the use of hydrogen chloride (Buchnea, 1971).

5.4.3.2 2-Acylglycerols

The two primary alcohol groups of glycerol are protected by condensing benzaldehyde with glycerol in catalytic amount of *p*-toluenesulphonic acid to yield 1,3-benzylideneglycerol. In this compound, only the free 2-hydroxyl is available for acylation (Mattson and Volpenhein, 1962). Benzylideneglycerol is crystallized and purified (Jensen and Pitas, 1976). The free 2-hydroxyl group is acylated with an acid chloride and the resulting acyl-1,3-benzylideneglycerol is crystallized. Deprotection of the benzylidene group is achieved by treatment with boric acid and water (Scheme 5.4.3 (i)). The presence of boric acid inhibits isomerization to the more stable 1-acylglycerol (the equilibrium ratio is 9:1 in favour of the 1-isomer). Small amounts of 2-acylglycerol can be prepared by hydrolysis of an appropriate triacylglycerol with pancreatic lipase. This enzyme is specific for the 1- and 3-postions and, thus, yields a 2-acylglycerol product. By keeping the incubation times short (e.g., 5 min), acyl migration is minimized. The 2-acylglycerol can then be purified by a boric acid TLC system (Jensen and Pitas, 1976).

5.4.3.3 1,3-Diacylglycerols

1,3-Diacylglycerols can be synthesised by acylation of a 1-acylglycerol with an acid chloride in the presence of pyridine and chloroform at room temperature for 24 h. The requisite product is crystallized from hexane or hexane-ethanol mixtures in 50 to 70% yield (Jensen and Pitas, 1976).

5.4.3.4 1,2-Diacylglycerols

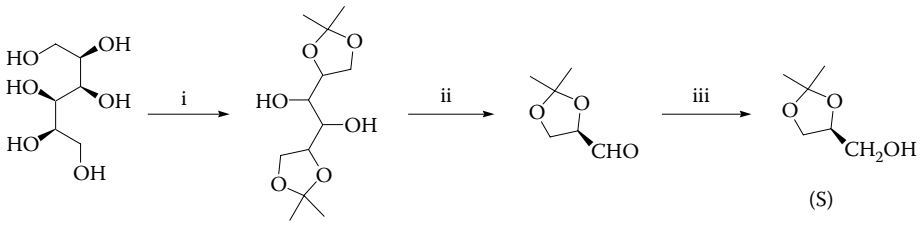
Several methods are available for synthesizing 1,2-diacylglycerols (Jensen, 1972). All procedures have to overcome the problems associated with the inherent instability of the isomer and its isomerization to 1,3-diacylglcyerol. In addition, it is difficult to crystallize 1,2-diacylglycrols because of their high solubility in appropriate solvents. Again, if only small amounts of 1,2-diacylglycerols are needed, then it is more convenient to use pancreatic lipase and to isolate 1,2-diacylglycerol by TLC (Jensen and Pitas, 1976). Also, passage of a 1,3-diacylglycerol isomer through neutral alumina will result in conversion of about 50% to the 1,2-diacylglycerol isomer, which can then be purified by TLC (Jensen et al., 1966).

A convenient method to prepare 1,2-diacylglycerol makes use of the tetrahydropyranyl ether of glycerol, which is obtained by reaction of dihydropyran with allyl alcohol to form allyl tetrahydropyranyl ether. The latter is oxidized with potassium permanganate to give tetrahydropyranylglycerol (Barry and Craig, 1955). Alternatively, glycerol carbonate can be converted to the tetrahydropyranyl ether and the carbonate removed with potassium hydroxide (Cunningham and Gigg, 1965). Examples are the preparation of glycerol 1,2-dioleate (Krabisch and Borgstrom, 1965; Turner et al., 1968) and glycerol 1,2-dipalmitate (Jensen and Pitas, 1976). Acylation of tetrahydropyranyl-glycerol is performed with an acid chloride. Purification of the tetrahydropyranyl protected 1,2-diacylglycerol is achieved by crystallization. The blocking ether can be removed by boric acid (Gigg and Gigg, 1967).

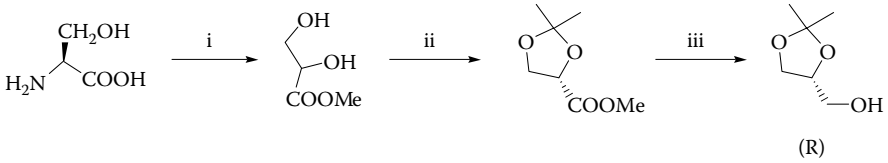
To prepare specifically structured triacylglycerols, methods to obtain the *R*- and *S*-enantiomers of the corresponding aldehyde of 1,2-glycerol acetonide have been developed (Jurczak et al., 1986). D-mannitol is converted to a 1,2,5,6-di-*O*-isopropylidene derivative and then oxidatively cleaved to yield an intermediate aldehyde, which is reduced with $NaBH_4$ to give the requisite (*S*)-1,2-O-isopropylidene glycerol (Scheme 5.4.3(ii)) (Eibl, 1981). Mikkilineni et al. (1988) prepared the (*S*)-(protected)-alcohol from D-isoascorbic acid.

To prepare the (*R*)-(protected)-alcohol, diazotization of L-serine gave chiral glyceric acid, which was then methylated. Acetonization of the latter compound was followed by lithium aluminum hydride reduction of the ester group to furnish the (*R*)-1,2-*O*-isopropylideneglycerol (Scheme 5.4.3(iii)) (Lok et al., 1976).

Methods for the preparation of the (*R*)-1,2-*O*-isopropylidene glycerol from ascorbic acid (Jung and Shaw, 1980) or from L-arabinose (Kanda and Wells, 1980; Kodali, 1987) were also reported. 1-Benzyl- and 3-benzyl-*sn*-glycerols can be formed from the appropriate 2,3- or 1,2-isopropylidene-*sn*-glycerol by reaction with benzyl



SCHEME 5.4.3 (ii) *Synthesis of (S)-1,2-O-isopropylidene glycerol from D-mannitol*. (Adapted from Eibl, H. (1981) *Chem. Phys. Lipids*, **28**, 1–5. With permission.)
Reagents: i, $ZnCl_2$/acetone; ii, $NaIO_4$, pH = 8; iii, $NaBH_4$.

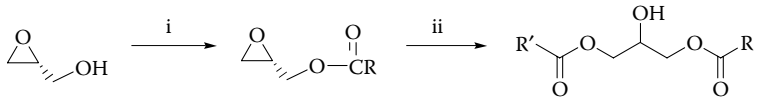


SCHEME 5.4.3 (iii) *Synthesis of (S)-1,2-O-isopropylidene glycerol from L-serine.* (Adapted from Lok, C.M. et al. (1976) *Chem. Phys. Lipids*, **16**, 115–122.)
Reagents: i, HNO_2, $(CH_3O)_2C(CH_3)_2$/*p*-TsOH; ii, acetone/*p*-TsOH; iii, $LiAlH_4$.

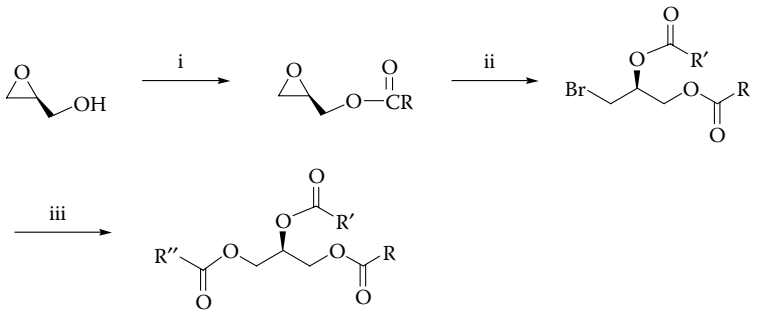
chloride under alkaline conditions (Buchnea, 1978). The 3-benzyl-1,2-isopropylidene- or 1-benzyl-2,3-isopropylidene-*sn*-glycerol products are separated by reduced pressure distillation and the isopropylidene protective group removed by hydrolysis with 10% acetic acid.

Glycidol is also used to prepare a variety of structurally specific diacylglycerols (Lok, 1978, Lok et al., 1985, Zlatanos et al., 1985). Glycidol ester was prepared from acid chlorides in pyridine, which was heated with carboxylic acids in the presence of quaternary ammonium salts to furnish 1,3-diacylglycerols (Scheme 5.4.3(iv)) (Lok et al., 1985). When enantiomers of glycidol are used, the configurational purity of the 1,3-diacylglycerols needs to be evaluated as acyl migration is likely to occur during the heating process in the above reaction.

To overcome acyl migration, Burgos et al. (1987) treated (*S*)-glycidol with stearic acid in the presence of $Ti(O\textit{i}\text{-}Pr)_4$ at 0°C to give (*S*)-1-stearoylglycerol. Sonnet's group (1991, 1994) reacted (*S*)-glycidol with an acyl chloride in pyridine at 0°C to form the chiral glycidol ester, which on treatment with an acid anhydride and LiBr gave a bromodiester intermediate. Villeneuve et al. (1994) used Bu_4NBr in addition to LiBr to improve ring opening of the epoxy ring. The bromodiester intermediate was then reacted with a carboxylate to displace the bromide to yield a chiral triacylglycerol (Scheme 5.4.3(v)).



SCHEME 5.4.3 (iv) *Synthesis of 1,3-diacylglycerol from glycidol*. (Adapted from Lok, C.M. et al. (1985) *Chem. Phys. Lipids*, **36**, 329–334.)
Reagents: i, RCOCl, pyridine; ii, R^1COOH, Et_4NBr.



SCHEME 5.4.3 (v) *Synthesis of chiral triacylglycerol from glycidol*. (Adapted from Sonnet, P.E. and Dudley, R.L. (1994) *Chem. Phys. Lipids*, **72**, 185–191.)
Reagents: i, RCOCl, pyridine; ii, $(R'CO)_2O$, LiBr, PhH; iii, CsO_2CR''.

5.4.3.5 Triacylglycerols

5.4.3.5.1 Monoacid

These triacylglycerols are readily prepared by reaction of glycerol with an appropriate acid chloride. Purification can be effected by crystallization or by TLC. They are often commercially available.

5.4.3.5.2 Diacid

The general principle of the synthesis of racemic diacid triacylglycerols is to react an appropriate monoacylglycerol or diacylglycerol with the desired acid chloride. Jensen and Pitas (1976) have detailed the formation of glycerol 1-palmitate-2,3-distearate; glycerol 1-palmitate-2,3-dioleate; glycerol 1-palmitate-2,3-dilinoleate; glycerol 1-oleate-2,3-dipalmitate; and glycerol 2-palmitate-1,3-dioleate.

For the acylation, additional amounts (50% molar excess) of pyridine and acid chloride are beneficial. The reaction takes from hours to days at room temperature. The triacylglycerol product can be crystallized with good purity in some cases, but in others it is necessary to use an alumina column in addition. Jensen and Pitas (1976) emphasize that, for the latter to be used efficiently, the triacylglycerol must be soluble in hexane-anhydrous diethyl ether. Many saturated triacylglycerols are not soluble in this mixed solvent and so have to be purified by crystallization alone. When the latter is used, then ethanol is included in the crystallization solvent so that excess acid chloride is converted to the ester, which remains in solution. When alumina columns are employed, care must be taken to eliminate all traces of alcohol, chloroform, or water from the eluting solvents, since these can ruin the separation. Alumina columns can be run rapidly and recoveries are 80 to 90% (Jensen et al., 1966).

Diacid triacylglycerols containing one acid in the 1-position and another in the 2- and 3-positions are prepared by acylation of the appropriate 1-mono-acylglycerol. When the single acid is at the 2-position, then the 1,3-diacylglycerol is acylated. The purity of the triacylglycerols is determined by TLC and the proper ratio of the acyl components can be established by GLC or determined by ^{13}C NMR spectroscopy (Lie Ken Jie et al., 1996a, 1996b).

5.4.3.5.3 Triacid

Jensen and Pitas (1976) have described the preparation of 1-palmitoyl-2-oleoyl-3-stearoylglycerol. The general principle is the same as for the synthesis of diacid triacylglycerols. In this case glycerol 1-palmitate-3-stearate is reacted with oleoyl chloride. After crystallization and purification on an alumina column, the triacylglcerol was recovered at better than 90% purity. By an appropriate selection of 1,3-diacylglycerols it is possible to prepare triacid triacylglycerols with any desired combination of acids.

5.4.4 Acylglycerols by enzymic processes

There are few lipid molecules, if any, which cannot be made by modern organic synthesis. Many common and rare lipid molecules (from unsaturated fatty acids to complex lipid metabolites, such as prostaglandins, leukotrienes, thromboxanes, and prostacyclins, very long chain fatty acids) have been produced in the laboratory. Some have found applications in the oleochemical industry (*viz*. surfactants and speciality triacylglycerols). Nevertheless, the application of traditional organic synthesis and production of some lipids suffers distinct drawbacks. Organic reactions accelerated by organic or inorganic catalysts do not always have a high degree of selectivity and require the use of complex multistep reactions to achieve stereospecificity and regioselectively in the final product.

The past 2 decades have witnessed a dramatic increase in the use of enzymes (lipases) as catalysts in lipid synthesis. Enzymes are highly selective in their catalytic action compared to organic or inorganic catalysts. Many of these enzymatic reactions are conducted at moderate temperatures and frequently in the absence of organic solvents. Immobilized lipases are readily available from commercial sources, which make the application of enzymic processes in the synthesis of acylglycerol molecules attractive, reliable and repeatable. Successful enzymatic methods have been developed to produce structured lipids.

"Structured lipids" are triacylglycerols that have been modified by incorporation of specific fatty acids to alter the fatty acid profile of natural triacylglycerols, by restructuring triacylglycerols through changing the positions of the acyl moieties within the same lipid molecule, or by enzymatic synthesis to yield a novel triacylglycerol. Structured lipids are modified from their natural state to achieve a desired nutritional, physical and chemical outcome. All these reactions involve a biocatalyst (lipase). Gandhi (1997), Lee and Akoh. (1998), Akoh (2001, 2002a, 2002b), Osborn and Akoh (2002), Iwasaki and Yamana (2000), Kim and Yoon (2003), Willis et al. (1998), McNeill (1999), and Xu (2000) have reviewed the synthesis and applications of structured lipids with medical, nutraceutical, and food applications.

Most common vegetable seed oils are composed mainly of saturated (palmitic and stearic acid), monounsaturated (oleic), and diunsaturated (linoleic acid) fatty acids. In view of the highly beneficial effects of polyunsaturated fatty acids (PUFA) to health, especially eicosapentaenoic (EPA), docosahexaenoic acid (DHA), arachidonic acid (AA), conjugated linoleic acid (CLA), α-linolenic acid (ALA), and γ-linolenic acid (GLA), great efforts have been directed to design enzymatic procedures to incorporate such polyunsaturated fatty acids into traditional triacylglycerols to yield nutraceuticals for supplementation in infant formula (Udell et al., 2005) or as food supplement for adults to enhance overall health (Gandhi, 1997). These designer lipids may replace conventional fats and oils in certain specialty applications because of their role as structure-health (nutraceuticals or medical lipids) and structure-function (functional lipids) attributes. The position of the highly unsaturated fatty acid in the glycerol moiety is key to their

functionality in foods and absorption when consumed. In most cases insertion of the desired highly unsaturated fatty acid at the *sn*-2 position of the triacylglycerol molecule will provide maximum nutritional benefits.

The best studied enzymes for lipid synthesis is the lipase family (triacylglycerol acylhydrolase, EC 3.1.1.3), which catalyze the hydrolysis of triacylglycerol to give mixtures of free fatty acids, diacylglycerols, monoacylglycerol, and glycerol when excess water is present. At low water activity, fatty acid and alcohols are esterified in the presence of lipases as exemplified by the esterification of glycerol and polyunsaturated fatty acids (PUFA) from fish oil. The main factors influencing the degree of esterification and triacylglycerol yield are the amount of enzyme, water content, temperature, and glycerol/fatty acid ratio. An example of reaction conditions established follows: 100 mg of Novozym 435 (from *Candida antartica*), 9 ml hexane, 50°C, glycerol/PUFA concentrate molar ratio 1.2:3 in the absence of water, 1 g molecular sieves added at the start of the reaction, and agitation at a rate of 200 rpm. Under these conditions, a triacylglycerol yield of 93.5% was obtained from cod liver oil polyunsaturated fatty acid concentrate. The product contained 25% eicospentaenoic acid (EPA) and 45% docosahexaenoic acid (DHA) (Robles Medina et al, 1999).

5.4.4.1 Esterification of fatty acids by lipases

Acid oil (a mixture of mainly free fatty acids and some triacylglycerols, FFA/TAG) is a by-product in the neutralization step of vegetable oil refining and an alternative source of biodiesel fuel. Watanabe et al. (2005a) describes a two-step enzymatic reaction of acid oil (FFA/TAG, 1:1, wt/wt) with methanol: (1) FFA/TAG, 10% wt methanol, and 0.5% wt immobilized lipase (*Candida antarctica)* at 30°C for 24 h resulted in the esterification of FFA; (2) the dehydrated reaction mixture, and 5% wt methanol using 6% wt immobilized lipase furnished methyl esters of fatty acids. The activity of the enzyme reached a maximum after six cycles and furnished >98.5% of methyl esters (biodiesel). A continuous production process involving a three-step flow reaction for the conversion of vegetable oil to 93% yield of methyl esters (biodiesel) using immobilized *C. antarctica* lipase was developed (Watanabe et al., 2000).

Immobilized lipase from *Rhizomucor miehei* is nonselective, but a lipase from *Geotrichum candidum* esterified the octadeca-9-*cis*,11-*trans*-dienoic acid (9*c*,11*t*-CLA) selectively (91%) leaving an unreacted free fatty acid fraction consisting of 82% of octadeca-10-*trans*,12-*cis*-dienoic acid (10*t*,12*c*-CLA) (McNeill et al., 1999).

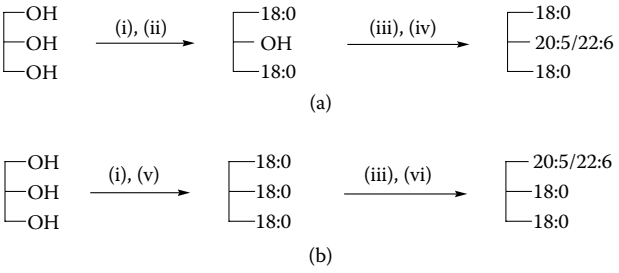
Esterification of a mixture of (9*c*,11*t*-CLA) and (10*t*,12*c*-CLA) with glycerol using lipase from *Penicillium camembertii* without dehydration gave equal amounts of the corresponding monoacyl- and diacylglycerols. Esterification with dehydration at 5mm Hg reduced pressure not only achieved a high degree of esterification (95%) but also suppressed the formation of diacyglycerol (6%) to give monoacylglycerol (90%) (Watanabe et al., 2005b).

Enzymatic synthesis of symmetrical 1,3-diacylglycerols by direct esterification of glycerol is exemplified by the reaction of oleic acid and glycerol in a solvent-free system with dehydration (3 mm Hg pressure), and Lipozyme (*Rhizopus miehei* lipase) at 25 to 45°C to give 1,3-diolein (61%) (Rosu et al., 1999). Using Novozym 435 and Lipase PS-D, Kristensen et al. (2005) prepared diacylglycerols in 60% yield from triacylglycerols by enzymatic glycerolysis. Lipase from *Rhizopus delemar* hydrolyzes common saturated and unsaturated acyl moieties, which are located exclusively at the *sn*-1 and *sn*-3 positions of triacylglycerols (Kosugi et al., 2004).

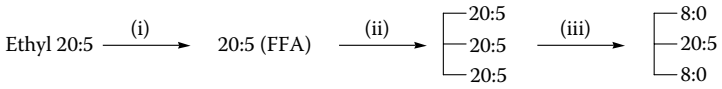
To prepare structured triacylglycerols containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) at the 1-/3- or position of the glycerol "backbone" of a triacylglycerol molecule, a chemo-enzymatic synthesis method for glycerol 2-EPA/DHA-1,3-distearate and by a two-step enzymatic approach for glycerol 1-EPA/DHA-2-EPA/DHA-3-stearate was designed (Haraldsson et al., 2000) (Scheme 5.4.4.1(i)).

5.4.4.2 Enzymatic enrichment of special polyunsaturated fatty acids

Fish oil contains about 10 to 15% of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Enzymatic enrichment procedures have been developed to enable these health benefiting fatty acids to be concentrated or isolated. The fish oil triacylglycerols are normally hydrolyzed to the free fatty acids. Lipases are then used to selectively esterify the common fatty acids (saturated, monoene, and diene fatty acids) with alcohols (methanol, ethanol, butanol, or lauryl alcohol) to give the corresponding esters, which can be readily isolated by solvent extraction (using *n*-hexane or other nonpolar organic solvents) leaving the free fatty acid fraction high in the desired polyunsaturated fatty acids (such as EPA and DHA).



SCHEME 5.4.4.1 (i) *Synthesis of structured triacylglycerols containing EPA/DHA at sn-specific positions of the triacylglycerol.* (Adapted from Haraldsson, G.G. et al. (2000) *J. Am. Oil Chem. Soc.*, **77**, 1139–1145.)
Reagents: i, stearic acid; ii, Rhizomucor miehei lipase; iii, eicosapentaenoic (EPA) or docosahexaenoic acid (DHA) acid; iv, 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide; 4-dimethylaminopyridine (DMAP); v, *Candida antarctica* lipase; vi, *Rhizomucor miehei* lipase.



SCHEME 5.4.4.3 (i) *Enzymatic synthesis of glycerol 1,3-dioctanoate-2-eicosapentaenoate.* (Adapted from Iwasaki, Y. et al. (2000) *J. Am. Oil Chem. Soc*., **77**, 501–506.)
Reagent: (i) Novozyme, H_2O; (ii) Novozyme, glycerol; (iii) ethyl octanoate, Lipozyme.

Esterification of the fatty acids in sardine cannery effluent with 1-butanol with *n*-hexane in the presence of lipozyme allowed up to 80% DHA enrichment (Schmitt-Rozieres et al., 2000). *Pseudomonas* lipases have high activity toward the saturated and monounsaturated fatty acids in fish oil and low activity toward EPA and DHA (with double bonds close to the acid/ester function). Thus, treatment of fish oil in anhydrous ethanol in the presence of *Pseudomonas* lipase resulted in the production of the ethyl esters of the saturated and monounsaturated fatty acids leaving EPA and DHA (total of 50%) unreacted in the residual mixture as mono-, di- and triacylglycerols (Haraldsson, et al., 1997).

Using the lipase *Rhizopus delemar* to esterify the fatty acids in hydrolysed tuna fish oil (containing 23% of DHA) with lauryl alcohol, the process allowed 84% DHA to be recovered from the unesterified fatty acid fraction (Shimada et al., 1997a). A two-step selective esterification of CLA isomers with lauryl alcohol and using *Candida rugosa* lipase that acted on 9*c*,11*t*-CLA more strongly than on 10*t*,12*c*-CLA, was reported by Nagao et al. (2002). The 9*c*,11*t*-CLA content as the lauryl ester increased to 73% with 79% recovery.

5.4.4.3 Incorporation of special fatty acids to triacylglycerols

Enzymatic transesterification (interesterification) has been widely used to incorporate health benefiting fatty acids into conventional (common) triacylglycerol molecules (Hunter, 2001). Lipase regioselectivity provides the ability to distinguish between the *sn-1,3* and *sn-2* position of the triacylglycerol molecule, which is important in the manufacture of structured lipids. Hence, taking advantage of the selectivity and specificity of lipases in their hydrolysis or esterification behaviour, numerous structured lipids have been produced.

Two immobilized lipases, IM60 (from *Rhizopus miehei*) and QLM (from *Alcaligenes sp.*), were used for the modification of the fatty acid composition of palm oil by incorporating EPA and DHA. Acidolysis and interesterification reactions were conducted in hexane. After a 24 h incubation in hexane, there was an average incorporation of 20.8% EPA and 15.6% DHA into palm oil. QLM discriminates against EPA (Fajardo et al., 2003).

γ-Linolenic acid (GLA) in borage oil was enriched by a three-step process: (1) selective hydrolysis in *iso*-octane by *Candida rugosa* lipase immobilized on microporous polypropylene, (2) selective esterification of borage fatty acids with 1-butanol by Lipozyme IM-20, and (3) acidolysis of the products of the previous reactions, that is, unhydrolyzed acylglycerols and unesterified free fatty acid, which gave 65% of GLA in their acylglycerols (Huang et al., 1997).

GLA can also be purified from borage oil (GLA content 22%) in two steps by hydrolysis with *Pseudomonas* sp. lipase (Liposam) to yield 91% of free fatty acids (GLA content 22.5%), which on selective esterification (74% yield) with lauryl alcohol by using *Rhizopus delemar* lipase gave the corresponding lauryl ester (GLA content 70%). To further elevate the GLA content, unesterified fatty acids were extracted and esterified in the same manner. GLA was purified to 93.7% with a recovery of 67% of its initial content (Shimada et al., 1997b). By a similar approach, DHA was purified from tuna oil using *Pseudomonas* sp. Lipase (Lipase-AK) was used in the hydrolysis step, while *Pseudomonas delemar* lipase was used for the esterification step. The DHA content was raised from 24 to 72% (yield of 83%) (Shimada et al., 1997c).

Glycerol 1,3-dioctanoate -2-eicosapentaenoate was synthesisedsynthesised by interesterification of glycerol trieicosapentaenoate with ethyl octanoate catalyzed by Novozyme and Lipozyme (Iwasaki et al., 2000, Kawashima et al., 2001; Irimescu et al., 2001) (Scheme 5.4.4.3(i)).

Similar approaches were adopted for the synthesis of glycerol 1,3-dioctanoate-2-γ-linolenate from borage oil using *Candida rugosa* lipase and *Rhizopus oryzae* lipase (Kawashima et al., 2002). Also prepared were glycerol 1,3-dilaurate-2-oleate using Lipozyme IM (Miura et al., 1999) and glycerol 1,3-dioleate-2-palmitate using Novozym 435 under reduced pressure (Chen et al., 2004).

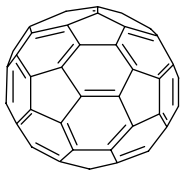
Lipase B (GCB) produced by the fungus *Geotrichum candidum* CMICC 335426 is known for its high specificity towards 9-*cis* unsaturated fatty acids. When sunflower oil was hydrolyzed in the presence of *G. candidum* lipase, the level of unsaturates (all of which have a 9-*cis* double bond) in the free fatty acid fraction was >99% w/w (Diks and Lee, 1999).

(See References for Section 5.4 at the end of Section 5.5.)

5.5 Fullerene lipids

During experiments aimed at understanding the mechanisms by which long-chain carbon molecules are formed in interstellar space, Kroto and coworkers (Kroto et al., 1985) vapourized graphite by laser irradiation under a helium atmosphere. A remarkable stable carbon cluster (in trace amount in the gas phase) was produced, which was shown by time-of-flight mass spectrometry to consist

of 60 carbon atoms. The structure for this extremely stable allotrope of carbon was predicted and subsequently proven as consisting of a truncated icosahedron, a polygon with 60 vertices and 32 faces, 12 of which are pentagonal and 20 hexagonal. The shape of this compound resembled a football or soccer ball (7).



(7)

As the naming of such a 60-carbon cluster would fill four lines according to the IUPAC nomenclature system, the discoverers coined the name "Buckminsterfullerene" for this type of carbon molecules. The name for this molecule stemmed from the resemblance of its shape to the dome structure built for the 1967 Montreal Exposition by the famous architect Buckminster Fuller. In current chemical literature, these carbon cage compounds are simply referred to as "fullerenes." To the laymen the word "bucky ball" has also been used to describe such molecules. To specify the number of carbon atoms in the cage structure of a fullerene molecule, a number inside the square bracket, for example, "[60]fullerene," denotes the number of carbon atoms (in this case 60) involved. The [60]fullerene is also referred to as "C_{60} fullerene."

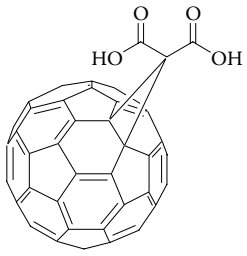
Fullerenes could have different sizes and consist of more than 60 carbon atoms. By mid 1990, a remarkable simple technique was developed for the production of a black soot by vaporizing inexpensive graphite electrodes in an atmosphere of helium (Krätschmer et al., 1990). The black soot contained about 5 to 10% of a mixture of fullerenes, consisting of C_{60} (about 80%), C_{70} (about 15%), and higher fullerenes (C_{76}, C_{78}, C_{84}, etc., about 5%). Recent experiments show the presence of giant fullerenes (C_{540}, C_{960} and C_{1500}) that could form by coalescence during condensation and soot agglomeration (Rietmeijer et al., 2004) and a reported giant C_{650} allotrope (Homann, 1998). [60]Fullerene and [70]fullerene are commercially available at reasonable prices. In recognition of the discovery and the pioneering work on the physics and chemistry of this unusual class of carbon allotropes, the 1996 Nobel Prize in Chemistry was awarded to Kroto, Smalley, and Curl.

Many books have been published during the past 20 years on the chemistry and physics of fullerenes (Kroto et al., 1993; Kadish and Ruoff, 2000; Guldi and Martin, 2002; Prassides, 2004; Hirsch and Brettreich, 2005). The chemistry of higher fullerenes (C_{70} and beyond) was reviewed by Thilgen et al. (1997).

The unique properties of fullerene and their derivatives (especially the hydrophobic nature, the spheroid structure, and the electronic properties) have attracted the attention of many life scientists, material technologists, and that of the pharmaceutical industry. In order to perform systematic testing *in vivo* or *in vitro*, the hydrophobic nature of fullerenes was overcome by encapsulation in special carriers (Jin et al., 1996; Cataldo, 2002), through suspension of fullerene derivatives in water or co-solvents (Mchedlov-Petrossyan et al., 1997; Scrivens et al., 1994) or by chemical functionalization through introduction of hydrophilic appendages to the fullerene moiety (Meier and Kiegiel, 2001; Isobe et al., 2003; Zhang et al., 2004; Cerar and Skerjanc, 2003; Goswami et al., 2004; Liu et al., 2005; Bar-Shir et al., 2005). Synthetic lipid bilayer membranes exhibit similar physicochemical properties to those of bio-membranes and can be immobilized as molecular lipid films (Fendler, 1994; Kuo and O'Brien, 1988).

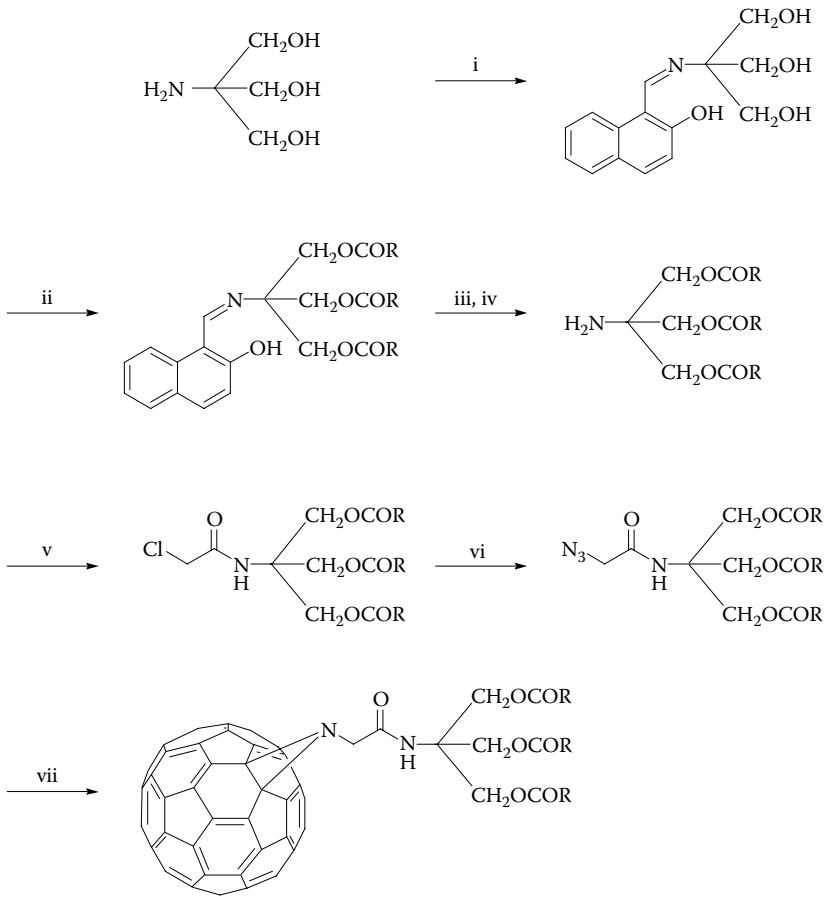
A triple-C_{18} fatty acid chain lipid containing a [60]fullerene moiety, forming multibilayer membrane films that possess main and subphase transitions with the subtransition regulating the electronic properties of the fullerene, was studied (Murakami et al., 1996; Nakanishi et al., 2002). Analogues of the same fullerene lipid containing different saturated and unsaturated fatty acid chains were also prepared (Lie Ken Jie and Cheung, 1999) to study the NMR properties (Scheme 5.5(i)).

Fullerene and its derivatives possess properties that hint at their use in biomedicine, such as the inhibition of HIV protease (HIV-P) by the C_{60} carbon cage that fits into the catalytic site of the proteases (Sijbesma et al., 1993; Friedman et al., 1993). The fullerene derivative (8) used was linked via a cyclopropane system to malonic acid to improve its water solubility.



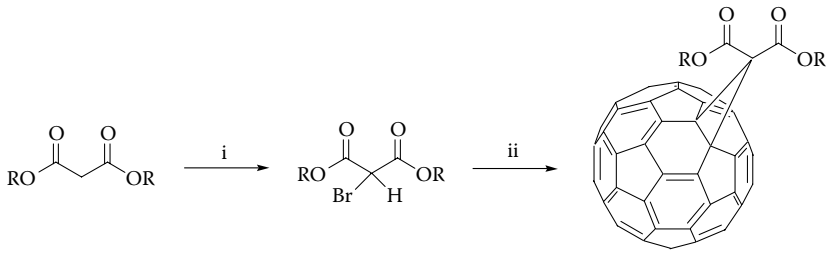
(8)

Utilizing the two carboxylic groups on the cyclopropane ring structure in compound **8**, Lie Ken Jie and Cheung (1998) esterified the carboxylic acid groups with long chain alcohols (from saturated, olefinic, and acetylenic fatty acids) to give a comprehensive series of fullerene lipids (*viz.* dialkyl 1,2-[6,6]-methano-[60]-fullerene dicarboxylate derivatives) (Scheme 5.5(ii)).



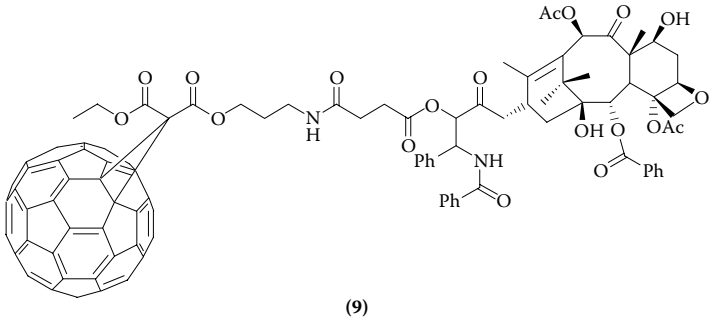
R = $(CH_2)_{10}CH_3$, $(CH_2)_{12}CH_3$, $(CH_2)_{14}CH_3$, $(CH_2)_{16}CH_3$
$(CH_2)_7CH{=}CH(CH_2)_7CH_3$, $(CH_2)_7CH{=}CHCH_2CH{=}CH\,(CH_2)_4CH_3$ or
$(CH_2)_7C{\equiv}C(CH_2)_7CH_3$

SCHEME 5.5 (i) *Synthesis of a triple-acyl chain [60]fullerene lipid.* (From Lie Ken Jie, M.S.F. and Cheung, S.W.H. (1999) Lipids, **34**, 1223–1230. With permission.)
Reagents: i, 2-hydroxynaphthaldeyde; ii, RCOOH, DCC, DMAP (DCC = 1,1'-dicyclohexyl carbodiimide; DMAP = 4-dimethylamino pyridine); iii, bromoacetic acid, THF; iv, n-butylamine; v, chloroacetyl chloride, Et_3N; vi, NaN_3; vii, C_{60}.



R = $(CH_2)_nCH_3$ where n = 1,3,5,7,8,9,17
$(CH_2)_9CH{=}CH_2$, $(CH_2)_5CH{=}CH(CH_2)_{10}CH_3$,
$(CH_2)_8CH{=}CH(CH_2)_7CH_3$,
$(CH_2)_8CH{=}CHCH_2CH{=}CH\,(CH_2)_4CH_3$,
$(CH_2)_5C{\equiv}C(CH_2)_{10}CH_3$
$(CH_2)_8C{\equiv}C(CH_2)_7CH_3$ or $(CH_2)_{12}C{\equiv}C(CH_2)_7CH_3$

SCHEME 5.5 (ii) *Synthesis of dialkyl 1,2-[6,6]-methano-[60]-fullerene dicarboxylate derivatives.* (From Lie Ken Jie, M.S.F. and Cheung, S.W.H. (1998) Lipids, **33**, 729–732. With permission.)
Reagents: i, Br_2; ii, NaH, C_{60}, toluene.



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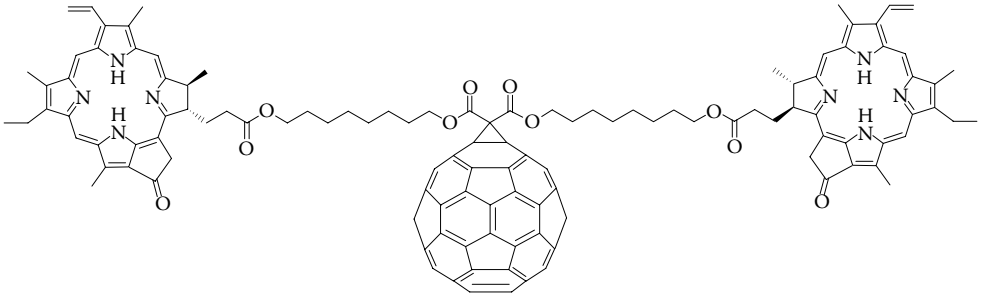

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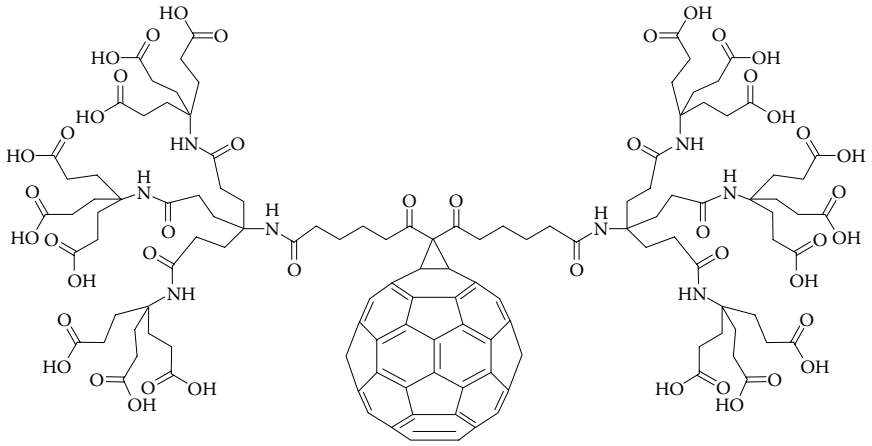
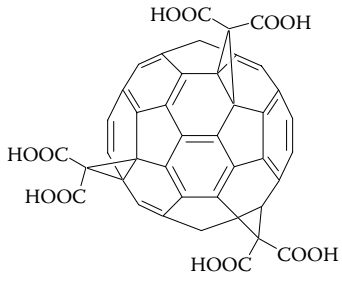


(11)

An interesting and potential lipophilic chemotherapeutic has been reported, which makes use of the carboxylic acid function of compound 8 to incorporate an anticancer drug, paclitaxel, in an effort to produce a slow-release drug-delivery system to combat lung cancer (compound 9) (Zakharian et al., 2005).

Two pyropheophorbide *a* moieties have also been linked to the two carboxylic acid functions of compound 8 to yield a sensitizer (compound 10**)** for photodynamic therapy (Rancan et al., 2005).

Dendritic methano[60]fullerene octadeca acid (compound 11) shows evidence for aggregation of the acid form in water. It has a high potential of scavenging deleterious radicals in biological systems (Quaranta et al., 2003; Krusic et al., 1991). This molecule exhibits antiviral activity against HIV-infected human lymphocytes *in vitro* in the micromolar range with no cell toxicity. Multiadducts are also produced when the carbene anion of various malonate esters are reacted with [60]fullerene to give hexakisadducts (Hirsch and Vostrowsky, 2001; Djojo et al., 2000, Felder-Flesch et al., 2005).



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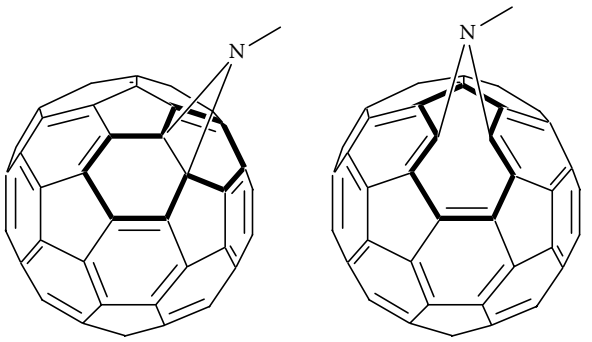
[60]Fullerene containing three methano-dicarboxylic acids (compound 12) was found to block apoptotic

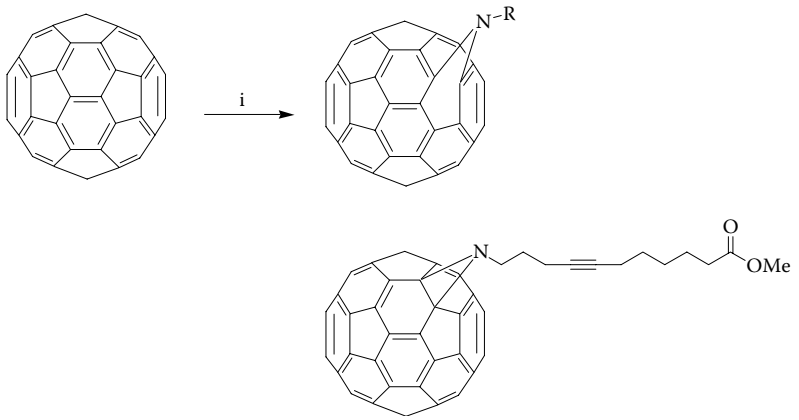


(13) **(14)**

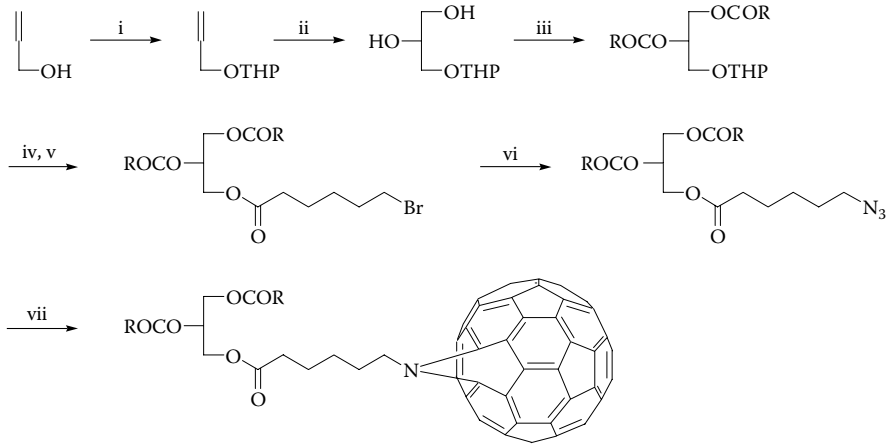
signaling of transforming growth factor-β in human hepatoma cells (Huang et al., 1998).

Reactions of various saturated ω-azido fatty esters with [60]fullerene gave fullerene lipids with a [5,6]-open substructure in the fullerene cage (Lie Ken Jie et al., 2001a). However, when methyl 11-azido-7-undecynoate was reacted with [60]fullerene, a mixture of [6,6]-closed substructure (13) and [5,6]-open type (14) in the fullerene cage was obtained (Scheme 5.5(iii)).

Several structured *rac*-triacylglycerols containing an acyl group with a [60]fullerene tethered to the distal end of the acyl chain at either the 1/3-acyl (Scheme 5.5(iv)) or 2-acyl position were synthesised (Lie Ken Jie et al., 2001b) (Scheme 5.5(v)).

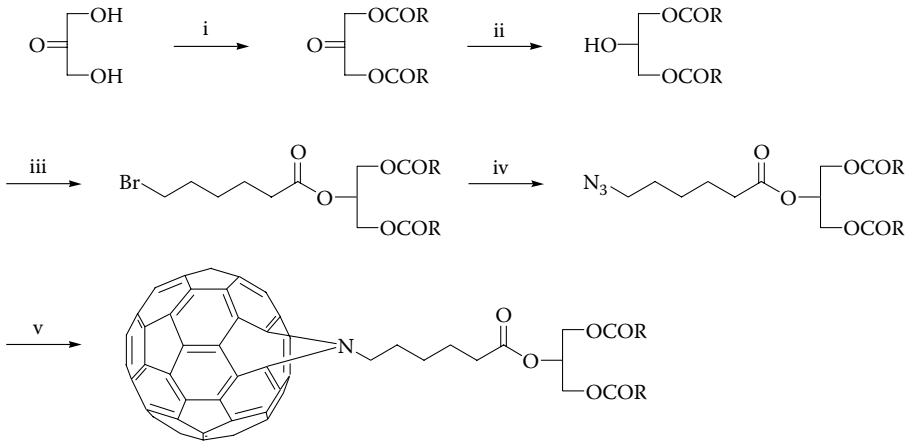


SCHEME 5.5 (iii) *Synthesis of nitrogen-bridged [60]fullerene fatty ester derivatives.* (From Lie Ken Jie, M.S.F. et al. (2001a) *Lipids*, **36**, 421–426. With permission.)
Reagents: i, N_3R where R = $(CH_2)_5COOMe$; $(CH_2)_7COOMe$; $(CH_2)_{11}COOMe$; $N_3(CH_2)_3C{\equiv}C(CH_2)_5COOMe$.



R = $(CH_2)_{16}CH_3$, $(CH_2)_7CH{=}CH(CH_2)_7CH_3$,
$(CH_2)_7CH{=}CHCH_2CH{=}CH\ (CH_2)_4CH_3$
or $(CH_2)7C{\equiv}C(CH_2)_7CH_3$

SCHEME 5.5 (iv) *Synthesis of structured triacylglycerols containing an aza-[60]fullerene unit at the 1/3-acyl position*. (From Lie Ken Jie, M.S.F. et al. (2001b) *Lipids*, **36**, 649–654. With permission.)
Reagents: i, dihydropyran, pyridinium, *p*-toluenesulfonate (PPTS), CH_2Cl_2; ii, $KMnO_4$, acetic anhydride, H_2O; iii, RCOOH, DCC (1,1'-dicyclohexylcarbodiimide), DMAP (4-dimethylaminopyridine), CH_2Cl_2; iv, PPTS, EtOH; v, $Br(CH_2)_5COOH$, DCC, DMAP; vi, NaN_3/DMF; vii, C_{60}, toluene.



R = $(CH_2)_{16}CH_3$, $(CH_2)_7CH{=}CH(CH_2)_7CH_3$,
$(CH_2)_7CH{=}CHCH_2CH{=}CH\,(CH_2)_4CH_3$ or $(CH_2)_7C{\equiv}C(CH_2)_7CH_3$

SCHEME 5.5 (v) *Synthesis of structured triacylglycerols containing an aza-[60]fullerene unit at the 2-acyl position.* (From Lie Ken Jie, M.S.F. et al. (2001b) Lipids, **36**, 649–654. With permission.)
Reagents: i, RCOOH, DCC, DMAP; ii, $NaBH_4$/THF, H_2O; iii, $Br(CH_2)_5COOH$, DCC, DMAP (DCC = 1,1'-dicyclohexyl carbodiimide; DMAP = 4-dimethylamino pyridine); iv, NaN_3/DMF; v, C_{60}, toluene.

References

Aasen, A.J. et al. (1970) Mass spectrometry of triglycerides: II. Specifically deuterated triglycerides and elucidation of fragmentation mechanisms. *Lipids,* **5**, 869–877.

Adlof, R. (1997) Preparation of methyl *cis*-9, *trans*-11- and *trans*-9, *trans*-11-octadecadienoate-17,17,18,18-d_4, two of the isomers of conjugated linoleic acid. *Chem. Phys. Lipids,* **88**, 107–112.

Adlof, R.O. (1999) Isotopically labelled fatty acids, in *Lipid Synthesis and Manufacture,* Ed. F.D. Gunstone, Sheffield Academic press, Sheffield, U.K., 46–93.

Akoh, C. (1995) Lipid-based fat substitutes. *Crit. Rev. Food Sci. Nutr.,* **35**, 405–430.

Akoh, C.C. (2001) Structured lipids containing omega-3 highly unsaturated fatty acids. ACS Symposium Series, 788 (Omega-3 fatty acids), 151–161.

Akoh, C.C. (2002a) Structured lipids. *Food Sc. Technol.* **117**, 877–908.

Akoh, C.C. (2002b) Structured lipids, in *Food Lipids, Chemistry, Nutrition, and Biotechnology,* Eds. C.C. Akoh and D.B. Min, Marcel Dekker, New York, pp. 877–909.

Akoh, C.C. Ed. (2005) *Handbook of Functional Lipids,* Taylor & Francis Group, Boca Raton, FL.

Akoh, C.C. and Lai, O.M. Eds. (2005) *Healthful Lipids,* Am. Oil Chem. Soc., Champaign, IL.

Akoh, C.C. and Min, D.B. Eds. *Food Lipids, Chemistry, Nutrition, and Biotechnology* (2002) Marcel Dekker, New York.

Anagnostopoulos, D. et al. (2005) Synthesis of all-*trans* arachidonic acid and its effect on rabbit platelet aggregation. *Bioorg. Med. Chem. Lett.,* **15**, 2766–2770.

Barley, G.C. et al. (1973) Natural acetylenes: Part XXXIX. Synthesis of methyl [1,9-^{14}C]-, [9-^{14}C]- and [10-^{3}H]-crepenynate, methyl [1,9-^{14}C]-, and [10-^{3}H]-linoleate and methyl [9-^{14}C]- and [10-^{3}H]-oleate. *J. Chem. Soc. (Perkin I)*, 151–154.

Barry, P.J. and Craig, B.M. (1955) Synthesis of symmetrical diglycerides from dihydroxy acetone and allyl alcohol. *Can. J. Chem.,* **33**, 716–721.

Bar-Shir, A. et al. (2005) Synthesis and water solubility of adamantyl-OEG-fullerene hybrids. *J. Org. Chem.*, **70,** 2660–2666.

Barve, J.A. and Gunstone, F.D. (1971) The synthesis of all the octadecynoic acids and all the *trans*-octadecenoic acids. *Chem. Phys. Lipids,* **7**, 311–323.

Batten, R.J. et al. (1980) A new method for removing the *t*-butyldimethylsilyl protecting group. *Synthesis,* **3**, 234–236.

Bhati, A. et al. (1980) Prospects and retrospects of glyceride synthesis, in *Fats and Oils: Chemistry and Technology,* Eds, R.J. Hamilton and A. Bhati), Applied Science, London, pp. 59–107.

Beerthuis, R.K. et al. (1971) Synthesis of a series of polyunsaturated fatty acids, their potencies as essential fatty acids and as precursors of prostaglandins. *Recl. Trav. Chim. Pays-Bas,* **90**, 943–960.

Bell, S.J. et al. (1997) The new dietary fats in health and disease. *J. Am. Diet Assoc.,* **97**, 280–286.

Belosludtsev, Y.Y. et al. (1986) A new synthesis of 5,8,11,14-eicosatetraynoic acid. *Bioorg. Khim.,* **12**, 1425–1426.

Bergelson, L.D. and Shemyakin, M.M. (1964) Synthesis of naturally occurring unsaturated fatty acids by sterically controlled carbonyl olefination. *Angew. Chem. Int. Ed.,* **3**, 250–260.

Bestmann, H.J. (1965) New reactions of alkylidenephosphoranes and their preparative uses. 1. Acid-base character of phosphonium salts and alkylidenephosphoranes. *Angew. Chem. Int. Ed.*, **4**, 583–587.

Bestmann, H.J. and Stransky, W. (1974) Reaktionen mit Phosphinalkylenen: XXXII. Herstellung und Reaktionen von Alkylidenphosphoranen (Phosphinalkylenen) in Hexamethylphosphorsauretriamid unter Verwendung von $OP[N(CH_3)_2]_2^-$ and $N(CH_3)_2^-$ als Base. *Synthesis*, 798–800.

Bestmann, H.J. et al. (1976) Darstellung lithiumsalzfreier Ylidlosungen mit Natrium-bis (trimethylsilyl) amid als Base. *Chem. Ber.,* **109**, 1694–1700.

Bestmann, H.J. and Vostrowsky, O. (1979) Synthesis of pheromones by stereoselective carbonyl olefination: a unitised construction principle. *Chem. Phys. Lipids,* **24**, 335–389.

Boswinkel, G. et al. (1996) Kinetics of acyl migration in monoglycerides and dependence on acyl chain length. *J. Am. Oil Chem. Soc.,* **73**, 707–711.

Bradshaw, R.W. et al. (1971) Natural acetylenes. A synthesis of crepenynic acid (octadec-9-en-12-ynoic acid). *J. Chem. Soc., C,* 1156–1158.

Broustal, G. and Loreau,. O. (2004) Stereocontrolled synthesis of (7E,9Z)-[9,10-^{2}H]-conjugated linoleic acid. *J. Labelled Cpd. Radiopharm.,* **47**, 875–880.

Brown, C.A. and Ahuja, V.K. (1973) "P2-Nickel" Catalyst with ethylenediamine, a novel system for highly stereospecific reduction of alkynes to *cis*-olefins. *J. Chem. Soc. Chem. Commun.,* 553–554.

Buchnea, D. (1967) Acyl migration in glycerides. A bimolecular resonant ion complex as intermediate in acyl migration of monoglycerides. *Chem. Phys. Lipids,* **1**, 113–127.

Buchnea, D. (1971) Synthesis of C-18 mixed acid diacyl-*sn*-glycerol enantiomers. *Lipids,* **6**, 734–739.

Buchnea, D. (1978) Stereospecific synthesis of enantiomeric acylglycerols, in *Handbook of Lipid Research,* Vol.1, Ed. A. Kuksis, Plenum, New York, pp. 233–287.

Buckman, B.O. et al. (1994) Synthesis and tissue biodistribution of [ω-^{11}C]palmitic acid. A novel PET imaging agent for cardiac fatty acid metabolism. *J. Med. Chem.,* **37**, 2481–2485.

Burgos, C.E. et al. (1987) A new, asymmetric synthesis of lipids and phospholipids. *J. Org. Chem.,* **52**, 4973–4977.

Cataldo, F. (2002) Encapsulation of C_{60} fullerene in γ-cyclodextrin: a new concept in the protection of organic substrates and polymers from ozone attack. Kinetic aspects on the reactivity between C_{60} and O_3. *Poly. Degrad. Stabil.*, **77**, 111–120.

Cerar, J. and Skerjanc, J. (2003) Water-soluble fullerenes. 3. Alkali salts of fullerenehexamalonic acid T_h-$C_{66}(COOH)_{12}$. *J. Phys. Chem. B.*, **107**, 8255–8259.

Chen, M.L. et al. (2004) Synthesis of the structured lipid 1,3-dioleoyl-2-palmitoylglycerol from palm oil. *J. Am. Oil Chem. Soc.***, 81**, 525–532.

Christophe, A.B. Ed. (1998) *Structural modified food fats: synthesis, biochemistry, and use.* Am. Oil Chem. Soc., Champaign, IL.

Corey, E.J. et al. (1980) *J. Am. Chem. Soc.,* **102**, 1436–1439.

Crombie, L. (1996) Synthesis in the isotopic labelling of plant fatty acids: their use in biosynthesis, in *Synthesis in Lipid Chemistry,* Ed. J.H.P. Tyman, Royal Society of Chemistry, Cambridge, U.K., pp. 34–56.

Cunningham, J. and Gigg, R. (1965) Glycerol 1,2-carbonate. *J. Chem. Soc.,* 1553–1554.

DasGupta, S.K. et al. (1982) Synthesis of isotopically labelled saturated fatty acids. *J. Lipid Res.,* **23**, 197–200.

Demmelmair, H. et al. (1997) New insights into lipid and fatty acid metabolism via stable isotopes. *Europ. J. Ped.,* **156**, S70–S74.

Descomps, B. (1995) Utilization of stable isotopes to study lipid metabolism in humans, in *New Trends in Lipid and Lipoprotein Analyses* Eds. J.L. Sébédio and E.G. Perkins, AOCS Press, Champaign, IL, 299–308.

Diks, R.M.M. and Lee, M.J. (1999) Production of a very low saturated oil based on the specificity of *Geotrichum candidum* lipase. *J. Am. Oil Chem. Soc.*, **76**, 455–462.

Dinh-Nguyen, N. et al. (1972) Perdeuteriated organic compounds. I. Normal long-chain saturated deuteriocarbons, monocarboxylic acids and methyl esters. *Chem. Scr.,* **2**, 171–178.

Djojo, F. et al. (2000) Fullerene dendrimers and lipofullerenes with an inherently chiral hexaaddition pattern. *Eur. J. Org. Chem.*, 1051–1059.

Dorset, D.L. (1987) Is the initial solid state acyl shift of 1,2-diglyceride intermolecular? *Chem. Phys. Lipids,* **43**, 179–191.

Dorset, D.L. and Pangborn, W.A. (1979) Solid state acyl shift of diglycerides: electron-diffraction study. *Chem. Phys. Lipids,* **25**, 179–189.

Dussault, P. and Lee, I.Q. (1995) A chemoenzymatic approach to hydroperoxy-eicosatetraenoic acids. Total synthesis of 5(*S*)-HPETE. *J. Org. Chem.,* **60**, 218–226.

Ege, S.N. et al. (1961) Synthesis of methyl 5,8,11,14-eicosatetraenoate (methyl arachidonate). *J. Am. Chem. Soc.,* **83**, 3080–3085.

Eibl, H. (1981) An improved method for the preparation of 1,2-isopropylidene-*sn*-glycerol. *Chem. Phys. Lipids,* **28**, 1–5.

Eibl, H. and Woolley, P. (1986) Synthesis of enantiomerically pure glyceryl esters and ethers. I. Methods employing the precursor 1,2-isopropylidene-*sn*-glycerol. *Chem. Phys. Lipids,* **41**, 53–63.

Emken, E.A. (1978) Synthesis and analysis of stable isotope- and radioisotope-labelled fatty acids, in *Handbook of Lipid Research — Fatty Acids and Glycerides,* Ed. A. Kuksis, Plenum, New York, pp. 77–121.

Eynard, T. et al. (1998) Synthesis of methyl (5Z,8Z,11Z,14Z,17Z)- and (5Z,8Z,11Z,14Z,17E)-[18-^{14}C] eicosapentaenoate. *J. Labelled Cpd. Radiopharm*, **XLI**, 411–421.

Fajardo, A.R. et al. (2003) Lipase-catalyzed incorporation of n-3-PUFA into palm oil. *J. Am. Oil Chem. Soc.***, 80**, 1197–1200.

Felder-Flesch, D. et al. (2005) Interfacial behavior and film-forming properties of an amphiphilic hexasubstituted [60]fullerene. *Tetrahedron Lett.,* **46**, 6507–6510.

Fendler, J.H. (1994) Membrane — Mimetic Approach to Advanced Materials. *Advances in Polymer Science*, Series 113, Springer-Verlag, Berlin.

Fischer, H.O.L. and Baer, E. (1937) Synthese optisch-activer Glyceride. *Naturwissenschaften,* **25**, 588–589.

Friedman, S.H. et al. (1993) Inhibition of the HIV-1 protease by fullerene derivatives: model building studies and experimental verification. *J. Am. Chem. Soc.,* **115**, 6506–6509.

Gandhi, N.N. (1997) Applications of lipase. *J. Am. Oil Chem. Soc.,* **74**, 621–634.

Gigg, J. and Gigg, R. (1967) The preparation of unsymmetrical diglycerides. *J. Chem. Soc. C,* 431–434.

Gilman, N.W. and Holland, B.C. (1974) Synthesis of some acetylenic acids. *Chem. Phys. Lipids*, **13**, 239–248.

Goerger, M.M. and Hudson, B.S. (1988) Synthesis of all-*trans*-parinaric acid-d_8 specifically deuterated at all vinyl positions. *J. Org. Chem.,* **53**, 3148–3153.

Golding, B.T. (1988) Synthesis and reactions of chiral C_3-units. *Chem. Ind.,* **19,** 617–621.

Goswami, T. H. et al. (2004) One-pot synthesis of a novel water-soluble fullerene-core starlike macromolecule via successive Michael and nucleophilic addition reaction. *Chem. Mater.*, **16**, 2442–2448.

Gravier-Pelletier, C. et al. (1990) Methods for the total synthesis of acyclic hydroxylated fatty-acids. *Progr. Lipid Res.,* **29**, 229–276.

Greene, T.W. and Wuts, P.G.M (1999) *Protective Groups in Organic Synthesis,* 3rd ed., John Wiley & Sons, New York.

Guldi, D.M. and Martin, N. (2002) *Fullerenes: from Synthesis to Optoelectronic Properties*, Kluwer Academic Pub., Dordrecht.

Gunstone, F.D., Ed. (2001) *Structured and modified lipids,* Marcel Dekker, New York.

Gunstone, F.D., Ed. (2003) *Lipids for functional foods and nutraceuticals*, The Oily Press, Bridgwater, U.K.

Gunstone, F.D. and Hornby, G.M. (1969) The conversion of alkenoic acids to alkynoic acids by bromination-dehydrobromination. *Chem. Phys. Lipids,* **3**, 91–97.

Gunstone, F.D. and Ismail, I.A. (1967) The synthesis of all the *cis* n-octadecenoic acids. *Chem. Phys. Lipids*, **1**, 209–224.

Gunstone, F.D. and Lie Ken Jie, M. (1970) The synthesis of ten octadecadiynoic acids and of the related *cis,cis*- and *trans,trans*-octadecadienoic acids. *Chem. Phys. Lipids,* **4**, 1–14.

Gunstone, F.D. et al. (1971) The synthesis and chromatographic and spectroscopic properties of some methyl *cis,cis*- dimethylene octadecadienoates. *Chem. Phys. Lipids,* **6**, 147–151.

Gunstone, G.D. and Norris, F.A. (1983) Synthesis of acylglycerols, in *Lipids in Foods,* Pergamon, Oxford, U.K., pp. 85–91.

Hall, D.R. et al. (1975) Synthesis of the major sex pheromone of the Egyptian cotton leafworm *Spodoptera littoralis* (Boisd). *Chem. Ind.,* 216–217.

Han, L. and Razdan, R.K. (1998) Total synthesis of methyl 14-hydroxy-(all-*cis*)-5,8,11-tetradecatrienoate: a useful intermediate for the synthesis of arachidonic acid analogues. *Tetrahedron Lett.,* **39**, 771–774.

Haraldsson, G.G. et al. (2000) Chemoenzymatic synthesis of structure triacylglcyerols containing eicosapentaenoic and docosahexaenoic acid. *J. Am. Oil Chem. Soc.*, **77**, 1139–1145.

Haraldsson, G.G. et al. (1997) The preparation of concentrates of eicosapentaenoic acid. *J. Am. Oil Chem. Soc.*, **74**, 1419–1424.

Haumann, B.F. (1997) Structured lipids allow fat tailoring. *INFORM,* **8**, 1004–1011.

Heitz, M.P. et al. (1989) Synthesis of *all-cis*-1-bromo-4,7,10,13-nonadecatetraene: A precursor to C-1 labelled arachidonic acid. *J. Org. Chem.,* **54**, 500–503.

Hermetter, A. and Paltauf, F. (1981) A new method for the detritylation of 1,2-diradyl-3-O-tritylglycerols. *Chem. Phys. Lipids*, **29**, 191–195.

Hirsch, A. and Brettreich, M. (2005) *Fullerenes — Chemistry and Reactions*, Wiley-VCH Verlag, Weinheim.

Hirsch, A. and Vostrowsky, O. (2001) C_{60} Hexakisadducts with an octahedral addition pattern – a new structure motif in organic chemistry. *Eur. J. Org. Chem.*, 829–848.

Homann, K.H. (1998) Fullerenes and soot formation — new pathways to larger particles in flames. *Angew. Chem. Int. Ed. Engl.*, **37**, 2434–2451.

Hsiao, C.Y. et al. (1974) Preparation of fully deuterated fatty acids by simple method. *Lipids,* **9**, 913–915.

Huang, F.C et al. (1997) Enrichment of γ-linolenic acid from borage oil *via* lipase-catalyzed reactions. *J. Am. Oil Chem. Soc.*, **74**, 977–981.

Huang, Y.L. et al. (1998) Blockage of apoptotic signaling of transforming growth factor-β in human hepatoma cells by carboxyfullerene. *Eur. J. Biochem.,* **254**, 38–43.

Huang, Y.S. and Sinclair, A.J. Eds. (1998) *Lipids in infant nutrition*, Am. Oil Chem. Soc., Champaign, IL.

Hunter, J.E. (2001) Studies on effects of dietary fatty acids as related to their position on triglycerides. *Lipids,* **36**, 655–668.

Irimescu, R. et al. (2001) Comparison of acyl donors for lipase-catalyzed production of 1,3-dicapryloyl-2-eicosapentenoylglycerol. *J. Am. Oil Chem. Soc.*, **78**, 65–70.

Isobe, H. et al. (2003) Synthesis of fullerene glycoconjugates through sulfide connection in aqueous media. *Org. Lett.*, **5**, 4461–4463.

Iwama, T. and Foglia, T.A. (1988) An improved procedure for the detritylation of 1-alkyl 2-acyl 3-trityl-*sn*-glycerols. *J. Am. Oil Chem. Soc.,* **65**, 954–958.

Iwasaki, Y. et al. (2000) Enzymatic synthesis of 1,3-dicapryloyl-2-eicosapentaenoylglycerol. *J. Am. Oil Chem. Soc.*, **77**, 501–506.

Iwasaki, Y. and Yamana, T (2000) Enzymatic synthesis of structured lipids. *J. Mol. Cat. B:* Enzym., **10,** 129–140.

Jeffery, T. et al. (1992) An efficient route to skipped diynes and triynes, (Z,Z) dienes and (Z,Z,Z) trienes, *Tetrahedron Lett.,* **33**, 5757–5760.

Jensen, R.G. (1972) Synthetic glycerides, in *Topics in Lipid Chemistry,* **3**, 1–35, ELEK Science, London.

Jensen, R.G. (1995) Discoveries in synthesizing glycerides. *INFORM*, **6**, 440–445.

Jensen, R.G. et al. (1966) Purification of triglycerides with an alumina column. *Lipids,* **1**, 451-452.

Jensen, R.G. and Pitas, R.E. (1976) Synthesis of some acylglycerols and phosphoglycerides, in *Advances in Lipid Research,* Vol. 14, Eds. R. Paoletti and D. Kritchevsky, Academic Press, New York, pp. 213–247.

Jin, C.Y. et al. (1996) Studies on novel cyclodextrans: Inclusion of C_{60} and C_{70}. *J. Inclusion Phenom. Mol. Recog. Chem.,* **24**, 301–310.

Jung, M.E. and Shaw, J.T. (1980) Total synthesis of (*R*)-glycerol acetonide and the antiepileptic and hypotensive drug (-)-gamma-amino-β-hydroxybutyric acid (GABOB): use of vitamin C as a chiral starting material. *J. Am. Chem. Soc.,* **102**, 6304–6311.

Jurczak, J. et al. (1986) (*R*)- and (*S*)-2,3-O-isopropylidene-glyceraldehyde in stereoselective organic synthesis. *Tetrahedron,* **42**, 447–488.

Kadish, K.M. and Ruoff, R.S. (2000) *Fullerenes — Chemistry, Physics and Technology*, John Wiley & Sons, New York.

Kanda, P. and Wells, M.A. (1980) Simplified procedure for the preparation of 2,3-O-isopropylidene-*sn*-glycerol from L-arabinose. *J. Lipid Res.,* **21**, 257–258.

Kawashima, A. et al. (2001) Enzymatic synthesis of high-purity structured lipids with caprylic acid at 1,3-positions and polyunsaturated. *J. Am. Oil Chem. Soc.*, **78**, 611–616.

Kawashima, A. et al. (2002) Production of structured TAG rich in 1,3-dicapryloyl-2-γ-linolenoyl glycerol from borage oil. *J. Am. Oil Chem. Soc.*, **79**, 871–877.

Kim, E.J. and Yoon, S.H. (2003) Recent progress in enzymatic production of structured lipids. *Food Sci. Biotechnol.,* **12**, 721–726.

Klemann, L.P. et al. (1994) Production of fat mixtures enriched with triacylglycerols bearing short, medium and long chain fatty acids. U.S. Patent 5,286,512, Feb. 15, 1994 (*Chem. Abstr.,* **122**, 54727y, 1994).

Klok, R. et al. (1974) Synthesis of 19-*cis*-docosenoic, 17-*cis*-eicosenoic and 15-*cis*-octadecenoic acid. *Recl. Trav. Chim. Pays-Bas,* **93**, 222–224.

Kodali, D.R. (1987) Improved method for the synthesis of 1- or 3-acyl-*sn*-glycerols. *J. Lipid Res.,* **28**, 464–469.

Kodali, D.R. and Duclos, R.I. Jr. (1992) Debenzylation and detritylation by bromodimethylborane: synthesis of mono-acid or mixed-acid 1,2- or 2,3-diacyl-*sn*-glycerols. *Chem. Phys. Lipids,* **61**, 169–173.

Kosugi, Y. et al. (2004) Use of Rhizopus delemar lipase as compared with other lipases for determination of *sn*-2 fatty acids in triacylglycerol. *J. Am. Oil Chem. Soc.*, **81**, 235–239.

Krabisch, L. and Borgstrom, B. (1965) Synthesis of racemic 1,2-diolein. *J. Lipid Res.,* **6**, 156–157.

Krätschmer, W. et al. (1990) Solid C-60 — A new form of carbon. *Nature*, **347**, 354–358.

Kristensen, J.B. et al. (2005) Diacylglycerol synthesis by enzymatic glycerolysis: screening of commercially available lipases. *J. Am. Oil Chem. Soc.*, **82**, 329–334.

Kroto, H.W. et al. (1993) *The Fullerenes*, Pergamon Press, Oxford, U.K.

Kroto, H.W. et al. (1985) C_{60}: Buckminsterfullerene. *Nature*, **318**, 162–163.

Krusic, P.J. et al (1991) Radical reactions of C_{60}. *Science*, **254**, 1183–1185.

Kunau, W.H. (1971a) Chemical synthesis of highly unsaturated fatty acids. I. Preparation of (*n-4*), (*n-1*)-alkadiynoic acids. *Chem. Phys. Lipids,* **7**, 101–107.

Kunau, W.H. (1971b) Chemical synthesis of highly unsaturated fatty acids, II. Preparation of substituted propargyl halides. *Chem. Phys. Lipids*, **7**, 108–120.

Kunau, W.H. (1973) Synthesis of unsaturated fatty acids. *Chem. Phys. Lipids,* **11**, 254–269.

Kuo, T. and O'Brien, D.F. (1988) Free standing polydiacetylene films cast from bilayer membranes. *J. Am. Chem. Soc.*, **110**, 7571–7572.

Labelle, M. et al. (1990) Synthesis of two analogues of arachidonic acid and their reactions with 12-lipoxygenase. *Tetrahedron,* **46**, 6301–6310.

Lam, C.H. and Lie Ken Jie, M.S.F. (1975) Synthesis of all the dimethylene-interrupted methyl octadecadiynoates and a study of their gas-liquid chromatographic properties. *J. Chromatogr.,* **115**, 559–570.

Lam, C.H. and Lie Ken Jie, M.S.F. (1976) The gas-liquid chromatographic properties of all dimethylene interrupted methyl *cis,cis*-octadecadienoates. *J. Chromatogr.,* **117**, 365–374.

Lee, K.T. and Akoh, C.C. (1998) Structured lipids: synthesis and applications, *Food Rev. Inter.,* **14**, 17–34.

Lie Ken Jie, M.S.F. and Cheung, S.W.H. (1998) Synthesis of dialkyl 1,2-[6,6]-methano-[60]-fullerene dicarboxylate derivatives. *Lipids,* **33**, 729–732.

Lie Ken Jie, M.S.F. and Cheung, S.W.H. (1999) Synthesis of C-60 fullerene derivatives bearing a long-chain saturated or unsaturated triester system. *Lipids,* **34**, 1223–1230.

Lie Ken Jie, M.S.F. et al. (2001a) Fullerene lipids: synthesis of novel nitrogen-bridged [60]fullerene fatty ester derivatives. *Lipids*, **36**, 421–426.

Lie Ken Jie, M.S.F. et al. (2001b) Fullerenoid lipids: first synthesis of structured triacylglycerols containing an aza-[60]fullerene unit. *Lipids,* **36**, 649–654.

Lie Ken Jie, M.S.F. and Choi, Y.C. (1992) Mass spectral studies of deuterium-labelled picolinyl fatty esters in the determination of double bond positions. *J. Am. Oil Chem. Soc.,* **69**, 1245–1247.

Lie Ken Jie, M.S.F. and Kalluri, P. (1998) Ultrasound in fatty acid chemistry: facile dehydrobromination of dibromo fatty esters to acetylenic ester derivatives. *Chem. Phys. Lipids,* **91**, 79–83.

Lie Ken Jie, M.S.F. and Lam, C.H. (1974) Synthesis of all the methyl undecynoates and the methyl *cis*-undecenoates and a study of their gas-liquid chromatographic properties. *J. Chromatogr.*, **97**, 165–173.

Lie Ken Jie, M.S.F. and Lam, C.C. (1995a) ^{1}H-Nuclear magnetic resonance spectroscopic studies of saturated, acetylenic and ethylenic triacylglycerols. *Chem. Phys. Lipids,* **77**, 155–171.

Lie Ken Jie, M.S.F. and Lam, C.C. (1995b) ^{13}C-Nuclear magnetic resonance spectroscopic studies of triacylglycerols of type AAA containing (*Z*)- and (*E*)-monoethylenic acyl groups. *Chem. Phys. Lipids,* **78**, 15–27.

Lie Ken Jie, M.S.F. and Lam, C.C. (1995c) ^{13}C-NMR studies of polyunsaturated triacylglycerols of type AAA and mixed triacylglycerols containing saturated, acetylenic and ethylenic acyl groups. *Chem. Phys. Lipids,* **78**, 1–13.

Lie Ken Jie, M.S.F. et al. (1996a) C-13 nuclear magnetic resonance spectroscopy analysis of the triacylglycerol composition of some margarines. *J. Am. Oil Chem. Soc.,* **73**, 1011–1017.

Lie Ken Jie, M.S.F. et al. (1996b) C-13 nuclear magnetic resonance spectroscopic analysis of the triacylglycerol composition of *Biota orientalis* and carrot seed oil. *J. Am. Oil Chem. Soc.,* **73**, 557–562.

Lie Ken Jie, M.S.F. et al. (1997) Fatty acids, fatty acid analogues and their derivatives. *Nat. Prod. Report,* **14**, 163–190.

Lindlar, H. (1952) Ein neuer Katalysator fur selektive Hydrierungen. *Helv. Chim. Acta,* **35**, 446–450.

Liu, Y. et al. (2005) Spectrophotometric study of fluorescence sensing and selective binding of biochemical substrates by 2,2'-bridged bis(b-cyclodextrin) and its water-soluble fullerene conjugate. *J. Phys. Chem. B.,* **109**, 23739–23744.

Lok, C.M. (1978) Versatile methods for the synthesis of mixed-acid 1,2-diacylglycerols. *Chem. Phys. Lipids,* **22**, 323–337.

Lok, C.M. et al. (1985) Synthesis of glycidol esters and mono/diacylglycerols from glycidol. *Chem. Phys. Lipids,* **36**, 329–334.

Lok, C.M. et al. (1976) Synthesis of chiral glycerides starting from D- and L-serine. *Chem. Phys. Lipids,* **16**, 115–122.

Loreau, O. et al. (2000) Large-scale preparation of (9Z,12E)-[1-^{13}C]-octadeca-9,12-dienoic acid, (9Z,12Z,15E)-[1-^{13}C]-octadeca-9,12,15-trienoic acid and their [1-^{13}C] all *cis*-isomers. *Chem. Phys. Lipids,* **106**, 65–78.

Marvell, E.N. and Li, T. (1973) Catalytic semihydrogenation of the triple bond. *Synthesis,* 457–468.

Mattson, A.W. and Volpenhein, R.A. (1962) Synthesis and properties of glycerides. *J. Lipid Res.,* **3**, 281–295.

Mattson, F.H. et al. (1964) Esterification of hydroxyl compounds by fatty acid anhydrides. *J. Lipid Res.,* **5**, 374–377.

Mazur, A.W. et al. (1991) Regio- and stereoselective enzymatic esterification of glycerol and its derivatives. *Chem. Phys. Lipids,* **60**, 189–199.

Mchedlov-Petrossyan, N.O. et al. (1997) Colloidal dispersions of fullerene C_{60} in water: some properties and regularities of coagulation by electrolytes. *J. Chem. Soc., Faraday Trans.,* **93,** 4343–4346.

McNeill, G.P. (1999) Enzymic processes in *Lipid Synthesis and Manufacture,* Ed. F.D. Gunstone, Sheffield Academic Press, Sheffield, pp. 288–320.

McNeill, G.P. et al. (1999) Enzymatic enrichment of conjugated linoleic acid isomers and incorporation into triglycerides. *J. Am. Oil Chem. Soc.,* **76**, 1265–1268.

Meier, M. S. and Kiegiel, J. (2001) Preparation and characterization of the fullerene diols 1,2-$C_{60}(OH)_2$, 1,2-$C_{70}(OH)_2$, and 5,6-$C_{70}(OH)_2$. *Org. Lett.,* **3**, 1717–1719.

Mikkilineni, A.B. et al. (1988) The chemistry of L-ascorbic and D-isoascorbic acids. 2. *R* and *S* glyceraldehydes from a common intermediate. *J. Org. Chem.,* **53**, 6005–6009.

Miura, S et al. (1999) A rapid method for enzymatic synthesis and purification of the structured triacylglycerol, 1,3-dilauroyl-2-oleoyl-glycerol. *J. Am. Oil Chem. Soc.,* **76**, 927–931.

Moss, R.A. et al. (1987) A convenient preparation of 1,2-diacylglycerols; *ortho*-iodobenzoyl as a protecting group. *Tetrahedron Lett.,* **28**, 5005–5008.

Murakami, H. et al. (1996) Fullerene lipid chemistry: self-organized multibilayer films of a C_{60}- bearing lipid with main and subphase transition. *J. Am. Chem. Soc.,* **118**, 4484–4485.

Murphy, P.J. and Lee, S.E. (1999) Recent synthetic applications of the non-classical Wittig reaction. *J. Chem. Soc. Perkin Trans.,* **1**, 3049–3066.

Nagao, T. et al. (2002) Fractionation and enrichment of CLA isomers by selective esterification with *Candida rugosa* lipase. *J. Am. Oil Chem. Soc.,* **79**, 303–308.

Nakanishi, T. et al. (2002) Structure and electrochemistry of self-organized fullerene-lipid bilayer films. *Chem. Eur. J.,* **8**, 1641–1648.

Neises, B. and Steglich, W. (1978) Simple method for the esterification of carboxylic acids. *Angew. Chem. Int. Ed. Engl.,* **17**, 522–524.

Noller, C.R. and Bannerot, R.A. (1934) The synthesis of unsaturated fatty acids. Synthesis of oleic and elaidic acids. *J. Am. Chem. Soc.,* **56**, 1563–1565.

Normant, J.F. and Alexakis, A. (1981) Carbometallation (*C*-metallation) of alkynes: stereospecific synthesis of alkenyl derivatives. *Synthesis,* 841–870.

Nunn, K. et al. (1992) Conjugate reduction vicinal to butadiene tricarbonyl iron complexes. Application to the synthesis of (+/-)-6,7-dihydro-LTB_4 methyl ester. *J. Org. Chem.,* **57**, 3359–3364.

Osbond, J.M. et al. (1961) Essential fatty acids. Synthesis of linoleic, γ-linolenic, arachidonic, and docosa-4,7,10,13,16-pentaenoic acid. *J. Chem. Soc.,* 2779–2787.

Osborn, H.T. and Akoh, C.C. (2002) Structured lipids — novel fats with medical, nutraceutical, and food applications. *Compr. Rev. Food Sci. Food Safety,* **1**, 93–103.

Pabon, H.J.J. et al. (1965) Synthesis of *all-cis* methyl 3,6,9,12-octadecatetraenoate, methyl 4,7,10,13-nonadecatetraenoate and methyl 5,8,11,14,17-eicosapentaenoate. *Recl. Trav. Chim. Pays-Bas,* **84**, 1319–1326.

Peng, S. et al. (2002) Synthesis of isotopically labelled arachidonic acids to probe the reaction mechanism of prostaglandin H synthase. *J. Am. Chem. Soc.,* **124**, 10786–10796.

Pfeiffer, F.R. et al. (1968) Glycerolipids I. Synthesis of D and L mono- and polyunsaturated 1,2-diglycerides via glycerol carbonates. *Tetrahredon Lett.*, **32**, 3549–3552.

Pichat, L. et al. (1969) Methylations of metalated acetylenic compounds in hexamethylphosphoric triamide solution and use of the Wittig reaction for synthesizing ^{14}C-labelled fatty acids. I. Synthesis of oleic-18-14C acid. *Bull. Soc. Chim Fr.*, **4**, 1198–1200.

Pomonis, J.G. and Hakk, H. (1990) Synthesis of ω9-tetracosynoic and ω9-octacosynoic acids as entries into tritiated metabolic precursors of *cis*-9-tricosene and *cis*-9-heptacosene in the housefly. *Lipids,* **25**, 821–826.

Prassides, K. Ed. (2004) *Fullerene-based Materials — Structure and Properties,* Springer-Verlag, Berlin.

Quaranta, A. et al. (2003) Photophysical properties of a dendritic methano[60]fullerene octadeca acid and its *tert*-butyl ester: evidence for aggregation of the acid form in water. *Phys. Chem. Chem. Phys.,* **5**, 843–848.

Quinn, J.G. et al. (1967) Synthesis of 100-gram quantities of highly purified mixed acid triglycerides. *J. Am. Oil Chem. Soc.,* **44**, 439–442.

Rachlin, A.I. et al. (1961) Synthesis of arachidonic acid. *J. Org. Chem.,* **26**, 2688–2693.

Rakoff, H. (1982) Preparation of fatty acids and esters containing deuterium. *Prog. Lipid Res.,* **21**, 225–254.

Rancan, F. et al. (2005) Fullerene-pyropheophorbide *a* complexes as sensitizer for photodynamic therapy: uptake and photo-induced cytotoxicity on Jurkat cells. *J. Photochem. Photobiol. B*, **80**, 1–7.

Raphael, R.A. and Sondheimer, F. (1950) The synthesis of long-chain aliphatic acids from acetylenic compounds. The synthesis of linoleic acid. *J. Chem. Soc.,* 2100–2103.

Reeves, L.W. et al. (1979) A deuterium and sodium nuclear magnetic resonance investigation into the hexadecylpyridinium/hexadecyltrimethyl ammonium liquid crystalline system. *Can. J. Chem.,* **57**, 747–753.

Rietmeijer, F.J.M. et al. (2004) C_{60} and giant fullerenes in soot condensed in vapors with variable C/H_2 ratio. *Fullerenes, Nanotubes, and Carbon Nanostruct.,* **12**, 659–680.

Robles Medina, A.R. et al. (1999) Lipase-catalyzed esterification of glycerol and polyunsaturated fatty acids from fish and microalgae oil. *J. Biotechnol.,* **70**, 379–391.

Rosu, R. et al. (1999) Enzymatic synthesis of symmetrical 1,3-diacylglycerols by direct esterification of glycerol in solvent-free system. *J. Am. Oil Chem. Soc.*, **76**, 839–843.

Sandri, J. and Viala, J. (1995) Direct preparation of (Z,Z)-1,4-dienic units with a new C6 homologating agent: synthesis of α-linolenic acid. *Synthesis*, 271–275.

Sauerwald, T. et al. (1996) Zur umbedenenklichkeit vo stabilen Isotopen in der padiatrischen Forschung und Diagnostick. *Monatsschr. Kinderheilkd,* **144**, 636–644.

Schlosser, M. and Christmann, K.F. (1966) Trans-selective olefin synthesis. *Angew. Chem. Int. Ed. Engl.,* **5**, 126.

Schmitt-Rozieres, M. et al. (2000) Enrichment of polyunsaturated fatty acids from sardine cannery effluents by enzymatic selective esterification. *J. Am. Oil Chem. Soc.,* **77**, 329–332.

Scrivens, W.A. et al. (1994) Synthesis of 14C-labelled C60, its suspension in water and its uptake by human keratinocytes. *J. Am. Chem. Soc.,* **116**, 4517–4518.

Shimada, Y. et al. (1997a) Purification of docosahexaenoic acid by selective esterification of fatty acids from tuna oil with *Rhizopus delemar* lipase. *J. Am. Oil Chem. Soc.,* **74**, 97–101.

Shimada, Y. et al. (1997b) Purification of γ-linolenic acid from borage oil by a two-step enzymatic method. *J. Am. Oil Chem. Soc.,* **74**, 1465–1470.

Shimada, Y. et al. (1997c) Purification of docosahexaenoic acid from tuna oil by a two-step enzymatic method: hydrolysis and selective esterification. *J. Am. Oil Chem. Soc.,* **74**, 1441–1446.

Sijbesma, R. et al. (1993) Synthesis of a fullerene derivative for the inhibition of HIV enzymes. *J. Am. Chem. Soc.,* **115**, 6510–6512.

Smith, C.R. Jr. (1972) Glyceride chirality, in *Topics in Lipid Chemistry,* Vol. 3, Ed. F.D. Gunstone, Elek Science, London, pp. 89–124.

Smith, R.E. et al. (1994) Overview of SALATRIM, a family of low-calorie fats. *J. Agric. Food Chem.,* **42**, 432–434.

Snyder, J.M. and Scholfield, C.R. (1982) *cis-trans* Isomerization of unsaturated fatty acids with *p*-toluenesulfinic acid. *J. Am. Oil Chem. Soc.,* **59**, 469–470.

Softly, B.J. et al. (1994) Composition of representative SALATRIM fat preparations. *J. Agric. Food Chem.,* **42**, 461–467.

Sonnet, P.E. (1991) A short highly regio- and stereoselective synthesis of triacylglycerols. *Chem. Phys. Lipids,* **58**, 35–39.

Sonnet, P.E. (1999) Synthesis of triacylglycerols in *Lipid Synthesis and Manufacture,* Ed. Gunstone, F.D., Sheffield Academic Press, Sheffield, pp. 162–184.

Sonnet, P.E. and Dudley, R.L. (1994) Stereospecific synthesis of selected triglycerides: comments on acyl migration and analysis of configuration. *Chem. Phys. Lipids,* **72,** 185–191.

Sprecher, H. (1977) The organic synthesis of polyunsaturated fatty acids, in *Polyunsaturated Fatty Acids,* Eds. W.H. Kunau and R.T. Holman, American Oil Chemists' Society, Champaign, IL, pp. 6–103.

Sprecher, H. (1979) The organic synthesis of unsaturated fatty acids. *Progr. Chem. Fats Other Lipids,* **15**, 219–254.

Subramanian, G.B.V. and Rastogi, A. (1991) A facile synthesis of 1-thiacontanol from threo-aleuritic acid. *Chem. Ind.,* 436–440.

Svatos, A.A. et al. (1994) Synthesis of deuterium labelled polyunsaturated fatty acids. *Tetrahedron Lett.,* **35,** 9497–9500.

Taber, D.F. et al. (1982) Preparation of deuterated arachidonic acids, in *Methods in Enzymology,* Academic Press, New York, pp. 366–369.

Taber, D.F. and You, K. (1995) New synthon for the convergent construction of skipped conjugation polyenes: synthesis of ethyl docosa-4,7,10,13,16,19-hexaenoate. *J. Org. Chem.,* **60**, 139–142.

Tamvakopoulos, C.S. and Anderson, V.E. (1990) Synthesis of ω-labelled fatty acids. *J. Labelled Cpd. Radiopharm.,* **28**, 187–191.

Thilgen, C. et al. (1997) The covalent chemistry of higher fullerenes: C70 and beyond. *Angew. Chem. Int. Ed. Engl.,* **36**, 2268–2280.

Thomas III, A.E. et al. (1965) Quantitative estimation of isomeric monoglycerides by thin-layer chromatography. *J. Am. Oil Chem. Soc.,* **42**, 789–792.

Tucker, W.P. et al. (1971) Synthesis of 11,11-dideutero-linoleic acid. *J. Labelled Cpd. Comp. Radiopharm.,* **7**, 11–15.

Tulloch, A.P. (1979) Synthesis of deuterium and carbon-13 labelled lipids. *Chem. Phys. Lipids,* **24**, 391–406.

Turner, D.L. et al. (1968) The total synthesis of phosphatidyl(dioleoyl)hydroxyl-L-proline and its activity in blood-clottting systems. *Lipids,* **3**, 228–233.

Udell, T. et al. (2005) The effect of α-linolenic acid and linoleic acid on the growth and development of formula-fed infants: a systematic review and meta-analysis of randomized controlled trials. *Lipids,* **40**, 1–11.

Valicenti, A.J. et al. (1985) Synthesis of octadecynoic acids and [1-^{14}C] labelled isomers of octadecenoic acids. *Lipids,* **20**, 234–242.

Van der Steen, D. et al. (1963) Synthesis of arachidonic acid and related higher unsaturated compounds. *Recl. Trav. Chim. Pays-Bas,* **82**, 1015–1025.

Vatèle, J-M. (1999) Polyene acids in *Lipid Synthesis and Manufacture,* Ed. F.D. Gunstone, Sheffield Academic Press, Sheffield, pp. 1–45.

Vedejs, E. and Peterson, M.J. (1994) Stereochemistrsy and mechanism in the Wittig reaction in *Topics in Stereochemistry*, Vol. 21, John Wiley & Sons, New York, pp. 1–56.

Verkade, P.E. (1953) Synthesis of glycerides. *Chim. Ind. (Paris),* **69**, 239–251.

Viala, J. and Sandri, J. (1992) Total stereospecific synthesis of all *cis*-5,8,11,14,17-eicosapentaenoic acid (EPA). *Tetrahedon Lett.,* **33**, 4897–4900.

Viala, J. and Santelli, M. (1988) Efficient stereoselective synthesis of methyl arachidonate via C3 homologation. *J. Org. Chem.,* **53**, 6121–6123.

Villeneuve, P. et al. (1994) Chiral synthesis of a triglyceride: example of 1-butyroyl-2-oleoyl-3-palmitoyl-*sn*-glycerol. *Chem. Phys. Lipids,* **72**, 135–141.

Voss, A. et al. (1991) The metabolism of 7,10,13,16,19-docosapentaenoic acid to 4,7,10,13,16,19-docosahexaenoic acid in rat liver is independent of a 4-desaturase. *J. Biol. Chem.,* **266**, 19995–20000.

Watanabe, Y. et al. (2005a) Production of FAME from acid oil model using immobilized *Candida antarctica* lipase. *J. Am. Oil Chem. Soc.,* **82**, 825–831.

Watanabe, Y. et al. (2005b) Production of MAG of CLA by esterification with dehydration at ordinary temperature using *Penicillium camembertii* lipase. *J. Am. Oil Chem. Soc.*, **82**, 619–623.

Watanabe, Y. et al. (2000) Continuous production of biodiesel fuel from vegetable oil using immobilized *Candida antarctica lipase. J. Am. Oil Chem. Soc.,* **77**, 355–360.

Westerman, P.W. and Ghrayeb, N. (1982) Synthesis of esters of tetradecanoic acid deuterated at the penultimate carbon: some general procedures for the synthesis of selectively deuterated fatty acids. *Chem. Phys. Lipids,* **29**, 381–387.

Willis, W.M. et al. (1998) Lipid modification strategies in the production of nutritionally functional fats and oils. *Crit. Rev. Food Sci. Nutr.,* **38**, 639–674.

Wüst, F. et al. (2000) A new approach for the synthesis of [^{11}C]-labelled fatty acids. *J. Labelled Cpd. Radiopharm.* **43**, 1289–1300.

Xu, X. (2000) Production of specific structured triacylglycerols by lipase-catalyzed reactions: a review. *Eur. J. Lipid Sci. Technol.,* **102**, 287–303.

Zakharian, T.Y. et al. (2005) A fullerene-paclitaxel chemotherapeutic: synthesis, characterization and study of biological activity in tissue culture. *J. Am. Chem. Soc.,* **127**, 12508–12509.

Zhang, X. et al. (2004) Iodo-controlled selective formation of pyrrolidino[60]fullerene and aziridino[60]fullerene from the reaction between C_{60} and amino acid esters. *J. Org. Chem.,* **69**, 5800–5802.

Zlatanos, S.N. et al. (1985) High yield monoglycerides preparation from glycidol and carboxylic acids. *J. Am. Oil Chem. Soc.,* **62**, 1575–1577.

5.6 Glycerophospholipids

5.6.1 General comments

Excellent reviews dealing with phospholipid synthesis are those of Rosenthal (1975), Slotboom and Bonsen (1970), and Paltauf and Hermetter (1994). Other references that deal with general aspects include a discussion of the effect of solvents and catalysts on acylation (Mangroo and Gerber, 1988), the synthesis of phospholipids using a phosphotriester approach (Woolley and Eibl, 1988), and uniformly cleavable β-hydrogenated protecting groups (Lemman et al., 1990). Stepanov and Slivets (1986) describe the formation of phosphoester bonds in phosphoglyceride synthesis and include discussion of various methods of phosphorylation used for phosphomono-, -di and –triester formation. Particular attention was paid to a comparison of the chlorophosphate versus silver salt methods.

Several papers deal with the preparation of derivatives of a number of phospholipids. An example of the synthesis of spin-labelled phospholipids is that of Lai et al. (1988), while Reynolds et al. (1991) describe the preparation of synthetic phospholipase substrates with *p*-nitrophenol linked to the *sn*-2 moiety of the phospholipid. Yu and Dennis (1991) detail the preparation of chiral thiophospholipids. Issacson et al. (1990) describe the synthesis of phospholipids with hydroxylated fatty acids at the *sn*-2 position, while Lebeau et al. (1988) have synthesised phospholipids linked to steroid hormone derivatives. General books on phospholipids are those of Hawthorne and Ansell (1982) and Ceve (1993).

5.6.2 General considerations

5.6.2.1 Sources of the glycerol backbone

Protected glycerol derivatives are convenient starting materials. The topic has been reviewed (Bittman, 1999). 2,3-*O*-Isopropylidene-*sn*-glycerol and its enantiomer 1,2-*O*-isopropylidene-*sn*-glycerol are available both commercially and from many natural sources. Analysis and precautions in their use are detailed in Paltauf and Hermetter (1994). Other well-known precursors of the glycerol backbone are vicinal diols, such as 1-*O*-benzyl-*sn*-glycerol or its enantiomer. Two other 3-*O*-protected glycerol derivatives are 3-*O*-(4-methoxyphenyl)-*sn*-glycerol and 3-*O*-(4-methoxybenzyl)-*sn*-glycerol (Bittman, 1999).

Other potential C-3 building blocks are reported by Paltauf and Hermetter (1994) although their individual use has, up until now, been limited. An exception is the use of racemic and optically active glycidols (and glycidol tosylates), which are commercially available. Epoxy alcohols, such as glycidol, can be easily ring-opened with nucleophiles to give 1,2-diols as the products. Regioselectivity can be improved by Lewis acid catalysis. A typical procedure might be that of Vinogradov et al. (1995). Alternatively, ring opening can be accomplished regiospecifically with primary or secondary alcohols in the presence of diisobutylaluminum hydride (Erukulla et al., 1995a) or with a sulfur nucleotide (see Bittman, 1999).

Ring opening of glycidol derivatives with fatty alcohols also generates 1-*O*-alkyl-*sn*-glycerols for making ether-linked phosphoglycerides. The advantages of using BF_3 to catalyse the ring opening have been discussed by Bittman (1999).

5.6.2.2 Diradylglycerols

Diradylglycerols are common intermediates for the synthesis of glycerophospholipids, though care has to be taken with acyl migration if there is an acyl group at the *sn*-2 position. Racemic diacylglycerols with identical saturated chains can be prepared from racemic benzylglycerol or from tetrahydropyranylglycerol. For diacylglycerols with one saturated acyl group at position-1 and a saturated or unsaturated group at positon-2, methods using allylesters of fatty acids can be utilised (Paltauf and Hermetter, 1994). For optically active diacylglycerols, methods similar to those for racemic compounds can be used, but with chiral adducts, such as 1- or 3-*O*-benzyl-*sn*-glycerol. A general route leading to various optically active saturated or unsaturated diaclyglycerols, with two different acyl chains starts from isopropylideneglycerol (Kodali and

Duclos, 1992). For other alternative methods of preparing diacylglycerols see Section 5.4 and Paltauf and Hermetter (1994).

5.6.2.3 Alkylacylglycerols

Reviews of methods for the synthesis of alkylacylglycerols are those of Paltauf (1983) and Paltauf and Hermetter (1991). These compounds are needed for the synthesis of alkylacylglycerophospholipids (which themselves are biochemical precursors for plasmalogens and platelet activating factor) or for making various model phospholipids.

For 1-*O*-alkenyl-2-acylglycerols, synthesis usually begins with racemic or optically active 1-*O*-alkylglycerols. Tritylation of the primary hydroxyl group of an alkylglycerol, acylation at position 2 and ditritylation with BF_3 or with bromomethylborane yields products of high isomeric purity (Paltauf and Hermetter, 1994).

1-Acyl-2-alkylglycerols can be produced by alkylation of benzylideneglycerol with alkylmethanesulphonates, followed by removal of the protecting group (acid-catalysed) and acylation of the 1-position. This gives racemic derivatives. For optically active compounds, 2-*O*-alkyl-1-acylglycerol-3-arenesulphonates can be prepared and used for the subsequent synthesis of glycerophospholipids with a saturated alkyl chain (Ali and Bittman, 1990). For the synthesis of optically active 1-acyl-2-*O*-alkylglycerides with saturated or unsaturated acyl and alkyl groups, a convenient route begins with commercially available 1- or 3-*O*-benzyl-*sn*-glycerol (see Paltauf and Hermetter, 1994).

Racemic di-*O*-alkylglycerols are usually made by alkylation of tetrahydropyranyl glycerol followed by acid-catalysed removal of the tetrahydropyranyl group. Those with saturated alkyl groups can also be made from 1- or 3-*O*-benzyl-*sn*-glycerols. For those compounds with different saturated or unsaturated alkyl chains, 1(3)-*O*-alkyl-3(1)-*O*-trityl-*sn*-glycerols can be used as starting material (see Paltauf and Hermetter, 1994, where methods for making 1,3-di-*O*-alkylglycerols will also be found).

5.6.2.4 Glycerophosphoesters as starting materials

Depending on the final glycerophospholipid required, a number of glycerophosphoesters could be utilised. Thus, *sn*-3-glycerophosphocholine can be used to make phosphatidylcholines and, by subsequent acyl exchange (Section 5.6.4.2), other glycerophospholipids. Although *sn*-3-glycerophosphocholine can be synthesised, it is often prepared from phosphatidylcholine by deacylation (Paltauf and Hermetter, 1994). *N*-Trityl-glycerophosphoethanolamine has been used to prepare phosphatidylethanolamines (Hermetter et al., 1983).

Details for other compounds, such as glycerol 3-phosphate or dihydroxyacetone phosphate that are useful substrates for phospholipid biosynthesis, are given in Paltauf and Hermetter (1994).

5.6.2.5 Transphosphatidylation

Plant or bacterial phospholipase Ds (Section 10.2.2) can catalyse transphosphatidylation under appropriate conditions. This allows relatively inexpensive phosphatidylcholines to be used to make equivalent glycerophospholipids. The subject has been reviewed by Ella et al. (1997). Phospholipase D-catalysed exchange has proven useful for the preparation of phosphatidylserine, phosphatidylethanolamine, phosphatidylglycerol as well as antitumor phospholipid-nucleoside conjugates (Bittman, 1999). The reaction proceeds in two steps with phosphatidic acid as a product of the first step. (In fact, phosphatidic acid would be formed naturally in aqueous systems.) The phosphatidic acid can then be used to prepare various glycerophospholipids by coupling it with the desired alcohol via a condensing agent such as trichloroacetonitride in dry pyridine (e.g., Ali and Bittman, 1989).

5.6.3 Phosphatidic acids

Phosphatidic acids can be synthesised by several routes (Figure 5.1). Paltauf and Hermetter (1994) consider that phosphorylation of diradylglycerols (see Section 5.6.2.2) is the most convenient.

5.6.3.1 Phosphorylation of diradylglycerols

Phosphorylation, using phosphorus oxychloride, is used to produce both saturated and unsaturated derivatives. A modification (Paltauf, 1976) of the method of Berecoechea et al. (1968) is recommended to give good yields. Several variants of the reaction conditions and phosphorylating agents are described by Paltauf and Hermetter (1994). One of these uses dimethylphosphorochloridate as the phosphorylating agent, in which case protecting methyl groups are removed with trimethylsilylbromide (Bittman et al. 1984).

5.6.3.2 Use of 1,2-diradylglycero-3-deoxy-3-iodo glycerols

Reaction of the above with silver salts of various phosphodiesters can be used and this should minimise the danger of acyl migration during phosphorylation. Silver salts of dibenzyl phosphate (Hessel et al. 1954), *p*-nitrobentylphosphates (de Haas and Van Deenen, 1961), or di-*p*-xylxylphosphate (Molotkovsky et al. 1976) are examples. The latter allows preparation of phosphatidic acids containing unsaturated acyl or alkyl groups.

5.6.3.3 Acylation of glycerol 3-phosphate

In this procedure, α-glycerol phosphate is acylated directly using acyl anhydrides (Lapidot et al. 1969). The acyl anhydrides are prepared with dicyclohexyl-carbodiimide (Rosenthal, 1975). Optically active or racemic phosphatidic acids can be prepared and either saturated or unsaturated acyl residues added. The method depends on the ability of the fatty acid salt to suppress the formation of mixed phosphoric-carboxylic anhydrides with the

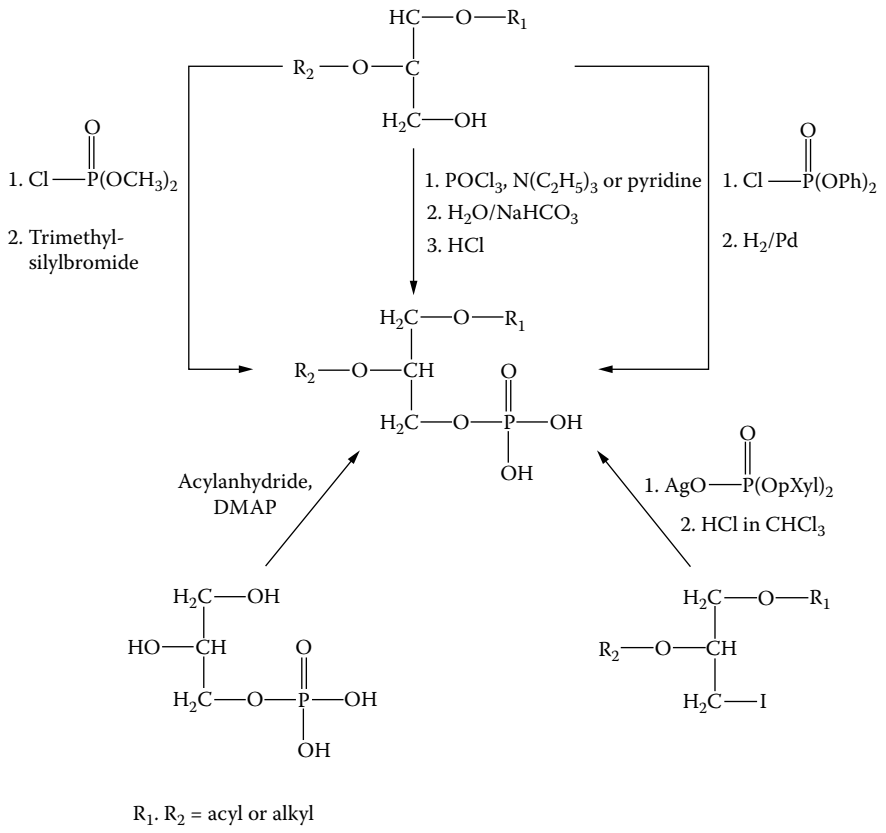


FIGURE 5.1 Synthesis of phosphatidic acid. (Adapted from Paltauf, F. and Hermetter, A. (1994) *Prog. Lipid Res.*, 33, 239-328.)

consequent phosphorylation of adjacent hydroxy groups. The use of acyl anhydrides rather than acyl chlorides probably prevents the formation of glycerol chlorhydrin esters (Aneja and Chadha, 1971a).

Diacylation of glycerol 3-phosphate may be the preferred route where radioactive labelling of the glycerol backbone is required. An improved method for acylation of the pyridinium salt of glycerol 3-phosphate is that of Gupta et al. (1977).

5.6.3.4 Derivatives of phosphatidic acid

Monacyl (lyso) phosphatidic acid can be made chemically or through phospholipase A_2 hydrolysis of phosphatidic acid (Van der Bend et al., 1992). Bis (diacylglycero) phosphoric acids (or bisphosphatidic acids) can be formed by condensation of 1,2-diacylglycerols with phenylphosphoryl dichloride followed by removal of the phenyl group via catalytic hydrogenolysis (Dang et al., 1982).

Various other derivatives, such as ether-containing and glycerol analogues of phosphatidic acid have been synthesised (Rosenthal, 1975). Other methods for synthesis of alkyl analogues of phosphatidic acid, by techniques similar to those for the preparation of phosphatidic acid, are given in Paltauf and Hermetter (1994). Methods for the synthesis of chiral vinylic phosphonolipid (Schwartz et al., 1989a) and α-hydroxyphosphonolipid analogues of phosphatidic acid (Schwartz et al., 1989b) have been described.

5.6.4 Phosphatidylcholines

General methods for the chemical synthesis of phosphatidylcholine (lecithin) involve the use of phosphatidic acid, glycerylphosphorylcholine or diradylglycerols.

5.6.4.1 Synthesis of phosphatidylcholine from phosphatidic acid

Readily available phosphatidic acids (see Section 5.6.3) can be esterified with a suitable choline salt. Suitable condensing agents include carbodiimides, trichloroacetonitrile and sulfonyl chlorides (Rosenthal, 1975). Not only are these procedures relatively straightforward, but they are also suitable for the preparation of phosphatidylcholines radio-labelled in the choline moiety.

The method of Aneja and Chadha (1971b) utilizes triisopropylbenzenesulfonyl chloride and is claimed to give products of better optical purity than other procedures. In this technique the phosphatidic acid is mixed and dried with choline acetate (in twice molar excess). The sulfonyl chloride derivative in dry pyridine is then added and, after reaction at 70°C for 1 h, the procedure is continued at room temperature for 4 h. Excess choline acetate can then be filtered off and the product purified, after passage through a mixed-bed resin column or by chromatography on a neutral alumina column.

5.6.4.2 Syntheses involving glycerophosphocholine

Acylation of glycerophosphocholine is the most straightforward method for phosphatidylcholine containing two identical saturated and unsaturated acyl groups. Gupta et al. (1977) used *N, N*-dimethyl-4-aminopyridine in chloroform for the acylation of a cadmium chloride complex of glycerophosphocholine with fatty acid anhydride. In contrast, acylation can occur with a twofold excess of acylimidazolide in the presence of methylsulfinylmethide (Warner and Benson, 1977). Modifications of reaction conditions and precautions are discussed by Paltauf and Hermetter (1994). For small scale preparation involving radioactive or other expensive fatty acids, a modification of the Gupta et al. (1977) method can be used (Mena and Djerassi, 1985).

For mixed acid phosphatidylcholines, procedures usually involve phospholipase A_2-removal of the acid from the *sn*-2 position followed by reacylation with a new fatty acid. Typically, fatty acyl anhydrides are used for the latter step (Gupta et al., 1977; Mason et al., 1981). Some improvements in the general methodology are discussed by Paltauf and Hermetter (1994). The acylation can also be carried out with 2-thiopyridylesters in the presence of silver ions (Nicholas et al., 1983).

A three-step procedure for the preparation of mixed acid phosphatidylcholines with both saturated and unsaturated chains is depicted in Figure 5.2.

The method by Hermetter et al. (1989) starts from a zinc chloride complex of glycerophosphocholine, which is converted to a 1-*O*-trityl derivative by reaction with triphenylmethylchloride, followed by acylation. The trityl group is then replaced by the chosen acyl moiety using acyl anhydride or acyl imidazolide.

5.6.4.3 Syntheses from diradylglycerols

Several ways of forming the phosphodiester bond are possible. A convenient method uses phosphatidic acid as an intermediate (see Section 5.6.4.1). An early procedure (Aneja and Chadha, 1971b) is simple, but has some drawbacks (Paltauf and Hermetter, 1994). An improved version, using the tetraphenylborate salt of choline, has been described (Harbison and Griffin, 1984). If the production of phosphatidate intermediates involves reaction of the diacylglycerols with phosphorus oxychloride then the dichlorophosphate intermediates can be condensed directly with choline chloride (Baer and Kindler, 1962). This method has been used very successfully by some (e.g., Brockerhoff and Ayengar, 1979), but may produce difficult-to-remove by-products (Paltauf and Hermetter, 1994).

Another method uses 2-bromoethyl dichlorophosphate (Hirt and Berchtold, 1958) and has been improved by Hansen et al. (1982) to reduce the reaction time and avoid side-reactions. Alternatively, the diradylglycerols can be condensed with 2-chloro-2-oxo-1,3,2-dioxaphospholane followed by treatment of the cyclic phosphates with trimethylamine (Chandrakumar and Hajdu, 1982). This type of synthesis, using dioxaphospholanes, tends to require long reaction times (for nucleophilic ring opening), but few by-products are formed. Improvements have been reported, such as the use of chloro-oxazaphospholane as phosphorylating agent or methods that use phosphatidylethanolamine (or *N*-methyl-phosphatidylethanolamine) as intermediate (Patel et al., 1979; Stumpf and Lemmen, 1990).

Phosphoamidite compling reactions, as utilised for nucleotides, have been used for phosphatidylcholine. For example, Bruzik et al. (1986) condensed dipalmitoylglycerol with chloro-(*N-N*-diisopropylamino) methoxyphosphine. However, subsequent steps are rather cumbersome and unsaturated derivatives are not possible. A different approach was taken by Hebert and Just (1990), who condensed 2-cyanoethyl (*N-N*-diisopropylamino) chlorophosphinite with bromoethanol to yield a dialkylphosphoramidite. The latter was then reacted with diacylglycerol in the presence of

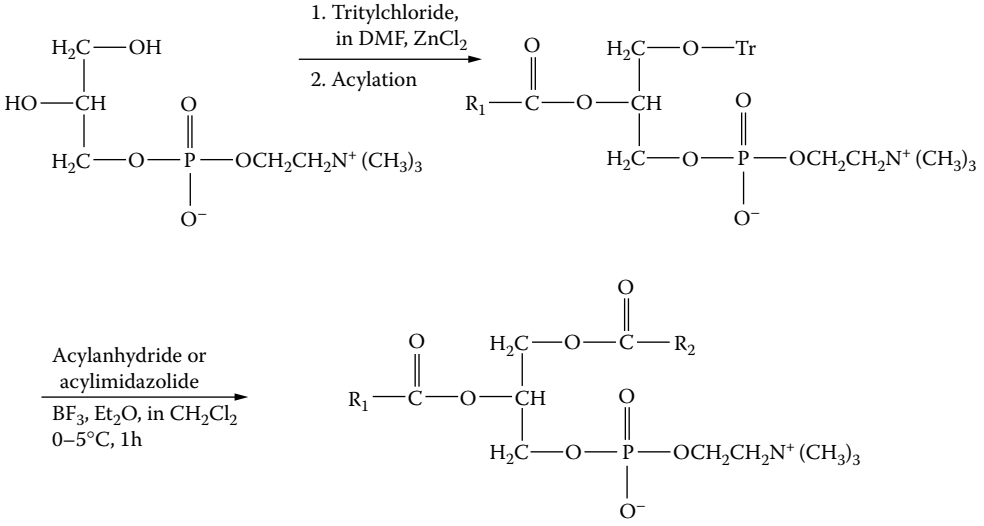


FIGURE 5.2 Synthesis of mixed acid, 1,2-diacyl-*sn*-glycero-3-phosphocholines. (Adapted from Paltauf, F. and Hermetter, A. (1994) *Prog. Lipid Res.,* 33, 239-328.)

TABLE 5.1 Phosphatidylcholine analogues (see Bonsen et al., 1972)

Type of Analogue	Compound
Stereoisomers of normal PC	1-*sn*-Phosphatidylcholine
Structural isomer of normal isomer	*rac*-1, 3-Diacylglycerol-2-phosphorylcholine
Modification of acyl chain at the 2 position of PC	2-Methyl derivatives of acyl chain 2,2-Dimethyl derivatives of acyl chain 3,3- Dimethyl derivatives of acyl chain *rac*-1, 2-Dibenzoylglycero-3-phosphorylcholine
Modification of ester linkages at the 2 position of PC	*rac*-1-Acyl-2-acylamido-2-deoxyglycero-3-phosphorylcholine 1-Acyl-2-alkanesulphonyl-*sn*-glycero-3-phosphorylcholine *rac*-1-Acyl-2-alkylglycerol-3-phosphorylcholine *rac*-1-Acyl-2-alkyl-2-deoxyglycero-3-phosphorylcholine
Compounds without an acyl group at the 2 position of PC	1-Acyl-*sn*-glycero-3-phosphorylcholine 1-Acyl-2-deoxyglycero-3-phosphorylcholine
Modification of glycerol backbone	1-Acylglycol-2-phosphorylcholine *rac*-1,2-Diacylbutanetriol-4-phosphorylcholine 2,2-(Diacylhydroxymethyl)propanol-1-phosphorylcholine 1,2-Diacyl-3-deoxy-*sn*-glycero-3-phosphorylcholine
Modification of the phosphate group	*rac*-1,2-Diacyl-3-deoxyglycero-3-sulfonic acid

tetrazole, followed by several steps to give phosphatidylglycerol in 65% yield.

5.6.4.4 Other phosphatidylcholine derivatives

The synthesis of other phosphatidylcholines (other than plasmalogens; see Section 5.6.10) has been well reviewed (Rosenthal, 1975; Paltauf 1983; Zeisig et al., 1990). Experimental details are given in Paltauf and Hermetter (1991) and further discussion will be found in Paltauf and Hermetter (1994). A large variety of other phosphate analogues have also been synthesised, and these are reviewed by Bonsen et al. (1972). A summary of the various compounds for which methods have been developed is made in Table 5.1.

For the preparation of mixed chain phosphatidylcholine analogues with bulky headgroups or with one acyl chain twice the length of the other, see Ali and Bittman (1989). Schwartz et al. (1989a) have described the synthesis of chiral vinylic phosphonolipid analogues, while Moschidis (1987) reported the preparation of 1-*O*-alkylethylene glycol phosphonic acid analogues of phosphatidylcholine. Thiol ester and thiol ether analogues are described by Hendrickson and Hendrickson (1990), while the formation of phosphatidylcholine labelled with 8-(2-anthroyl)-octanoic acid, for use as a fluorescent probe, is detailed by Perochon and Tocanne (1991). General methods for fluorophore-labelled phospholipid substrates, including derivatives of phosphocholine, are given by Hendrickson (1991).

Phosphatidylcholine analogues containing acylamino, alkylamino, or carbamyl groups have been made. They can have a variety of uses including potential inhibitors of phospholipase A_2, as PAF analogues with PAF agonistic, antagonistic, or cytostatic activity or as phospholipid analogues for basic membrane biophysical studies. Details of their syntheses are given in Paltauf and Hermetter (1994), who also discuss other phosphatidylcholine derivatives, such as cyclopentano or sulfonium analogues or 1,2-dialkylmethylidene derivatives.

5.6.5 Phosphatidylethanolamines

Phosphatidylethanolamine is usually synthesised from phosphatidic acid or from iodohydrin diesters. The analogous route to that of phosphatidylcholine from glycerophosphorylcholine is not usually used because it is difficult to obtain the substrate in a sufficiently pure state. Care must be taken during the synthesis of phosphatidylethanolamines to protect the unsubstituted amino function.

5.6.5.1 Synthesis of phosphatidylethanolamine from phosphatidic acid

The first problem encountered in this route is the blocking of the primary amino group by a reagent that can be removed easily and under conditions that are not accompanied by ester hydrolysis or fatty acyl modification. The trityl group can be used for this purpose. In a typical procedure (Aneja et al., 1970) *N*-tritylethanolamine is condensed with a phosphatidic acid by means of 2,4,6-triisopropylbenzenesulphonyl chloride. The reaction is fast and, after heating for 1 min at 60°C to dissolve the phosphatidic acid, proceeds in 90% yield at room temperature in 1.5 h. The product is purified by triethylamine impregnated silicic acid chromatography. Removal of the trityl group can be accomplished by hydrogenolysis (not appropriate

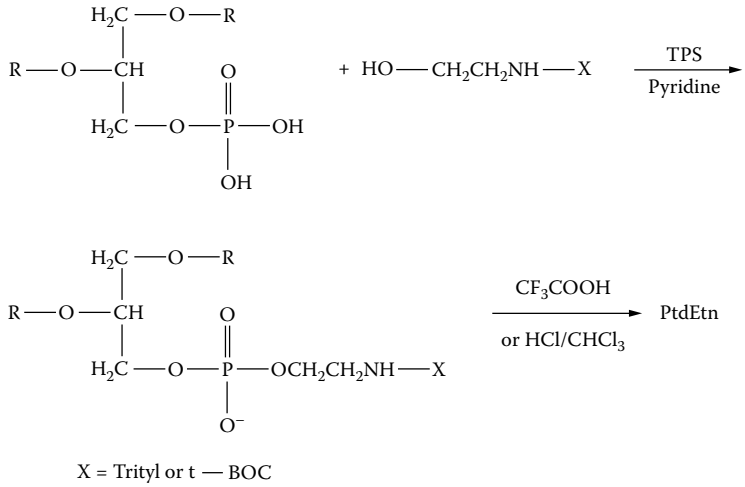


FIGURE 5.3 Synthesis of ethanolamine glycerophospholipids from phosphatidic acids. (Adapted from Paltauf, F. and Hermetter, A. (1994) *Prog. Lipid Res.,* 33, 239–328.)

for unsaturated acyl groups) or by very mild acid hydrolysis (Rosenthal, 1975). Alternative condensing agents to sulphonyl chlorides include carbodiimides and trichloroacetonitrile.

A depiction of the synthesis of phosphatidylethanolamines from phosphatidic acids is shown in Figure 5.3. Paltauf and Hermetter (1994) summarize alternative methodologies, including the use of phosphatidic acid dichloride for the condensation with ethanolamine (Eibl, 1978).

5.6.5.2 Synthesis of phosphatidylethanolamine from α-iododeoxyglycerol diesters

This method is especially attractive for the synthesis of mixed-acid phosphatidylethanolamines because of the ease of preparation of specific diesters of α-iododeoxyglycerol. The phosphorylethanolamine function is introduced via the silver salt of a substituted derivative. As in the preparation of phosphatidylethanolamine from phosphatidic acid, it is essential that the protective groups can be easily removed. Silver *t*-butyl (*N*-*t*-butyloxycarbonyl-2-aminoethyl) phosphate is the most useful reagent because it allows the simultaneous removal of both phosphoester and amino-protecting groups under conditions that do not affect unsaturation or ester groups (Rosenthal, 1975). Yields of about 50% for both the preparation of the silver salt of the substituted phosphorylethanolamine and for the condensation with the iododeoxyglycerol can be expected.

5.6.5.3 Synthesis of phosphatidylethanolamine from glycerylphosphorylethanolamine

As mentioned above, this method is not often used. The amino nitrogen is blocked with phthaloyl or trityl groups before acylation to yield a phosphatidylethanolamine with two identical fatty acids. Mixed-acid phosphatidylethanolamines can be synthesised by deacylation (phospholipase A)-reacylation (Slotboom and Bonsen, 1970).

5.6.5.4 Synthesis of phosphatidylethanolamine from diacylglycerols

Reaction of diacylglycerols with a number of reagents can be used for the formulation of phosphatidylethanolamines. These include bromoethyl dichlorophosphate (Eibl and Nicksch, 1978; Figure 5.4), phosphorus oxychloride (see Figure 5.4) (via phosphatidic acid dichloride) as described by Eibl (1978), phosphorus acid-1,1,1,trichloro-2-methyl-2-propylester-chloride-dimethylamide (Lemmen et al. 1990), phosphorus trichloride (Lindh and Stawinski, 1989), and chloro-(*N,N*-diisopropylamino) methoxylphosphine (Bruzik et al. 1986). Details of these methods and other pertinent discussion on the use of particular methods for specific purposes are given in the excellent review by Paltauf and Hermetter (1994).

5.6.5.5 Other phosphatidylethanolamine derivatives

The synthesis of ether phosphatidylethanolamines is analogous to that for the formation of the corresponding phosphatidylcholines (Rosenthal, 1975). Relevant literature has been reviewed (Paltauf, 1983) and laboratory methods have been published (Paltauf and Hermetter, 1991). The *N*-methyl and *N, N*-dimethyl derivatives have been synthesised from their corresponding phosphatidic acids (Aneja et al., 1970). See also Calderon and Yague (1986) for the preparation of *N, N*-dimethylphosphatidylethanolamines. An often-used method utilises phospholipase D to catalyse transphosphatidylation of the corresponding phosphatidylcholine in the presence of *N*-methyl or dimethylethanolamine (e.g., Sisk and Huang, 1992). The formation of *N*-acylphosphatidylethanolamines and other analogues is detailed by Slotboom and Bonsen (1970) and that of ethanolamine plasmalogens in Section 5.6.10.

Synthesis of 1-palmitoyl-2-diphenylhexatrienylpropanoylphosphatidylethanolamine for use as a fluorescent probe is given by Beck et al. (1990), while dialkylphosphatidylethanolamines are detailed by Ruocco and Makriyannis

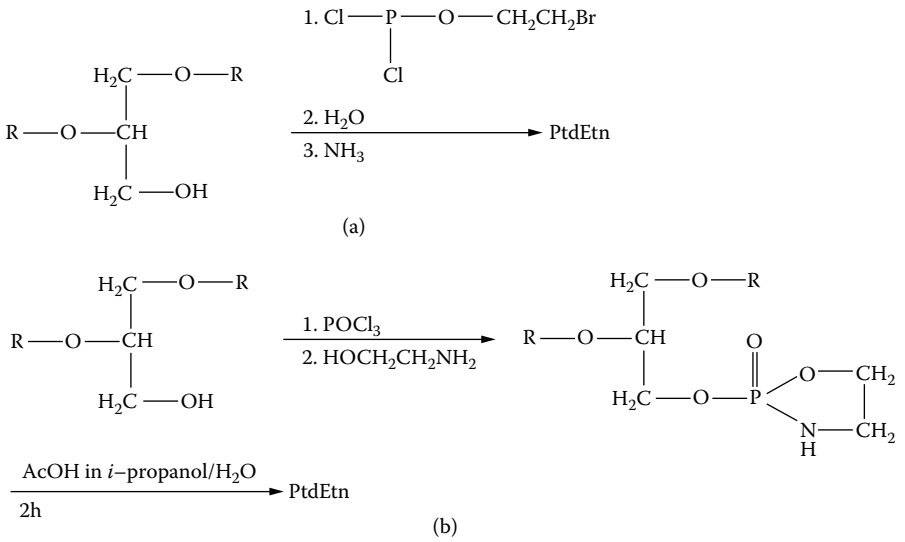


FIGURE 5.4 Synthesis of ethanolamine glycerophospholipids from diradylglycerols. (Adapted from Paltauf, F. and Hermetter, A. (1994) *Prog. Lipid Res.,* 33, 239–328.)

(1989). Moschidis (1987) described the preparation of 1-*O*-alkylethylene glycol phosphonic acid analogues of phosphatidylethanolamine. Other derivatives include *N*-biotinyl phosphatidylethanolamine, carboxyacyl- and *P*-maleimidophenyl butyryl- compounds (Paltauf and Hermetter, 1994).

5.6.6 Phosphatidylserines

Three basic methods that can be used are discussed below.

5.6.6.1 Synthesis of phosphatidylserine from phosphatidic acid

One simple procedure allows the synthesis of phosphatidylserine by condensation of phosphatidic acid with an amino- and carboxy-protected serine. *N*-Carbobenzoxyl-DL-serine benzyl ester was condensed with the phosphatidic acid in the presence of triisopropylbenzenesulphonyl chloride. The protecting groups were then removed by hydrogenation, which limits the method to saturated phosphatidylserines. However, the use of different protecting groups should allow this method to be used for unsaturated compounds. An alternative procedure involves the introduction of the phosphate and serine functions via a complex silver salt to glycerol iodohydrin diesters (de Haas et al., 1964).

5.6.6.2 Synthesis of phosphatidylserine from substituted glycerophosphorylserine

1,2-Isopropylideneglycerol is treated successively with phosphorus oxychloride and *N*-phthalylserine. Hydrolysis of the ketal and the phosphomonochloride groups then yields glycero-3-phosphoryl-*N*-phthalylserine as the barium salt after neutralization with barium hydroxide. Acylation with an excess of acid chloride then furnishes the desired phosphatidylserine. In addition, the acylation can be accomplished in a stepwise manner to give mixed-acid phosphatidylserines (Shvets et al., 1967).

5.6.6.3 Use of phosphotriester intermediate

Woolley and Eibl (1988) have described the preparation of enantiomerically pure phosphatidylserines. They used a procedure that is applicable to the formation of many phospholipids. Their method was to make an appropriate diacylglycerol (or dialkylglycerol), to react this with phosphorus oxychloride, then an alkyl donor and finally use methanolysis to create a phosphate triester. The nature of the alkyl donor determined the headgroup of the final phospholipid, while demethylation was used at a later stage. In the case of phosphatidylserine the yield was 95%.

5.6.6.4 Compounds related to phosphatidylserine

Phosphatidylthreonine has been prepared by a method involving phosphorylation of isopropylideneglycerol with phosphorus oxychloride and a sulphonyl chloride-activated condensation (Moore and Szelke, 1970).

5.6.7 Phosphatidylglycerols

Phosphatidylglycerol can be synthesised using 2,3-dibenzyl-*sn*-glycerol as one of the starting materials (Saunders and Schwartz, 1966). The blocking groups must be removed by catalytic hydrogenolysis, so the method can only be used for saturated molecular species. Furthermore, the use of phosphorus oxychloride also has disadvantages (Slotboom and Bonsen, 1970).

Of the other reported syntheses of phosphatidylglycerol, the method of Bonsen et al. (1966) is the most widely used. It allows preparation of saturated or unsaturated mixed-acid and same-acid phosphatidylglycerols of any configuration. It should be borne in mind that

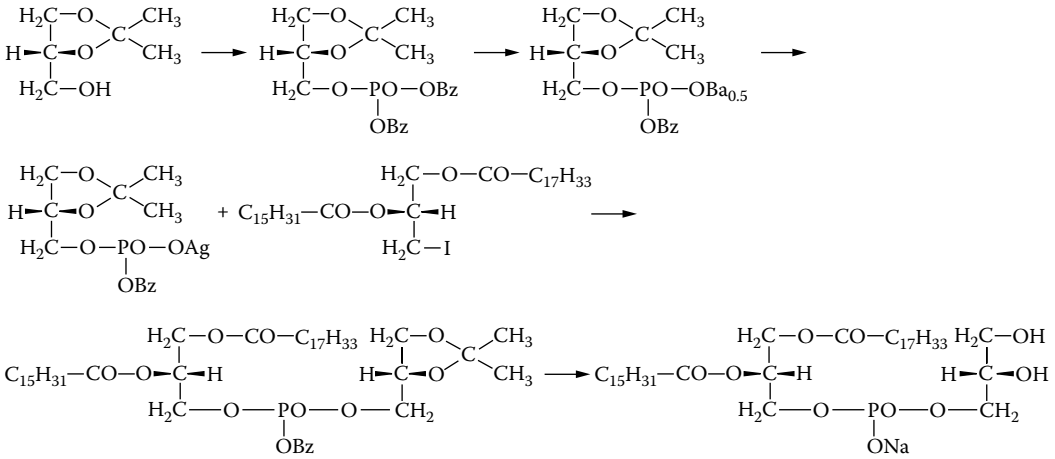


FIGURE 5.5 Synthesis of a mixed-acid phosphatidylglycerol; Bz = benzyl. (Scheme adapted from Slotboom, A.J. and Bonsen, P.P.M. (1970). *Chem. Phys. Lipids*, 5, 301-397.)

stereochemistry is especially important for this phospholipid since, in the natural compound, the unacylated glycerol has the *sn*-1 configuration. Therefore, 2,3-isopropylidene-*sn*-glycerol is a starting material (Figure 5.5). The critical stage is the coupling of a silver salt and an iodo derivative. For various reasons (Slotboom and Bonsen, 1970), 1,2-diacyl-3-iododeoxy-*sn*-glycerol and the silver salt of *sn*-glycerol-1-phosphate, containing appropriate blocking groups, were used.

Dibenzylphosphoric acid and dicyclohexylcarbodiimide were used to generate tetrabenzyl phosphate. This could then be reacted with 2,3-isopropylideneglycerol in the presence of imidazole to yield 2,3-isopropylidene-*sn*-glycerol-1-dibenzyl phosphate. Debenzylation of the latter was carried out with barium iodide and the barium salt was converted to the silver salt, which was reacted with 3-iododeoxy-1-oleoyl-2-palmitoyl-*sn*-glycerol. The fully protected phosphatidylglycerol was debenzylated, followed by reaction with boric acid in trimethyl borate, which affected removal of the isopropylidene group (Rosenthal, 1975). Yields of 30% are obtained (Slotboom and Bonsen, 1970).

Rosenthal (1975) provides notes on certain parts of the preparative procedure. This includes preparation of dibenzylphosphoric acid and of 2,3-isopropylidene-*sn*-glycerol.

The preparation of enantiomerically pure phosphatidylglycerol is described by Woolley and Eibl (1988), while Calderon and Yague (1988) reported the preparation of phosphatidylglycerols from diacylglycerols. Halophilic bacteria contain a diphytanyl analogue of phosphatidylglycerol with the opposite stereo-chemical configuration of the diphytanylglycerol moiety. Using diphytanylglycerol (from a natural source) as starting material, Joo and Kates (1969) prepared the iodo derivative. Condensation with the silver salt of di-*p*-nitrobenzyl phosphate was followed by debenzylation to give the silver salt of 2,3-diphytanyl-*sn*-glycerol-1-(mono-*p*-nitrobenzyl) phosphate. The latter was reacted with 1-benzyl-3-iododeoxy-2-*t*-butyl-*sn*-glycerol or its enantiomer, and the protecting groups were released by catalytic hydrogenolysis and then treatment with hydrogen chloride. Careful optical rotation measurements established that the natural compound was 1-*sn*-phosphatidyl-3-*sn*-glycerol (i.e., both glycerol moieties had the opposite configurations to the glycerol moieties of the diacyl phospholipid from other bacteria or from plants). For a fuller discussion of diether structural analogues of phoshatidylglycerol, phosphatidylglycerophosphate and phosphatidylglycerol sulphate, see Kates (1978).

Methods have been reported for the synthesis of aminoacyl esters (Bonsen et al., 1965) and glucosaminyl esters (Verheij et al., 1971) of phosphatidylglycerol.

Phosphatidylglycerol phosphate was synthesised by Bonsen and de Haas (1967). 1,3-Diiodo-2-*O*-*t*-butylglycerol was reacted with the silver salt of 1,2-diacyl-*sn*-glycerol-3-(monobenzyl) phosphate to produce the mono condensation product. This was reacted with the silver salt of di-*t*-butyl phosphate, and then anionic debenzylation and treatment with hydrogen chloride yielded 3-*sn*-phosphatidyl-1-*rac*-glycero-3 phosphate.

The diether analogue of phosphatidylglycerol phosphate, which occurs in halobacteria, was synthesised by Joo and Kates (1968, 1969).

5.6.8 Diphosphatidylglycerols (cardiolipins)

1,3-Diphosphatidylglycerol appears to be the structure of natural cardiolipin, but this has only been proven in a few cases (Slotboom and Bonsen, 1970). Some derivatives of diphosphatidylglycerol have also been isolated in high yields from beef heart using chromatography (cf. Slotboom and Bonsen, 1970) or by recrystallization as the barium salt followed by chromatography on ammonia-treated silicic acid (Takahashi et al., 1967).

Several methods of synthesizing diphosphatidylglycerol have been described. De Haas and van Deenen (1965) reacted 1 mol of 1,3-diiodo-2-*O*-*t*-butyloxypropane with 2 mol of the silver salt of 1-stearoyl-2-oleoyl-*sn*-glycerol-3-(monobenzyl) phosphate. Anionic debenzylation and then hydrogen chloride successively released the blocking groups (see Figure 5.6; Rosenthal, 1975).

Inoue and Nojima (1968) used a similar method for the synthesis of racemic diphosphatidylglycerol. These workers used a benzyl-protecting group instead of the *t*-butyl group and, therefore, had to use catalytic hydrogenolysis for its removal. Consequently, their method can be used only for saturated molecular species.

A semisynthetic approach for making diphosphatidylglycerol with two identical acyl chains at the *sn*-2 position of both phosphatidyl groups is that of Dale and Robinson (1988). They blocked the free hydroxyl group of bovine heart cardiolipin with a tetrahydropyranyl group and then used phospholipase A_2 followed by acylation.

Saunders and Schwartz (1966) used 1,2-distearoyl-*sn*-glycerol, phosphorus oxychloride and 2-benzyl-glycerol as starting materials. Because they also removed the benzyl group by catalytic hydrogenolysis, only saturated derivatives could be made.

de Haas and van Deenen (1963) have prepared acyl derivatives of diphosphatidylglycerol. A series of diphosphatidylalkanediols have been synthesised by Inoue and Nojima (1968). Moschidis (1988) has described the preparation of phosphono analogues of diphosphatidylglycerol, while Fowler et al. (1988) reported the synthesis of various photoreactive analogues.

5.6.9 Phosphatidylinositols and other inositol lipids

The asymmetry of the inositol moiety poses a considerable problem for the synthetic chemist. A synthetic asymmetrically substituted inositol derivative must, therefore, be used, and this should contain an optically active inositol moiety (the natural isomer being *myo*-inositol).

Unsaturated phosphatidylinositol has been synthesised by Molotkovsky and Bergelson (1971) using an optically active glycerol derivative, but a racemic *myo*-inositol moiety, which was added from an acetylated inositol phosphate benzyl ester. Klyashchitski et al. (1971) prepared a completely optically active dipalmitoylphosphatidylinositol. However, because they used benzyl and phenyl protecting groups, their method is not suitable for unsaturated phosphatidylinositols.

In the procedure of Molotkovsky and Bergelson (1971) *rac*-2,3,4,5,6-penta-*O*-acetyl-*myo*-inositol was first converted to *rac*-1-dibenzylphosphoryl-2,3,4,5,6-penta-*O*-acetyl-*myo*-inositol-1-benzyl phosphate, which in turn was made into a silver salt. The latter was reacted with 3-iododeoxy-1-lauroyl-2-oleoyl-*sn*-glycerol and the resultant triester was debenzylated with sodium iodide. The product was purified, reacted with hydrazine hydrate and the phosphatidylinositol finally purified by chromatography. Care was necessary to avoid deacylation of the product, and the method adopted seemed to avoid the danger of unprotected *myo*-inositol phosphotriesters undergoing positional migration. For details, refer to Rosenthal (1975) and Bergelson (1980).

The synthesis of enantiomerically pure lyso- and alkylphosphatidylinositols is described by Filthath and Eibl

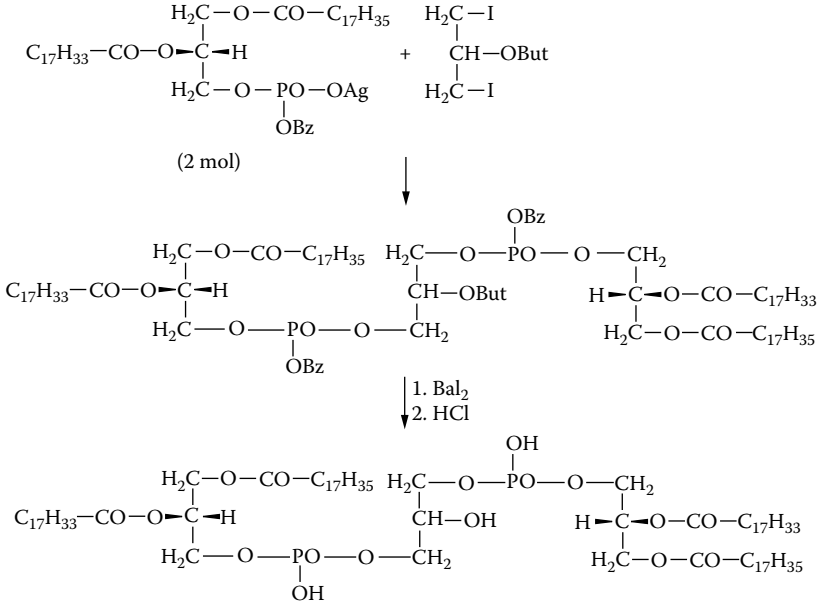


FIGURE 5.6 Synthesis of mixed-acid unsaturated diphosphatidylglycerol by the method of de Haas and van Deenen (1965). (Adapted from Slotboom, A.J. and Bonsen, P.P.M. (1970). *Chem. Phys. Lipids*, 5, 301–397.)

(1992). A chromatogenic substrate for phosphatidylinositol-specific phospholipase C enzymes has been prepared by Shashidhar et al. (1991). A method for the synthesis of isopropylidene derivatives of phosphatidylinositol is given by Noda and Keenan (1990).

The higher inositides, diphosphoinositide (1-(3-*sn*-phosphatidyl)-L-*myo*-inositol 4-phosphate) and triphosphoinositide (1-(3-*sn*-phosphatidyl)-L-*myo*-inositol 4,5-*bis*phosphate) can be isolated from natural sources. Great care must be taken at several stages in order to obtain reasonable yields of material (cf. Schacht, 1981).

Following the synthesis of a readily available chiral protected *myo*-inositol derivative for the synthesis of inosital phosphates, the derivative can be used to form a basis for making a whole series of phosphatidylinositol phosphates. The overall methods are depicted in Figure 5.7 and details will be found in Desai et al. (1996) and references therein. Of particular note were the 3-phosphorylated phosphatidylinositols since there is much current interest in the activity and function of phosphatidylinositol 3-kinase (Payrastre, 2004). Alternative ways of making phosphatidylinositol-3-4,5-*tris*phosphate are described by Watanabe et al. (1994) and by Gou and Chen (1994). Synthesis of phosphatidylinositol phosphates in general is described by Desai et al. (1996).

Paltauf and Hermetter (1994) described other methods for the production of phosphatidylinositol (often with different fatty acid combinations) by the use of semisynthetic

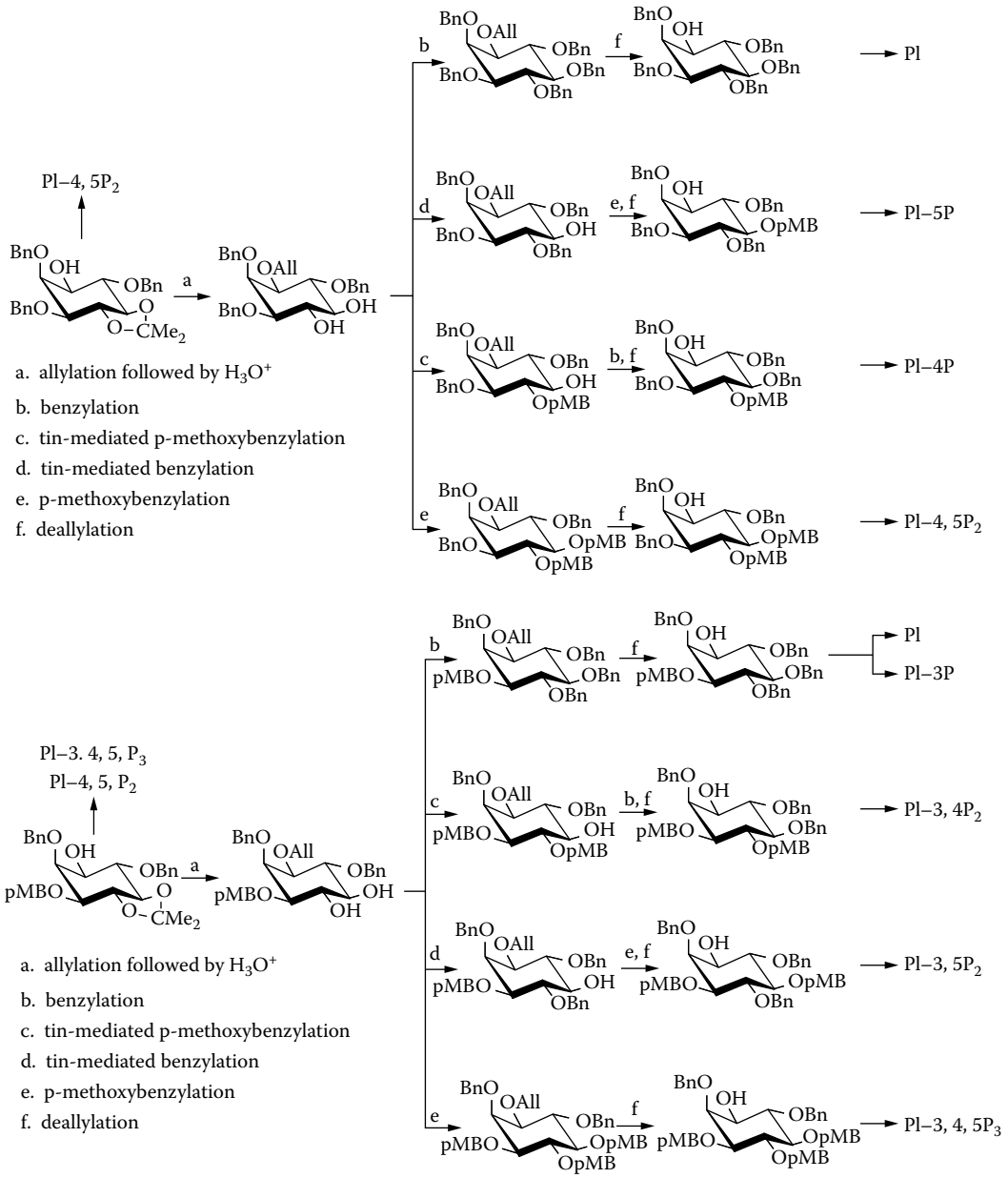


FIGURE 5.7 Two overall methods for synthesizing phosphatidylinositol phosphates. (From Desai, T. et al. (1996) In *Synthesis in Lipid Chemistry* (Ed., J.H.P. Tyman), Royal Soc. of Chemistry, Cambridge, U.K., pp. 67–93.)

routes. In one method, total chemical synthesis from phosphatidic acid yielded phosphatidylinositol in a 40% yield (Ward and Young (1988), and the diisopropylidene derivative could be converted to phosphatidylinositol 4-phosphate (Jones et al., 1989). Other methods for these lipids are also given in Paltauf and Hermetter (1994) and a method for preparing phosphatidylinositol-4,5-*bis*phosphate in 32% overall yield is that of Dreef et al.(1988).

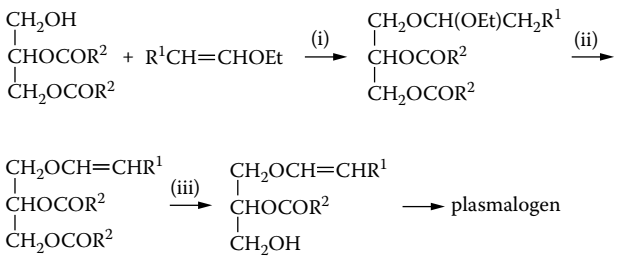
Verheij et al. (1970) have synthesised phosphatidylglucose and Lukyanov et al. (1965) have made a phosphatidylinositol aminoethyl phosphotriester isomer. Synthesis of glycosylphosphatidylinositol anchors is detailed by Gigg and Gigg (1997).

5.6.10 Plasmalogens

Plasmalogens are a group of phosphoglycerides that are phosphorylated derivatives of 1-(alk-*cis*-1-enyl)-2-acyl-*sn*-glycerol. The similarity in properties between plasmalogens and the corresponding 1,2-diacyl phospholipids has made isolation procedures difficult. Moreover, chemical synthesis is made difficult by the presence of both alkali-labile fatty acid ester bonds and a reactive, acid-labile *cis*-vinyl ether bond.

The synthesis of 1-alkenylglycerols, 1-alkenyl-2,3-diacylglycerols (neutral plasmalogens) or 1-alkenyl-2-acyl-3-halodeoxyglycerols is detailed by Slotboom and Bonsen (1970) and Paltauf (1983). These syntheses are carried out in five ways: (1) methods involving debromination of cyclic glycerol-(1-bromoalkyl) acetals; (2) syntheses involving partial reduction of a triple bond; (3) procedures involving thermal elimination of 1,2-diacylglycerol from *bis*(1,2-diacylglycerol) acetals; (4) methods involving elimination of *p*-toluenesulphonic acid or hydrogen iodide; and (5) syntheses involving elimination of hydrogen from 1-chloroethers.

Methods for the synthesis of choline plasmalogens have been reviewed (Paltauf, 1983). Synthesis of a choline plasmalogen has been reported by Slotboom et al. (1967). An addition reaction between hexadec-1-enyl ethyl ether and *rac*-1,2-dipalmitoylglycerol was catalysed by *p*-toluenesulphonic acid to yield a mixed acetal (Figure 5.8). Thermal elimination of ethanol in the presence



Reagents: I, *p*-$CH_3C_6H_4SO_3H$; ii, sulphanilic acid (thermal elimination); iii, pancreatic lipase.

FIGURE 5.8 Synthesis of choline plasmalogen.

of sulphanilic acid gave a neutral plasmalogen, which was modified so that the palmitic acid at position-2 was replaced with oleic acid. (This was because natural plasmalogens usually contain an unsaturated moiety at position-2). The modified neutral plasmalogen was hydrolysed with pancreatic lipase, which yielded 1-hexadec-1′-enyl-2-oleoyl-glycerol. (The advantages of using pancreatic lipase, which does not attack vinyl ether linkages, is fully discussed by Slotboom and Bonsen (1970).) The above derivative was then converted to the choline plasmalogen by the method of Hirt and Berchtold (1958).

Interestingly, although total chemical synthesis of choline plasmalogen is well described (see Paltauf and Hermetter, 1994), most researchers seem to use semisynthetic methods. This is especially true for plasmalogens with defined alkenyl/acyl chain composition or for radio- or fluorescent-labelled compounds.

Methods for the total chemical synthesis of ethanolamine plasmalogens have been reported (Paltauf, 1983). In addition, ethanolamine plasmalogens with special moieties (e.g., fluorescent or radioactive acyl chains at the *sn*-2 position) can be made from the appropriate phosphatidylcholine using phospholipase D (Loidl et al., 1990).

Radioactive ethanolamine plasmalogen can be conveniently prepared and isolated from protozoan cell cultures (Achterberg et al., 1986a). Radioactive ethanolamine plasmalogen can also be used to prepare dimethylethanolamine and choline analogues by means of a phospholipase D-catalysed reaction (Achterberg et al., 1986b).

A simple method for producing lysoethanolamine plasmalogen uses mild alkaline hydrolysis, if necessary of a mixture of natural phosphatidylethanolamine and its plasmalogen (Hannahan et al., 1990). Appropriate use of protective groups during acylation then allows formation of plasmalogens with defined acyl groups (Hermetter and Paltauf, 1982). A partial synthesis scheme has been used for the formation of 1-(alk-*cis*-1′-enyl)-2-acyl-*sn*-glycero-3-phosphoric acid (Eibl and Lands, 1970).

A phosphotriester derivative of 1-dodec-1′-enyl-2-stearoylglycerol has been made, but this was not converted into a plasmalogen. However, the 1-alkenyl-2-acyl-3-bromodeoxyglycerol can be used as a starting compound to form the *N, N*-dimethyl derivative of 1-hexadec-1′-enyl-2-stearoylglycero-3-phosphorylethanolamine. (See Rosenthal (1975) for more details.)

Chacko et al. (1967) have reported the preparation of a synthetic plasmalogen analogue; an acetal phosphatidylcholine. The synthesis of plasmalogens and a number of substituted or double-labelled derivatives has also been summarized by Horrocks and Sharma (1982).

Dialkylphosphatidylcholines and –phosphatidylethanolamines can be made by a method detailed by Abdelmageed et al. (1989). General methods for the preparation of alkyl ether and vinyl ether substrates for phospholipases are given by Paltauf and Hermetter (1991). These authors

also describe the preparation of the biologically active platelet-activating factor (PAF) (1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) (see Section 5.6.12), while Wang and Tai (1990) reported the synthesis of an aldehydic analogue of PAF for use in the preparation of antibodies.

5.6.11 Monoacylphosphoglycerides

Since 1- and 2-monoacylphosphoglycerides are important metabolic intermediates, there has been some interest in their chemical synthesis. In addition, specific phospholipases can be used to prepare either the 1- or 2-monoacyl compounds. Thus, pancreatic lipase attacks the 1-position of a phosphoglyceride to yield the 2-monoacyl product, while snake venom phospholipase removes only the 2-acyl moiety (see Section 10.2.2). For the chemical methods, a major problem is acyl migration. This is particularly rapid for the 2-monoacylphosphoglyceride, since the primary ester is more stable. Therefore, the primary hydroxyl must be protected throughout the synthesis and the blocking group only released at the final stage.

So far, methods are available for saturated monoacylphosphoglycerides. These are discussed by Slotboom and Bonsen (1970). For production of a 1-monoacyl compound, the 2 position of glycerol can be protected with a benzyl ether group.

Slotboom et al. (1963, 1967) prepared *rac*-1-stearoyl-lyso-phosphoglycerides starting from *rac*-1-stearoyl-2-benzyl-3-iododeoxyglycerol (cf. Rosenthal. 1975). Other methods are detailed in Slotboom and Bonsen (1970). In addition, Fujiwara et al. (1967) have reported that 80 to 90% of the 2-acyl ester of phosphatidylcholine can be cleaved with laurylamine or certain alkoxides.

Several methods for the synthesis of 2-monoacyl-phosphoglycerides are described in Slotboom and Bonsen (1970). These use benzyl or trityl groups to protect the free hydroxyl group and the blocking group is removed by hydrogenolysis. If this is carried out in neutral medium, then acyl migration is minimized. A representative method is that of de Haas and van Deenen (1965), who prepared four out of the six possible monoacylphosphatidylcholines.

Billimoria and Lewis (1968) used trityl groups for blocking, but showed that removing them by refluxing with 90% aqueous acetic acid for 10 min rather than by hydrogenolysis gave 100% of the 1-monoacyl product. Although this complete migration demonstrated the importance of neutral solutions in the final step, it does suggest that their method could be used for the preparation of unsaturated 1-monoacylphosphoglycerides (cf. Rosenthal, 1975).

Eibl et al. (1970) formed a D-α-monoacylphosphatidylcholine using a 1,2,5,6-D mannitol tetrabenzyl ether intermediate. Arnold et al. (1967) described a method for the preparation of DL-α and β-ether monoacylphosphatidylcholines, while *O*-methylated and acetylated monoacylphosphatidylcholines were synthesised by Weltzein and Westphal (1967). Enantiomerically pure ester and other lysophospholipids were prepared by Eibl and Woolley (1988), while Filthuth and Eibl (1992) reported the synthesis of lysophosphatidylinositol.

5.6.12 Platelet activating factor (PAF) and analogues

Platelet activating factor (PAF) is most easily made by semisynthetic procedures beginning from choline plasmalogen. The acyl group is removed from the *sn*-2 position and acetylation then uses acetic anhydride (Figure 5.9; see Demopoulos et al., 1979). Alternative natural sources of ether lipids that can be used as starting materials include ethanolamine plasmalogen (Kumar et al., 1984) or 1-*O*-alkylglycerols from ratfish liver oil (Muramatsu et al., 1981).

Complete chemical synthesis of PAF has been accomplished with either saturated or unsaturated alkyl groups (Figure 5.10; see Paltauf and Hermetter, 1994). An alternative method (Surles et al., 1985) allows the synthesis

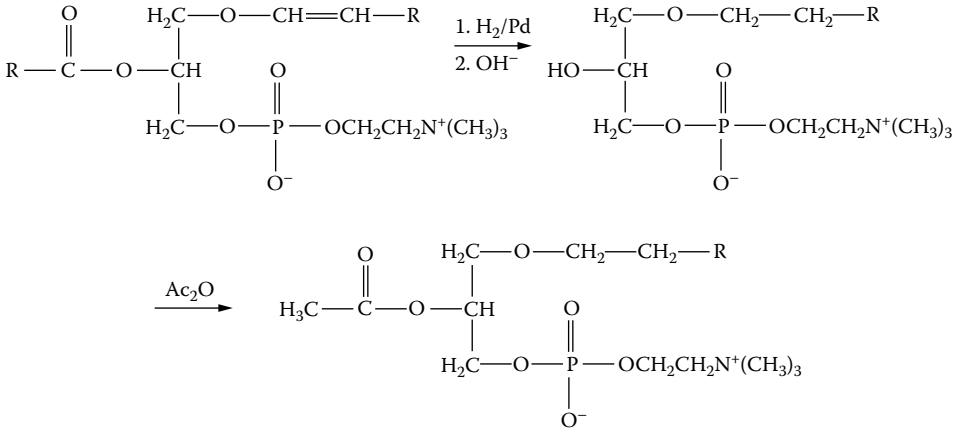


FIGURE 5.9 Semisynthetic preparation of PAF from natural choline plasmalogen. (Adapted from Paltauf, F. and Hermetter, A. (1994) *Prog. Lipid Res.,* 33, 239–328.)

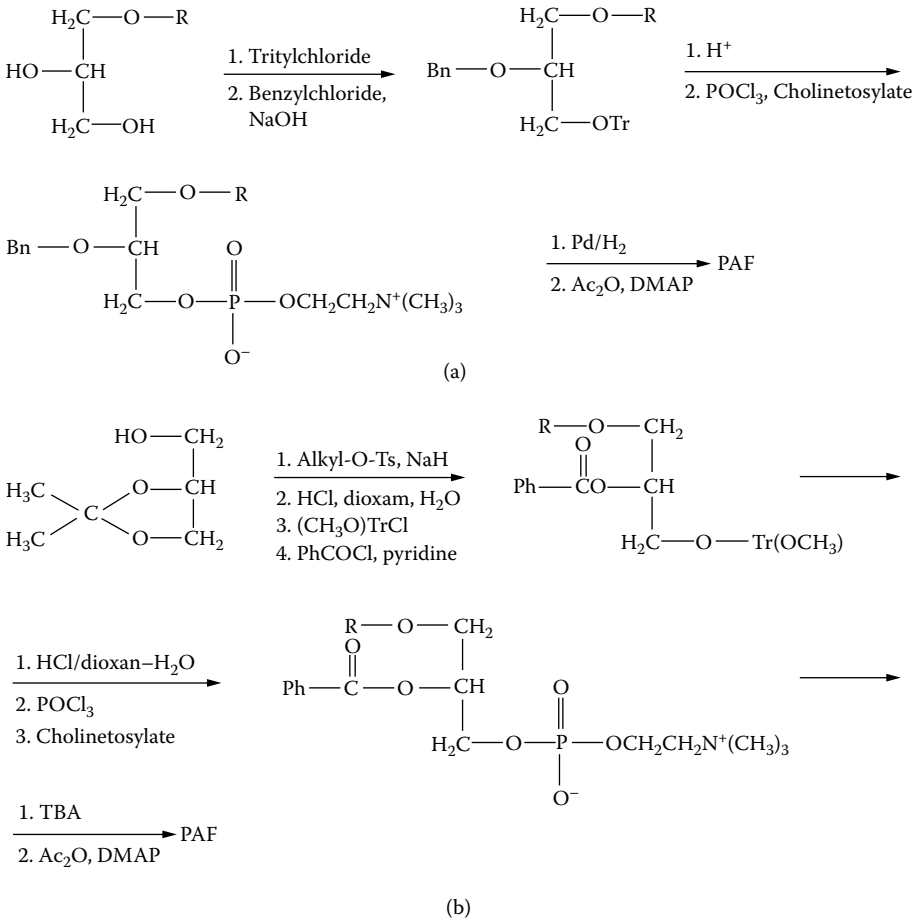


FIGURE 5.10 Total chemical synthesis of PAF. (Adapted from Paltauf, F. and Hermetter, A. (1994) *Prog. Lipid Res.,* 33, 239–328.) R = 9(Z)octadecenyl; Tr(OCH$_3$) = 3-Methoxytrityl; TBA = Tetrabutylammoniumhydroxide.

of racemic and optically active PAF containing an unsaturated alkyl chain in a rather short reaction series.

A number of chiral adducts other than glycerol derivatives, including D- mannitol and D-or L-tartaric acids, have been used as starting materials for the synthesis of PAF (Paltauf and Hermetter, 1994). These authors also describe the synthesis of a whole series of PAF analogues. Such compounds are of great current interest as PAF agonists or antagonists where they can have clinical value, e.g., for the treatment of bronchial asthma. Analogues include PAF modified in the alkyl chain, those containing *N*- or S- substitutes, those with modifications of the glycerol backbone and those with alterations in the head group. In addition, over a hundred derivatives of PAF with nonhydrolyzable substituents on the *sn*-2 position of glycerol have been synthesised (Zeisig et al., 1990). Many have potential as anticancer compounds and some have been investigated in clinical trials.

5.6.13 Phosphonolipids

These compounds occur naturally in a large number of organisms, particularly sea anemones, aquatic molluscs and ciliates (see Section 1.2.3.4). Methods have been reported for the synthesis of: (1) phosphonolipids with the C-P bond between the phosphorus moiety and the polar headgroup, i.e., diacyl-*sn*-glycerol-3(*X*)-phosphonates; (2) phosphonolipids derived from 1,2-propanediol with a phosphonic acid attached to C-3, i.e., 1,2-diacyloxy- (or alkoxy-) propylphosphonic acid (or ester); (3) the phosphono analogue of sphingolipids, i.e., ceramide-(*X*)-phosphonate; and (4) phosphinic acid analogues with two C-P bonds, i.e., 1,2-diacyloxy- (or alkoxy-)propyl-(*X*)-phosphinate. These structures are indicated in Figure 5.11. For details of the synthesis of such compounds, see Slotboom and Bonsen (1970) and Rosenthal (1975). Hori and Nozawa (1982) have reviewed phosphonolipids in detail.

A number of phosphonolipid analogues of phospholipids have been prepared. These include 1-*O*-alkylethylene glycol phosphonic acid analogues of phosphatidylcholine and phosphatidylethanolamine (Moschidis, 1987), a phosphono analogue of diphosphatidylglycerol (Moschidis, 1988), chiral vinylic analogues of phosphatidic acid and phosphatidylcholine (Schwartz et al., 1988a) and α-hydroxyphosphonolipid analogues of phosphatidic acid (Schwartz et al., 1989b).

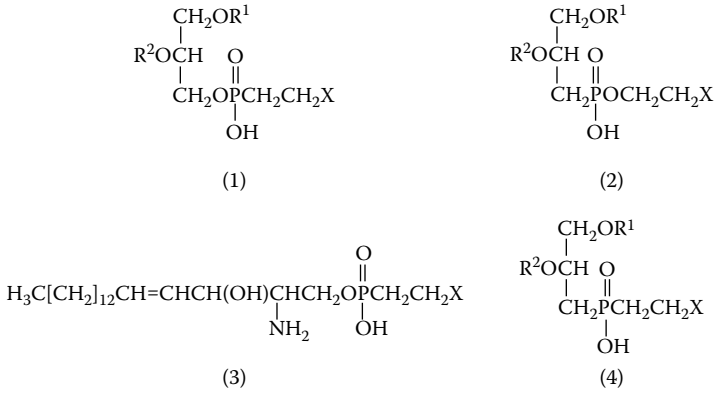


FIGURE 5.11 General structures of types of phosphonolipids that have been synthesised. X represents a polar head-group in each case. Note the two C-P bonds for compounds in Group 4.

5.6.14 Phospholipid analogues

Phospholipid analogues are unnatural compounds with high metabolic stability, but which are readily incorporated into cell membranes (Geilen et al., 1994). Disruption of plasma membrane signalling pathways by such membrane-active compounds could represent a new approach to chemotherapy (Arthur and Bittman, 1988). Most currently used analogues are based on lysophosphatidylcholine (lyso PC) or on lyso-PAF. Structures synthesised include glycerol-containing *sn*-2 substituted analogues, long-chain, glycerol-free phosphobase agents, sugar-containing analogues of lysoPC or lysoPAF and phosphocholine-containing analogues of sphingomyelin with truncated acyl side chains. References to their synthesis, and details of their anticancer effects and possible mechanisms of action are given in Wieder et al. (1999).

Many analogues of glycerophospholipids are useful in basic science. These include fluorescent, photoactivate, and isotopically or spin-labelled compounds. There are some extremely useful compendia for such analogues and references to their synthesis in Paltauf and Hermetter (1994). In addition, these authors give information about the synthesis of polymerisable phospholipids, for use in stable liposomes (Ringsdorf et al., 1988) or in the ultra-thin membranes of sensors or biomedical devices (Nakaya et al., 1990).

References

Abdelmageed, O.H. et al.(1989). Syntheses of ^{2}H- and ^{13}C-labelled 1,2-di-*O*-hexadecyl-*sn*-glycero- 3-phosphoethanolamines and 1,2-di-*O*-hexadecyl-*sn*-glycero-3-phosphocholines. *Chem. Phys. Lipids,* **50**, 163–169.

Achterberg, V. et al. (1986a). Biosynthetic preparation of radioactively labeled ethanolamine plasmalogen (1-*O*-[1-14C]octadec-1′-enyl-2-acyl-*sn*-glycero-3-phosphoethanolamine) using a protozoan cell culture. *Chem. Phys. Lipids,* **41**, 137–145.

Achterberg, V. et al. (1986b). Conversion of radiolabelled ethanolamine plasmalogen into the dimethylethanolamine and choline analogue via transphosphatidylation by phospholipase D from cabbage. *Chem. Phys. Lipids,* **41**, 349–353.

Ali, S. and Bittman, R, (1989) Mixed-chain phosphatidylcholine analogues modified in the choline moiety: preparation of isomerically pure phospholipids with bulky head groups and one acyl chain twice as long as the other. *Chem. Phys. Lipids.,* **50**, 11–21.

Ali, S., and Bittman, R. (1990). Synthesis of optically active 1-acyl-2-*O*-alkyl-*sn*-glycero-3-phosphocholine via 1-*O*-benzyl-*sn*-glycerol 3-arenesulfonate. *Biochem. Cell Biol.,* **68**, 360–365.

Aneja, R. and Chadha, J.S. (1971a). Acyl-chloro-deoxyglycerophosphorylcholines: structure of the so-called cyclic lysolecithins. *Biochim. Biophys, Acta,* **239**, 84–91.

Aneja, R. and Chadha, J.S. (1971b). A total synthesis of phosphatidylcholines. *Biochim. Biophys. Acta,* **248**, 455–457.

Aneja, R., Chadha, J.S. and Davies, A.P. (1970). A general synthesis of glycerophospholipids. *Biochim. Biophys. Acta,* **218**, 102–111.

Arnold, D. et al. (1967). Concerning the synthesis of lysolecithin and its ether analogs. *Justus Liebigs Ann. Chem.,* **40**, 234–239.

Arthur, G. and Bittman, R. (1998). The inhibition of cell signalling pathways by antitumor ether lipids. *Biochim. Biophys, Acta,* **1390**, 85–102.

Baer, E. and Kindler, A. (1962). L-α-(Dioleoyl)lecithin. Alternate route to its synthesis. *Biochemistry,* **1**, 518–521.

Barlow, P.N. et al. (1988). Synthesis and some properties of constrained short-chain phosphatidylcholine analogues: (+)- abd (–)-(1,3/2)-1-*O*-(phosphocholine)-2,3,-*O*-dihexyanoylcyclopentane-1,2,3-triol. *Chem. Phys. Lipids,* **46**, 157–164.

Beck, A. et al. (1990). 1-Palmitoyl-2-[3-(diphenylhexatrienyl) propanoyl]-*sn*-glycero-3-phosphoethanolamine as a fluroscent membrane probe. Synthesis and partitioning properties. *Chem. Phys. Lipids,* **55**, 13–24.

Berecoechea, J. et al. (1968). Phospholipid ethers. Synthesis of dipalmityl-1,2-oxy-3-glyceryl-phosphoric acid and dipyro (dipalmityl-1,2-oxy-3-glyceryl phosphoric) acid. *J. Bull. Soc. Chim. Biol.,* **50**, 1561–1567.

Bergelson, L.D. (1980) *Lipid Biochemical Preparations,* Elsevier, Amsterdam.

Billimoria, J.D. and Lewis, K.O. (1968). The synthesis of phospholipids. I. Phosphatidyl and lysophosphatidylethanolamines. *J. Chem. Soc.,* [Perkin 1], **12**, 1404–1412.

Bittman, R. (1999). Chemical synthesis of glycerophospholipids and their analogs. In *Lipid Synthesis and Manufcture,* Ed., F.D. Gunstone, Sheffield Academic Press, Sheffield, U.K., pp. 185–207.

Bittman, R. et al. (1984). Synthesis of phospholipids via dimethylphosphoryl chloride. *Chem. Phys. Lipids,* **34**, 201–205.

Bonsen, P.P.M. and de Haas, G. H. (1967). Silver di-*t*-butyl phosphate, a useful reagent in the synthesis of phosphonolipids. Synthesis of mixed-acid phosphatidic acid and phosphatidyl glycerophosphate. *Chem. Phys. Lipids,* **1**, 100–109.

Bonsen, P.P.M. et al. (1965). Synthesis and enzymic hydrolysis of an *O*-alanyl ester of phosphatidyl glycerol. *Biochim. Biophys. Acta.* **106**, 93–105.

Bonsen, P.P.M. et al. (1966). The synthesis of 3-phosphatidyl-1-glycerol. *Chem. Phys. Lipids*,. **1**, 33–40.

Bonsen, P.P.M. et al. (1972). Chemical synthesis of some lecithin analogues—potential inhibitors of phospholipase A. *Chem. Phys. Lipids,* **8**, 199–220.

Brockerhoff, H. and Ayengar, N.K.N. (1979). Improved synthesis of choline phospholipids. *Lipids,* **14**, 88–89.

Brockerhoff, H. and Yurkowski, M. (1965). Simplified preparation of L-α-glyceryl phosphoryl choline. *Can. J. Biochem.,* **43**, 1777.

Bruzik, K.S. et al. (1986). A general method for the synthesis of glycerophospholipids. *J. Org. Chem.,* **51**, 2368–2370.

Caldern, J. and Yague, P. (1986). Phosphoric acid derivatives. XV. Phosphatidylethanolamines and N, N-dimethyl-phosphatidylethanolamines from diacylglycerol (*O*-acetoxyphenyl hydrogen phosphates). *Chem. Phys. Lipids,* **41**, 147–157.

Caldern, J. and Yague, P. (1988). Phosphoric acid derivatives. XVI. Phosphatidylglycerols from diacylglycerol (*O*-acetoxyphenyl hydrogen phosphates). *Chem. Phys. Lipids,* **46**, 121–125.

Ceve, G. Ed. (1993) *Phospholipid Handbook,* Marcel Dekker, New York.

Chacko, G.K. et al. (1967). Plasmalogen synthesis. Use of l-alkynylglycerols and the production of allenic ethers. *J. Org. Chem.,* **32**, 3698–3700.

Chandrakumar, N.S. and Hajdu, J. (1982). A new method for the stereospecific synthesis of ether phospholipids. Preparation of the amide analog of platelet-activating factor and related derivatives. *Tertrahedron Lett.,* **23**, 1043–1046.

Cubero Robles, E. and Van Den Berg, D. (1969). Synthesis of lecithins by acylation of *O*-(*sn*-glycero-3-phosphoryl) choline with fatty acid anhydrides, *Biochim. Biophys. Acta,* **187**, 520–526.

Dale, M. P., and Robinson, N.C., (1988). Synthesis of cardiolipin derivatives with protection of the free hydroxyl: its application to the study of cardiolipin stimulation of cytochrome c oxidase. *Biochemistry,* **27**, 8270–8275.

Dang, Q.Q. et al. (1982). Synthesis and identification of *bis* (diacylglycero) phosphoric acid and *bis* (monoacylglycero) phosphoric acid. *Lipids,* **17***,* 798–802.

de Haas, G.H. and van Deenen, L.L.M. (1961). Synthesis of enantiomeric mixed-acid phosphatides. *Rec. Trav. Chim.,* **80**, 951–970.

de Haas, G.H. and van Deenen, L.L.M. (1963). Cardiolipin and derivatives. 1. Synthesis of an acyl derivative of diphosphatidylglycerol. *Rec. Trav. Chim.,* **82**, 1163–1172.

de Haas, G.H. and van Deenen, L.L.M. (1965). Structural identification of isomeric lysolecithins, *Biochim. Biophys. Acta,* **106**, 315–325.

de Haas, G.H. et al. (1964). Synthesis of mixed-acid phosphatidylserine containing unsaturated fatty acids. *Rec. Trav. Chim.,* **83**, 99–1168.

Demopoulos, C.A. et al. (1979). Platelet-activating factor. Evidence for 1-*O*-alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine as the active component (a new class of lipid chemical mediators). *J. Biol.Chem.,* **254**, 9355–9358.

Desai, T. et al. (1996) Synthesis of phosphatidylinositol phosphates. In *Synthesis in Lipid Chemistry*, Ed., J.H.P. Tyman, Royal Soc. of Chemistry, Cambridge, U.K., pp. 67–93 .

Dreef, C.E. et al. (1988). Synthesis of 1-*O*-(1,2-di-*O*-palmitoyl-*sn*-glycero-3-phospho)-D-myo-inositol 4,5-*bis*phosphate an analog of naturally occurring PtdIns(4,5) P_2. *Tetrahedron Lett.,* **29**, 6513–6516.

Eibl, H. (1978). Phospholipid synthesis: oxozaphospholanes and dioxaphospholanes as intermediates. *Proc. Natl. Acad. Sci. U.S.A.,* **75**, 4074–4077.

Eibl, H. and Lands, W.E.M. (1970). Phosphorylation of 1-alkenyl-2-acylglycerol and preparation of 2-acylphosphoglycerides. *Biochemistry,* **9**, 423–428.

Eibl, H. and Nicksch, A. (1978). The synthesis of phospholipids by direct amination. *Chem. Phys. Lipids,* **22**, 1–8.

Eibl, H. and Woolley, P. (1988). A general synthetic method for enantiomerically pure ester and ether lysophospholipids. *Chem. Phys. Lipids,* **47**, 63–68.

Eibl, H. et al. (1970). Synthesis of choline phosphatides, 6.3-palmitoyl-glycerol-1-phosphorylcholine (D-α-lysolecithin) and its inactivity as substrate for acyltransferases. *Liebig's Ann. Chem.,* **738**, 161–169.

Ella, K.M. et al. (1997). Utilization of alcohols by plant and microbial phospholipase D. *Biochem. Mol. Biol. Intl.,* **41**, 715–724.

Erukella, R.K. et al. (1995). Stereospecific and regiospecific ring opening of glycidol with primary and secondary alcohols mediated by diisobutyl aluminium hydride. *J. Chem. Soc. Perkin Trans. I.,* **18**, 2199–2200.

Filthuth, E. and Eibl, H. (1992). Synthesis of enantiomerically pure lysophosphatidylinositols and alkylphosphoinositols. *Chem. Phys. Lipids,* **60**, 253–261.

Fowler, W.T. et al. (1988). Photoreactive cardiolipin analogues. *Chem. Phys. Lipids,* **47**, 261–271.

Fujiwara, Y. et al. (1967). The preferential cleavage of the β-ester linkage of egg yolk lecithin by treatment with alkoxides and laurylamine. *Bull. Chem. Soc. Japan,* **40**, 230–231.

Geilen, C.C. et al. (1994). Uptake, subcellular distribution and metabolism of the phospholipid analog hexadecylphosphocholine in MDCK cells. *Biochim. Biophys. Acta,* **1211**, 14–22.

Gigg, R. and Gigg, J. (1997). Synthesis of glycosylphosphatidylinositol anchors. In *Glycopeptides and Related Compounds*, Eds., D.G. Large and C.D. Warren, Marcel Dekker, New York, pp. 327–392.

Gou, D-M. and Chen, C-S. (1994). Synthesis of L-phosphatidyl-D-myo-inositol 3,4,5-trisphosphate, an important

intracellular signalling molecule. *J. Chem. Soc. Chem. Commun.,* 2125–2126.

Gupta, C.M. et al. (1977). Glycerophospholipid synthesis: Improved general method and new analogs containing photoactivable groups. *Proc. Natl. Acad. Sci. U.S.A.,* **74**, 4315–4319.

Hanahan, D.J. et al. (1990). Novel route to preparation of high purity lysoplasmenylethanolamine. *J. Lipid Res.,* **31**, 2113–2117.

Hansen, W.J. et al. (1982). An improved procedure for the synthesis of choline phospholipids via 2-bromoethyl dichlorophosphate. *Lipids*, **17**, 453–459.

Harbison, G.S. and Griffin, R.G. (1984). Improved method for the synthesis of phosphatidylcholines. *J. Lipid. Res.,* **25**, 1140–1142.

Hebert, N. and Just, G. (1990). Synthesis of phospholipids using an inverse phosphate triester approach. *J. Chem. Soc. Chem. Commun.,* 1497–1498.

Hendrickson, H.S. (1991). Phospholipase A_2 assays with fluorphore-labelled lipid substrates. *Methods in Enzymology,* New York, **197**, 90–94.

Hendrickson, H.S. and Hendrickson, E.K. (1990). A facile asymmetric synthesis of glycerol phospholipids via trityl-glycidol prepared by the asymmetric epoxidation of allyl alcohol. Thiolester and thioether analogs of phosphatidylcholine. *Chem. Phys. Lipids,* **53**, 115–120.

Hermetter, A. et al. (1983). An improved method for the preparation of "mixed-chain" phosphatidylethanolamines. *Chem. Phys, Lipids,* **32**, 145–152.

Hermetter, A. et al. (1987). 1-*O*, *N*-ditritylglycerophosphoethanolamine, a novel intermediate for the facile preparation of mixed-acid phosphatidylethanolamines. *Chem. Phys. Lipids,* **43**, 69–77.

Hermetter, A. et al. (1989). 1-*O*-trityl-*sn*-glycero-3-phosphocholine: a new intermediate for the facile preparation of mixed-acid 1,2-diacylglycerophosphocholines. *Chem. Phys Lipids.*, **50**. 57–62.

Hessel, L.W. et al. (1954). Phosphatidic acids and derivatives. IV. Synthesis of iodohydrin. *Recueil. Trav. Chim. Pays-Bas Belgique*, **73**, 150–156.

Hirt, R. and Berchtold, R. (1958). Zur synthese der phosphatide. 2. Eine neue synthese der lecithine. *Pharm. Acta Helv.*, **33**, 349–356.

Hori, T. and Nozawa, Y. (1982). Phosphonolipids. In *Phospholipids,* Eds., J.N. Hawthorne and G.B. Ansell, Elsevier, Amsterdam, pp. 95–128.

Horrocks, L.A. and Sharma, M. (1982). Plasmalogens and *O*-alkyl glycerophospholipids. In *Phospholipids,* Eds., J.N. Hawthorne and G.B. Ansell, Elsevier, Amsterdam, pp. 51–93.

Inoue, K. and Nojima, S. (1968). Immunochemical studies of phospholipids. II. Syntheses of cardiolipin and its analogues. *Chem. Pharm. Bull. (Tokyo),* **16**, 76–81.

Isaacson, Y. et al. (1990). The synthesis and molecular dynamics of phospholipids having hydroxylated fatty acids at the *sn*-2 position. *Chem. Phys, Lipids,* **52**, 217–226.

Jones, M. et al. (1989). Improved syntheses of inositol phospholipid analogs. *Tetrahedron Lett.,* **30**, 5353–5356.

Joo, C.N. and Kates, M. (1968). Synthesis of the phytanyl diether analogs of phosphatidyl glycerophosphate and phosphatidyl glycerol present in *Halobacterium cutirubrum. Biochim. Biophys. Acta,* **152**, 800–803.

Joo, C.N. and Kates, M. (1969). Synthesis of the naturally occurring phytanyl diether analogs of phosphatidyl glycerophosphate and phosphatidyl glycerol. *Biochim. Biophys. Acta,* **176**, 278–297.

Kates, M. (1978). The phytanyl ether-linked polar lipids and isoprenoid neutral lipids of extremely halophilic bacteria. *Prog. Chem. Fats Other Lipids,* **15**, 301–342.

Klyashchitski, B.A. et al. (1971). Derivatives of asymmetrically substituted myoinositol. VI. Synthesis of 2,3,4,5,6- and 1,2,4,5,6-penta-0-benzyl-sn-myo-inositols. Total synthesis of 1-0-(1, 2-dipalmitoyl-sn-glyceryl-phosphoryl)sn-myo-inositol. *Zh. Obshch. Khim.,* **41**, 1386–1393.

Kodali, D.R. and Duclos, R.I. Jr. (1992) Debenzylation and detritylation by bromodiemethylborane: synthesis of mono-acid or mixed-acid, 1,2- or 2,3-diacyl-*sn*-glycerols. *Chem. Phys. Lipids,* **61**, 169–173.

Kumar, R. et al. (1984). A facile route to semi-synthesis of acetyl glycerylether phosphoethanolamine and its choline analogue. *J. Lipid Res.,* **25**, 198–208.

Lai, C. et al. (1988). Spin-label assay for phospholipase A_2. *Anal. Biochem.,* **172**, 397–402.

Lapidot, Y. et al. (1969). The synthesis of diacyl-DL-(and –L-)α-glycerol phosphates. *Chem. Phys. Lipids,* **3**, 125–134.

Lebeau, L. et al. (1988). Synthesis of phospholipids linked to steroid hormone derivatives. *Chem. Phys. Lipids,* **46**, 57–62.

Lemmen, P. et al. (1990). A synthesis of phospholipids using a phosphite-triester approach and uniformly cleavable-halogenaed protecting groups. *Chem. Phys. Lipids*, **53**, 65–75.

Lindh, I. and Stawinsky, J. (1989). A general method for the synthesis of glycerophospholipids and their analogs via H-phosphonate intermediates. *J. Org. Chem.*, **54**, 1338–1342.

Loidl, J. et al. (1990). Uptake of fluorescent plasmalogen analogs by cultured human skin fibroblasts deficient in plasmalogen. *Biochim. Biophys. Acta,* **1049**, 75–84.

Lukyanov, A.V. et al. (1965). Complex lipids. Synthesis of α-(α', α-distearoyl)glycerylphosphoryl-(1-myo-inositol)ethanolamine. *Zh. Or.Khim*., **36,** 1029–1031.

Mangroo, D. and Gerber, G.E. (1988). Phospholipid synthesis: effects of solvents and catalysts on acylation. *Chem. Phys. Lipids,* **48**, 99–108.

Mason, J.T. Broccoli, A.V. and Huang, C-H. (1981). Method for the synthesis of isomerically pure saturated mixed-chain phosphatidylcholines. *Anal. Biochem.,* **113**, 96–101.

Mena, P.L. and Djerassi, C. (1985). Synthesis of 5,9-hexacosadienoic acid phospholipids. 11. Phospholipid studies of marine organisms. *Chem. Phys. Lipids,* **37**, 257–270.

Molotkovsky, J.G. and Bergelson, L.D. (1971). The resolution of racemic alcohols through the acid oxalates. A new synthesis of optically active *myo*-inositol 1-phosphates. *Tetrahedron Lett.* **50**, 4791–4794.

Molotkovsky, J.G. et al. (1976). A new protective group in the synthesis of phospholipids. *Chem. Phys. Lipids,* **17**, 108–110.

Moore, J.W. and Szelke, M. (1970). Synthesis of threonine phosphoglycerides. *Tetrahedron Lett.,* **50**, 4423–4426.

Moschidis, M.C. (1987). Synthesis of 1-*O*-alkylethylene glycol phosphonic acid analogues of lecithin and cephalin. *Chem. Phys. Lipids,* **43**, 39–45.

Moschidis, M.C. (1988). Synthesis of a phosphono analogue of cardiolipin and study of its silicic acid column chromatographic behaviour. *Chem. Phys. Lipids,* **46**, 253–257.

Muramatsu, T, et al. (1981). A facile method for the preparation of 1-O-alkyl-2-O-acetoyl-sn-glycero-3-phosphocholines (platelet activating factor). *Chem, Phys, Lipids,* **29**, 121–127.

Nakaya, T. et al. (1990). Polymerized phospholipid Langmuir-Blodgett multilayer films. *Langmuir,* **6**, 291–293.

Nicholas, A.W. et al. (1983). Synthesis of mixed-acid phosphatidylcholines and high-pressure liquid chromatographic analysis of isomeric lysophosphatidylcholines. *Lipids,* **18**, 434–438.

Noda, N. and Keenan, R.W. (1990). Phosphatidylinositol isopropylidene derivatives: synthesis, characterization and use as synthetic intermediates. *Chem. Phys. Lipids,* **53**, 53–63.

Paltauf, F. (1976). An improved synthesis of 1-O-[3H]-alkyl-2-acyl-sn-glycerol-3-phosphorylethanolamine with an unsaturated acyl chain. *Chem. Phys. Lipids,* **17**, 148–154.

Paltauf, F. (1983). Biosynthesis of 1-*O*-(1-alkenyl) glycerolipids (plamalogens). In *Ether Lipids,* Eds., H. K. Mangold and F. Paltauf, Academic Press, New York, pp. 107–128.

Paltauf, F. and Hermetter, A. (1991). Preparation of alkyl ether and vinyl ether substrates for phospholipases. *Meth.Enzymol.,* **197**,134–148.

Paltauf, F. and Hermetter, A. (1994) Strategies for the synthesis of glycerophospholipids. *Prog. Lipid Res.,* **33**, 239–328.

Patel, K.M., Morrisett, J.D. and Sparrow, J.T. (1979). The conversion of phosphatidylethanolamine into phosphatidycholine labeled in the choline group using methyl iodide, 18-crown-6 and potassium carbonate. *Lipids*, **14,** 596–597.

Payrastre, B. (2004) Phosphoinositides. In *Bioactive Lipids* (Eds., A. Nicolaou and G. Kokotos), The Oily Press, Bridgewater, U.K., pp. 63–84.

Perochon, E. and Tocanne, J.-F. (1991). Synthesis and phase properties of phosphatidylcholine labelled with 8-(2-anthroyl)-octanoic acid, a solvatochromic fluorescent probe. *Chem. Phys, Lipids,* **58**, 7–17.

Reynolds, L.H., Washburn, W.N., Deems, R.A. and Dennis, E.A. (1991). Assay strategies and methods for phospholipases. *Meth. Enzymol.,* **197,** 3–23.

Ringsdorf, H., Schlarb, B. and Venzmer, J. (1988). Molecular architecture and function in polymeric oriented systems. Models for the study of organization, surface recognition, and dynamics in biomembranes. *Angew. Chem. Int. Ed., Engl.,* **27**, 113–158.

Roberts, M.F. (1991). Nuclear magnetic resonance spectroscopy to follow phospholipase kinetics and products. *Meth. Enzymol.,* **197**, 31–48.

Rosenthal. A. F. (1975). Chemical synthesis of phospholipids and analogues of phospholipids containing carbon-phosphorus bonds. *Meth. Enzymol.,* **35**, 429–529.

Saunders, R.M. and Schwartz, H.P. (1966). Synthesis of phosphatidylglycerol and diphosphatidylglycerol. *J. Am. Chem. Soc.,* **88**, 3844–3847.

Schacht, J. (1981). Extraction and purification of polyphosphoinositides. *Meth. Enzymol.,* **72**, 626–631.

Schwartz, P.W. et al. (1989a). Phosphonolipids. The synthesis of chiral vinylic phosphonolipid analogues of phosphatidic acid and phosphatidyl choline. *Chem. Phys. Lipids,* **49**, 1–7.

Schwartz, P.W. et al. (1989b). Phosphonolipids. 2. α-Hydroxyphosphonolipid analogues of phosphatidic acid. *Chem. Phys. Lipids,* **49**, 131–134.

Shashidhar, M.S. et al. (1991). A chromogenic substrate for phosphatidylinositol-specific phospholipase C: 4-nitrophenyl myo-inositol-1-phosphate. *Chem. Phys. Lipids,* **60**, 101–110.

Shvets, V.I. et al. (1967). Complex lipids. XXVIII. Synthesis of highly unsaturated phosphatidylserines. *Zh. Obschch Khim.,* **37**, 1454–1458.

Sisk, R. B. and Huang, C-H. (1992). Calorimetric studies on the influence of N-methylated headgroups on the mixing behavior of diheptadecanoyl phosphatidylcholine with 1-behenoyl-2-lauroylphosphatidylcholine. *Biophys. J.,* **61**, 593–603.

Slotboom, A.J. and Bonsen, P.P.M. (1970). Recent developments in the chemistry of phospholipids. *Chem. Phys. Lipids,* **5**, 301–397.

Slotboom, A.J. et al. (1963). On the synthesis of isomeric lysophosphatides. *Rec. Trav. Chim.,* **82**, 469–486.

Slotboom, A.J. et al. (1967). On the synthesis of plasmalogens. *Chem. Phys. Lipids*, **1**, 192–208.

Stepanov, A.E. and Shvets, V.I. (1986). Formation of phosphoester bonds in phosphoglyceride synthesis. *Chem. Phys. Lipids,* **41**, 1–51.

Stumpf, R. and Lemmen, P. (1990). Syntheses of phospholipids via oxazaphospholanes. *Z.Naturforsch B.,* **45**, 1729–1731.

Surles, J.R. et al. (1985). Facile synthesis of platelet-activating factor and racemic analogs containing unsaturation in the sn-1-alkyl chain. *J. Med. Chem.,* **28,** 73–78.

Takahashi, T. et al. (1967). Rapid method for the preparation of cardiolipid by ion exchange. *Bull. Soc. Chim. Biol.,* **49**, 65–70.

Van der Bend, R.L. et al. (1992). Metabolic conversion of the biologically active phospholipid, lysophosphatidic acid, in fibroblasts. *Biochim. Biophys. Acta,* **1125**, 110–112.

Verheij, H.M., Smith, P.F., Bonsen, P.P.M. and van Deenen, L.L.M. (1970). The chemical synthesis of a phosphatidylglucose. *Biochim. Biophys. Acta,* **218**, 97–101.

Verheij, H.M. et al. (1971). The chemical synthesis of two isomers of glucosaminylphosphatidylglycerol. *Chem. Phys. Lipids.,* **6**, 46–57.

Vinogradov, S.V. et al. (1995) Synthesis of phospholipid-inhibitor conjugates by enzymatic transphosphoatidylation with phospholipase. D. *J. Am. Chem. Soc.,* **115**, 10487–10491.

Wang, C.-J. and Tai, H.-H. (1990). A facile synthesis of an aldehydic analog of platelet activating factor and its use in the production of specific antibodies. *Chem. Phys. Lipids,* **55**, 265–273.

Ward, J.G. and Young, R.C. (1988). Synthesis of 1,2-dipalmitoyl-sn-glycer-3-yl-D-myo-inositol 1-phosphate. *Tetrahedron Lett.,* **29**, 6013–6016.

Warner, T.G. and Benson, A.A. (1977). An improved method for the preparation of unsaturated phosphatidylcholines: acylation of sn-glycero-3-phosphorylcholine in the presence of sodium methylsulfinylmethide. *J. Lipid. Res.,* **18**, 548–552.

Watanabe, Y., Hirofuji, H. and Ozaki, S. (1994). Synthesis of ε. phosphatidylinositol 3,4,5-trisphosphate. *Tetrahedron Lett.,* **35**, 123–124.

Weltzein, H.A. and Westphal, O. (1967). *O*-Methylated and *O*-acetylated lysolecithin. *Justus Liebig's Ann. Chem.,* **709**, 240–243.

Wieder, T., Reutter, W., Orfanos, C.E. and Geilen, C.C. (1999) Mechanism of action of phospholipid analogs as anticancer compounds. *Prog. Lipid. Res.,* **38**, 249–259.

Woolley, P. and Eibl, H. (1988). Synthesis of enantiomerically pure phospholipids including phosphatidylserine and phosphatidylglycerol. *Chem. Phys. Lipids,* **47**, 55–62.

Yu, L. and Dennis, E.A (1991). Thio-based phospholipase assay. *Meth. Enzymol,* **197,** 65–75.

Zeisig, R., Arndt, D. and Brachitz, H. (1990). Ether lipids — synthesis and application in tumor therapy. *Die Pharmazie,* **45**, 809–818

5.7 Sphingolipids

Two general reviews of the chemistry and synthesis of sphingolipids are those of Hakomori (1983) and of Shapiro (1969). Table 5.2 indicates general methods that have been used for the formation of sphingosines or glycosphingolipids. Specific references for the various methods are shown in the table and in the text below.

Gigg and Gigg (1966) and Gigg et al. (1966) have described two ways of synthesizing 4-D-hydroxysphinganine from D-galactosamine and 4-galactose. Gaver and Sweeley (1966) and Mendershausen and Sweeley (1969) prepared 3-oxo derivatives of sphinganine, *N*-acetyl-4-*trans*-sphingenine, and *N*-carbobenzosphinganine. Stoffel

TABLE 5.2 Summary of methods for the chemical synthesis of sphingosines and glycosphingolipids

Compound	Method
Sphingosines	
DL-Sphingosine	Reduction of DL-*erythro*-2-acetamido-3-*keto*-4-*trans*-octadecanoic acid
Synthesis and separation of D- and L-sphingosines	Resolution of DL-*erythro*-2-acetamido-3-hydroxy-4-*trans*-octadecanoate through L(+)-acetylmandeloyl ester
Differential synthesis of *trans*, *erythro* and *threo*; *cis, erythro* and *threo*	Condensation of 2-hexadecynal and 2-nitroethanol, followed by reduction
Dihydrosphingosine	
Dihydrosphingosine, DL-*threo*-, *erythro*-mixture	Oxamination of 3-*keto*-octadecanoate, followed by reduction with $LiAlH_4$
Dihydrosphingosine	Condensation of palmital and β-nitroethanol
D-*Erythro*- dihydrosphingosine	$LiAlH_4$ reaction of β-benzamido-β-hydroxyoctadecanoic acid methyl ester
D-*Erythro*- or D-*threo*-dihydrosphingosine	Ammonolysis of *trans* or *cis* epoxide
D-*Erythro*- dihydrosphingosine	Reduction of β-*N*-acetylamino-β-*keto*-octanoate by $LiAlH_4$ followed by treatment with methyl dichloroacetate and differential crystallization
^{14}C-labelled DL-dihydrosphingosine followed by racemic separation	Separation of DL-sphingosine into D and L diastereoisomers
Phytosphingosine	
D-*Ribo*-D-amino-1,3,4-trihydroxysphinganine	Glucosamine derivative (methyl-α-2-benzamido-2-deoxy-glucofuranoside) is converted to the oxazoline, the 2-allosamine derivative; compound is converted by oxidation to aldehyde, which is then coupled to olefin by Wittig reagent-triphenyltridecylphosphonium bromide; resulting product is reduced by catalytic hydrogenation
Cerobrosides	*N*-Fatty acyl-3-*O*-benzoylsphingosine condensed with
(Cerasine, phrenosine and glucocerebroside)	acetobromo sugars by Koenig-Knor type reaction; $Hg(CN)_2$ as a catalyst
Lactosylceramide	N-Fatty acyl-3-O-benzoylsphingosine condensed with
(cytolipin H)	heptaacetyllactosyl bromide; $Hg(CN)_2$ as a catalyst
Gangliotriaosylceramide	Condensation of decaacetylgangliotriaosyl chloride and N-stearoyl-3-O-benzoylsphingosine
(Tay-Sachs' globoside)	
Sulfatide	Reaction of 2,4,6-tri-O-acetyl-α-D-galactopyranosyl bromide
(3-O-sulphate galactosylceramide)	with N-octadecanoyl-3-O-benzoyl-DL-dihydrosphingosine
Glycolipid analogues	
Gal-β(1→6) Gal-β(1→152)ceramide	Reaction analogous as for synthesis of lactosylceramide
Gal-β(1→3) Gal-β(1→1)ceramide	(see above)

See Hakomori, (1983) for details.

and Sticht (1967) have described a simple method for the synthesis of 3-dehydrosphinganine hydrochloride. For the preparation of radiolabelled sphinganines and sphingenines, refer to Stoffel and Sticht (1967) and to Stoffel et al. (1971) for the synthesis of radiolabelled sphingomyelin. For a summary of these syntheses and other chemical preparations of sphingolipids, see Shapiro (1969).

A very thorough review of the synthesis of racemic sphingosines, chiral pool syntheses, and enantiomeric syntheses is given by Jung and Schmidt (1999). In addition to sphingosine, methods for the preparation of sphinganine, L-*erythro*-sphingosine and threo-sphingosine are also detailed. Furthermore, racemic and enantioselective syntheses of phytosphingosine are also described (Jung and Schmidt, 1999). The authors recommend the use of azido derivatives of sphingosine and phytosphingosine for the production of glycosphingolipids (see also Castro-Polomino et al., 1997; Kratzer and Schmidt, 1995). This is also described in detail in Schmidt (1996).

Barenholz and Gatt (1975) have described techniques for the separation of sphingosine bases by silicic acid chromatography. Bovine spinal cord lipids are used as starting material and hydrolysed in acid-methanol. Sphingosine and dihydrosphingosine can then be isolated from the hydrolysate by chromatography. For the isolation of phytosphingosine and dihydrosphingosine, a fraction obtained from the growth medium of the yeast *Hansenula ciferri* was used.

Ceramides are often prepared from sphingomyelins by phospholipase C digestion (cf. Bergelson, 1980). Hay and Gray (1969) prepared ceramides by such methods. These were then tritylated and the 3-*O*-benzoyl ester was formed. After detritylation, the 3-*O*-benzoylceramide was condensed with the appropriate acetobromo sugar to form various neutral ceramides. Flowers (1967) described a two-step method for lactosylceramide preparation. Individual species of ceramides can be made by *N*-acylation of DL-sphinganine or sphingenine with stearic acid or with DL-2-acetoxypalmitic acid in the presence of a mixed carbodiimide. The free hydroxyl acid ceramide can then be generated by alkaline hydrolysis from the acetate and separation of diastereoisomers by preparative silica gel thin layer chromatography (TLC) (Hammarstrom, 1971).

The ceramides prepared by one of the above methods can be conveniently analysed by TLC and characterized by enzymic hydrolysis. Particular points to pay attention to during these syntheses are summarized by Bergelson (1980).

Galactosylceramide can be conveniently isolated from bovine brain tissue. If this organ is extracted successively with acetone, diethyl ether and then hot aqueous ethanol, sphingolipids are obtained in the final fraction. These precipitate on cooling and the residue is washed with acetone and dissolved in hot acetic acid. On cooling they can again be precipitated, dissolved in pyridine and passed through an aluminum oxide column to separate cerebrosides from sulphatides and gangliosides. After reprecipitating the cerebrosides, these are benzoylated and the benzoate separated by silicic acid chromatography into fractions containing nonhydroxy and hydroxy fatty acids. Finally, the benzoyl groups are hydrolysed with methanolic sodium methoxide (Bergelson, 1980).

Glucosylceramide can be synthesised by condensation of acetobromoglucose with 3-*O*-benzoylceramide. Benzoylceramide can be prepared by the method of Hay and Gray (1969) and is condensed with acetobromoglucose in the presence of mercuric cyanide catalyst (Bergelson, 1980). The benzoyl group is removed with methanolic sodium methoxide and the *O*-β-D-glycosyl (1→1) ceramide purified by silicic acid column chromatography.

Fluharty et al. (1974) have reported the isolation of ^{35}S-labelled cerebroside sulphate. They injected the brains of young rats intracerebrally with [^{35}S]-sulfate and sacrificed the animals 3 days later. Total lipids were extracted and glycerolipids destroyed by alkaline hydrolysis. TEAE-cellulose column chromatography was then used to purify [^{35}S]-sulphatide. Jatzkewitz and Nowoczek (1967) have synthesised D-galactose 3-sulfate and shown it to be identical to the galactose sulgate present in brain sulfatides.

Sulfatide can also be made by sulfation of 2,4,6-tri-*O*-acetylgalactopyranosylceramide followed deacetylation (Ishizuku et al., 1980). In addition, cerebroside 6-sulfate has been prepared by sulfation of cerebroside (Holmgren et al., 1980).

Psychosine (1-*O*-galactosylsphingosine) was prepared by Radin (1974) starting from *O*-β-D-galactosyl (1→1) ceramide. The latter was deacylated by refluxing with aqueous potassium hydroxide in butanol. The potassium hydroxide was removed by precipitation as potassium perchlorate and fatty acids with hexane extraction. Finally, a chloroform/methanol/water mixture allows partition of excess perchloric acid into the upper phase, the chloroform phase containing the psychosine product (cf. Bergelson, 1980).

Sphingomyelin can be obtained from bovine brain following acetone treatment, by ethanol and petroleum ether extraction. It is separated from cerebrosides by aluminum oxide column chromatography and phosphoglycerides are removed by mild alkaline hydrolysis. Silicic acid column chromatography allows final purification (Hanahan, 1961). DL-2-*N*-Stearoyldihydrosphingomyelin was synthesised by Zvonkova et al. (1974). They started with DL-*N*-stearoylsphinganine and tritylated the primary hydroxyl group on C-1. The hydroxyl on C-3 was then benzoylated, when the trityl group could be removed with hydrogen chloride. The unmasked hydroxyl group was then phosphorylated with β-chloroethylphosphodichloride to yield 1-*O*-(β-chloroethylphospho)-3-*O*-benzochloride. The latter was treated with trimethylamine to generate the quaternary ammonium of choline. Removal of the benzoyl group then yielded DL-2-*N*-stearoyl-dihydrosphingomyelin.

Stoffel (1975) has reported a method for the chemical synthesis of choline-labelled sphingomyelins. These

compounds are demethylated to the corresponding ceramide-1-phosphoryl-*N,N*-dimethylethanolamines. The purified compounds are then quaternized in high yield with [^{14}C]- or [^{13}C]- methyl iodide to yield the respective sphingomyelins, which are labelled in their choline moieties. The technique allows the preparation of sphingomyelins of high specific radioactivity regardless of their degree of unsaturation.

Because many of the previous methods to make sphingomyelin were either lengthy or resulted in racemic mixtures, the use of azidosphingosine as starting material has been employed (Dong and Butcher, 1993; Schmidt, 1996). The method could be used to make ceramide-1-phosphate and sphingomyelin. Using 3-*O*-protected azidosphingosine, an analogous sequence could be used for sphingosine-1-phosphate and lysosphingomyelin (Kratzer and Schmidt, 1995).

The isolation of gangliosides from brain has been reviewed by Kanfer (1969) and by Bergelson (1980). Other specific references are Suzuki (1965), Svennerholm et al. (1972) and Laine et al. (1974), and general comments on ganglioside separations will be found in Kates (1986) and Christie (2003).

A typical isolation procedure makes use of the great hydrophilicity of gangliosides. Thus, a chloroform-methanol extract, when subjected to Folch partition, has most of the gangliosides in the upper phase. Phospholipid contaminants of this phase can be removed by alkaline hydrolysis and the gangliosides precipitate as their barium salts. From these the free acids can be generated and then the gangliosides can be crystallized from solution. Other methods of isolating total gangliosides use gel filtration and DEAE-cellulose chromatography for purification (cf. Bergelson, 1980).

Some specific isolations are as follows. Haematoside (G_{M3}) can be obtained from rat liver by Folch partition, dialysis, Sephadex G-50 filtration and preparative TLC on silica gel G. Chloroform/methanol/2.5 M NH_4OH (60/35/8, by volume) is used as solvent and the zones are detected with iodine vapour or, better, with a nondestructive spray, such as primuline (Skipski, 1975). Haematoside is the least polar of the gangliosides and has a R_f of about 0.5. It can be eluted from the silica gel with chloroform/ methanol/ water (60/35/8, by volume).

Monosialyltetrahexosylceramide (G_{H1}) and disialyltetrahexosylceramide ($G_{D1\alpha}$) were isolated from bovine brain total gangliosides by silicic acid column chromatography (Svennerholm et al., 1972). Ganglioside $G_{D1\alpha}$ has also been tritiated. The method involves catalytic addition of tritium gas to the unsaturated centres of the gangliosides isolated from beef brain. Because this results in a partial destruction of the ganglioside, the tritiated $G_{D1\alpha}$ has to be purified by Folch partition, dialysis, and preparative TLC (Bergelson, 1980). Alternatively, purified beef brain ganglioside $G_{D1\alpha}$ can be oxidized selectively in its *N*-acetylneuraminic acid residue with sodium metaperiodate. The oxidized ganglioside is then dialysed and reduced with tritiated borohydride. After dialysis, the product is passed through a Dowex-50 column and freeze-dried (Bergelson, 1980).

Successful synthesis of ganglio-*N*-triosylceramide (Tay-Sachs' globoside) was based on the successful synthesis of ganglio-*N*-triose. After the trisaccharide had been made, it was converted to octaacetyl bromide and, subsequently, condensed with 3-*O*-benzoyl-*N*-stearoylsphingosine (see Hakomori, 1983). Analogous procedures can be used to synthesise various neutral ceramides (Flowers, 1966). Gangliosides with fluorescent or paramagnetic probes on the lipid moiety have also been synthesised chemically (Acquotti et al., 1986).

The Tay-Sachs' ganglioside (G_{M2}) has been prepared, which is specifically labelled in either the *N*-acetylneuraminosyl or *N*-acetylgalactosaminyl portions (Tallman et al., 1975). For the first type of labelling, G_{H1} is an intermediate and, for the second, G_{H3} is isolated from erythrocytes first and then converted to G_{M2}.

Methods for the preparation of inositol-containing sphingolipids are given in Schmidt (1996). These include D-*erythro*-ceramide-1-phosphoinositol and ceramide-containing GPI (glycosyl phosphatidylinositol) anchors.

Two useful review articles covering the isolation and purification of sphingolipids are those of Rouser et al. (1969) and Kundu (1981). Rouser et al. cover the use of DEAE- and TEAE-cellulose column chromatography, while Kundu gives a thorough summary of TLC methods. The latter includes solvent systems, detection methods, analytical and preparative methods, and radio-autography (fluorography). Skipski (1975) and Christie (2003) also discuss separation methods. Skipski (1975) recommends the use of primuline as a nondestructive spray for sphingolipids because it is sensitive and does not interfere with the subsequent analysis of carbohydrate, fatty acids, and sphingosines by gas-liquid chromatography (GLC) or with the colorimetric determination of sphingosines by the method of Yamamoto and Rouser (1970).

Various analogues of sphingolipids have been synthesised, particularly those in which the glycosidic linkages are different from natural compounds (see Hakomori, 1983).

References

Acquotti, D. et al. (1986). A new chemical procedure for the preparation of gangliosides carrying fluorescent or paramagnetic probes on the lipid moiety. *Chem. Phys. Lipids,* **40**, 71–86.

Barenholz, Y. and Gatt, S. (1975). Separation of sphingosine bases by chromatography on columns of silica gel. *Meth. Enzymol.,* **35**, 529–533.

Bergelson, L.D. (1980) *Lipid Biochemical Preparations,* Elsevier, Amsterdam.

Castro-Palomino, J.C. et al. (1997) Efficient synthesis of ganglioside GM_2 for use in cancer vaccines. *Angew. Chem. Int. Ed. Engl.,* **36**, 1998–2001.

Christie, W.W. (2003). *Lipid Analysis,* 3rd ed., The Oily Press, Bridgwater, U.K.

Dong, Z., and Butcher, J.A. (1993). An efficient route to *N*-palmitoyl-D-erythro-sphingomyelin and its carbon-13-labelled derivatives. *Chem. Phys. Lipids*, **66**, 41–46.

Flowers, H.M. (1966). Substituted cerebrosides. II. Synthetic dihydrosulfatides. *Carbo. Res.,* **2**, 371-379.

Flowers, H.M. (1967). Substituted cerebrosides. III. 1-*O*-(4-*O*-β-D-hexopyranosyl--D-glucopyranosyl) dihydroceramides. *Carb.. Res.,* **4**, 42–48.

Fluharty, A.L. et al. (1974). Simplified procedure for preparation of ^{35}S-labelled brain sulfatide. *Lipids*, **9**, 865–869.

Gaver, R.C. and Sweeley, C.C. (1966). Chemistry and metabolism of sphingolipids. 3-Oxo derivatives of N-acetylsphingosine and N-acetyldihydrosphingosine. *J. Am. Chem. Soc.,* **88**, 3643–3647.

Gigg, J. and Gigg, R. (1966). A synthesis of phytosphingosines from D-galactose. *J. Chem. Soc.,* **20**, 1876–1879.

Gigg, J. et al. (1966). A synthesis of phytosphingosines from D-glucosamine. *J. Chem. Soc.,* **20**, 1872–1876.

Hakomori, S.-I. (1983). Chemistry of glycosphingolipids. In *Sphingolipid Biochemistry* (Eds., J. N. Kanfer and S I. Hakomori), Plenum, New York, pp. 1–64.

Hammarstrom, S. (1971). A convenient procedure for the synthesis of ceramides. *J. Lipid Res.*, **12**, 760–765.

Hanahan, D.G. (1961). Sphingomyelin. *Biochem. Prep.*, **8**, 121–124.

Hay, J.B. and Gray, G.M. (1969). The partial synthesis of some naturally occurring glycosphingolipids with special reference to O-β-D-galactosyl-(1→4)-O-β-D-galactosyl-(1→1)-ceramide. *Chem. Phys. Lipids,* **3**, 59–69.

Holmgren, J. et al. (1980). Sendai virus receptor: proposed recognition structure based on binding to plastic-absorbed gangliosides. *Proc. Natl. Acad. Sci. USA,* **77**, 1947–1950.

Ishizuku, I. et al. (1978). Sulfated glyceroglycolipids in rat brain. Structure, sulfation *in vivo*, and accumulation in whole brain during development. *J. Biol. Chem.,* **253**, 898–907.

Jatzkewitz, H. and Nowoczek, G. (1967). Synthesis of sulphur-35-labelled D-galactose sulphate and ceramide D-galactose sulphate (cerebroside sulphate). *Chem. Ber.,* **100**, 1667–1674.

Jung, K.H., and Schmidt, R.R. (1999). Sphingolipids. In *Lipid Synthesis and Manufacture,* Ed., F.D. Gunstone, Academic Press, Sheffield, U.K., pp. 208–249.

Kanfer, J.W. (1969). Preparation of gangliosides. *Meth. Enzymol.,* **14**, 660–664.

Kates, M. (1986) *Techniques in Lipidology,* 2nd ed., Elsevier, Amsterdam.

Kratzer, B. and Schmidt, R. R. (1995). Efficient synthesis of sphingosine-1-phosphate, ceramide-1-phosphate, lysosphingomyelin and sphingomyelin, *Liebig's Ann*., 957–963.

Kundu, S.K. (1981). Thin-layer chromatography of neutral glycosphingolipids and gangliosides. *Meth. Enzymol.,* **72**, 185–204.

Laine, R.A. et al. (1974). Isolation and characterization of membrane glycosphingolipids. *Meth. Membr. Biol.,* **2**, 205–244.

Mendershausen, P.B. and Sweeley, C.C. (1969). Chemistry and metabolism of sphingolipids. Chemical synthesis of 2-amino-1-hydroxyoctadecan-3-one(3-ketosphinganine). *Biochemistry*, **8,** 2633–2635.

Radin, N.S. (1974). Preparative isolation of cerebrosides (galactosyl and glucosyl ceramide). *J. Lipid Res.,* **17**, 290–293.

Rouser, G. et al. (1969). Diethylaminoethyl and triethylaminoethyl cellulose column chromatographic procedures for phospholipids, glycolipids and pigments. *Meth. Enzymol.,* **4**, 272–317.

Schmidt, R.R. (1996). Sphingosines, phosphosphingolipids and glycosphingolipids. In S*ynthesis in Lipid Chemistry,* Ed., J.H.P. Tyman, Royal Soc. Chem., Cambridge, pp. 93–118.

Shapiro, D. (1969). *Chemistry of Sphingolipids,* Hermann, Paris.

Skipski, V.P. (1975). Thin-layer chromatography of neutral glycosphingolipids. *Meth. Enzymol.*, **35**, 396–425.

Stoffel, W. (1975). Chemical synthesis of choline-labelled lecithins and sphingomyelins. *Meth. Enzymol.*, **35**, 533–541.

Stoffel, W. and Sticht, G. (1967). Metabolism of sphingosine bases, II. Studies on the degradation and transformation of [3-14C]erythro-DL-dihydrosphingosine, [7-3H]erythro-DL-dihydrosphingosine in rat liver. *Hoppe-Seyler's Z. Physiol. Chem*., **348**, 1345–1351.

Stoffel, W. et al. (1971). A simple chemical method for labelling phosphatidylcholine and sphingomyelin in the choline moiety. *Hoppe-Seyler's Z. Physiol. Chem.,* **352**, 1058–1064.

Suzuki, K. (1965). The pattern of mammalian brain gangliosides. II. Evaluation of the extraction procedures, postmortem changes and the effect of formalin preservation. *J. Neurochem.,* **12**, 629–638.

Svennerholm, L. et al. (1972). Gangliosides, isolation. *Methods Carb. Chem.* **6**, 464–474.

Tallman, J.F. et al. (1975). The preparation of Tay-Sachs ganglioside specifically labelled in either the *N*-acetylneuraminosyl or *N*-acetylgalactosaminyl portion of the molecule. *Meth. Enzymol.,* **38**, 541–548.

Yamamoto, A. and Rouser, G. (1970). Spectrophotometric determination of molar amounts of glycosphingolipids and ceramide by hydrolysis and reaction with trinitrobenzenesulfonic acid. *Lipids,* **5**, 442–444.

Zvonkova, E.N. et al. (1974). Method for synthesis of sphingomyelins from ceramides. *Khim Prir. Sojed,* **5,** 553–558.

5.8 Glycosylglycerides

Analytical separation and small-scale isolation of galactosylglycerides can be carried out conveniently by TLC on silica gel G (cf. Hitchcock and Nichols, 1971; Harwood, 1980). Sastry (1974) has discussed the various solvent systems in some detail, and the method of Khan and Williams (1977) is particularly useful. For larger-scale preparations, column chromatography by the method of O'Brien and Benson (1964) will be found to be good for the isolation of all three major plant glycosylglycerides. Yields of the two galactosylglycerides may be improved by the use of repeated chromatography on silica gel or on carbon-Celite columns (van der Veen et al., 1967). A rapid method of batch elutions from silica gel has been reported by de Stefanis and Ponte (1969). Most of the fractions obtained by these methods are slightly contaminated, but can be conveniently cleaned up by TLC or, in the case of phospholipid contaminants, by passage through Amberlite MB-3 (Sastry and Kates, 1964). Different methods of isolation and good advice on various techniques for the separation of glycosylglycerides are described by Heinz (1996).

A useful reference for general chemical procedures to synthesise glycoglycerolipids is that of Gigg and Gigg (1990). They cover the synthesis of mono-, di- and triglycosyldiacylglycerols, glycosylalkylacylglycerols, glucosyldialkylglycerols, and some phosphatidylglucosyldiacylglycerols.

Wehrli and Pomeranz (1969) prepared the chiral β-galactosyldiacylglycerol by a method shown in Figure 5.12. Condensation of acetobromogalactose with the glycerol derivative, bis(1-acyl-2-methylene-glycerol), was followed by acid hydrolysis to give the acetylated galactosylmonoacylglycerol. Reaction with acid chloride then gave acetylated galactosyl-diacylglycerol. Hydrazinolysis was used to remove acetyl groups and yield monogalactosyldiacylglycerol stereochernically identical to the naturally occurring plant lipid. The same route could be used to prepare galactosyldiacylglycerols with two different fatty acids (Gigg and Gigg, 1990).

Various β-glycosyldiacylglycerols have been prepared by an orthoacetate glycosidation procedure to diacylglycerols (see Shvets et al*.,* 1973). Glycosides of 2-deoxy-D-glucose with racemic diacylglycerols and glucosyl- or mannosyldiacylglycerols containing fluorescent labels in the lipid and sugar portions have been made (see Gigg and Gigg, 1990). A saturated β-galactosyidiacylglycerol was prepared via an intermediate formed by condensation of aceto-bromogalactose with 1,2-di-*O*-benzyl-*sn*-glycerol (Gent and Gigg, 1975). The latter compound was also used by Batrakov et al. (1976) for the preparation of β-galactosyl-, β-glucosyl-, and α-mannosyldiacylglycerols. Improvements in their procedure resulting in high yields (70 to 75%) have been reported (Mannock et al*.,* 1987; van Boeckel et al., 1985).

Seminolipids (sulfated galactosylacylalkylglycerols occurring in testes and brain of animals) (see Murray and Narasimhan, 1990), α-glucosyldiacylglycerols (occurring in bacteria) (see Kates, 1990b), and monoglycosyldialkylglycerols have all been synthesised chemically (see Gigg and Gigg, 1990, for more details).

Glycosylglycerols have been synthesised by several workers. Wickberg (1958) reported the synthesis of *O*-α-D-galactopyranosyl- and *O*-β-D-galactopyranosyl (1→1)-D-glycerols using a condensation reaction between tetra-*O*-acetyl-α-D-galactopyranosyl bromide and methylene-bis-2-*O*-(3-*O*-benzoyl-D-glycerol). He also prepared the corresponding L-glycerol galactosides. A second method was used by Charlson et al. (1957) for the formation of *O*-α-D-galactopyranosyl- and *O*-β-D-galactopyranosyl(1→2)glycerols. Brundish and Baddiley (1968) have reported the formation of *O*-α-D-glucopyranosyl- and *O*-β-D-glucopyranosyl(1→1)-D-glycerols and their anomeric L-glycerol galactosides. They have also synthesised four positional isomers of diglucosylglycerols. The major glycosylglyceride of *Pneumococcus,* *O*-α-D-galactopyranosyl(1→2)-*O*-α-D-glucopyranosyl(1→1)-D-glycerol, was synthesised by Brundish et al. (1967). Sulfoquinovosylglycerol has been synthesised by Miyano and Benson (1962).

Wehrli and Pomeranz (1969) reported the synthesis of 1,2-dipalmitoyl- and 1-palmitoyl-2-linoleoyl-3-*O*-(-β-D-galactopyranosyl)-*sn*-glycerols. They acylated the primary hydroxyl groups of 2,5-methylene-D-mannitol and then cleaved the mannitol moiety between C-3 and C-4 with lead tetraacetate. The resultant aldehyde was reduced and galactose attached by the Koenigs–Knorr reaction. The acetal was then hydrolysed and the hydroxyl group acylated.

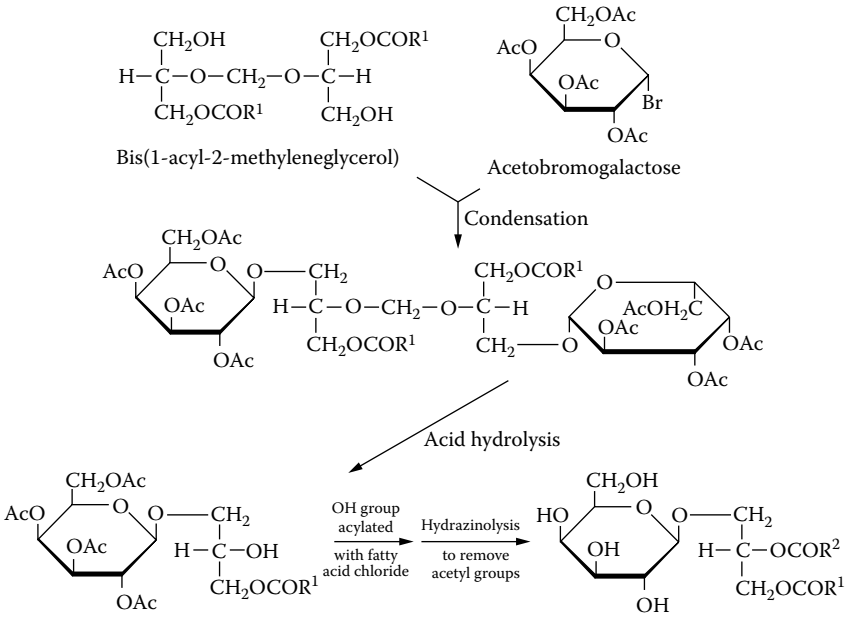


FIGURE 5.12 Synthesis of galactosyldiacylglycerol. Ac = CH_3CO. See Wehrli and Pomeranz (1969).

Hydrazinolysis of the acetylated glycolipid completed the procedure. A simpler method, but which involved loss of optical activity at C-2 of the glycerol, was reported by Bashkatova et al. (1971a). They formed 1,2-dipalmitoyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerol as well as the mannose equivalent. Their procedure has also been used to synthesise diglycosyldiacylglycerols (cf. Bashkatova et al., 1971b; Shvets et al., 1973).

A useful technique described by Heinz (1971) has been used to prepare different molecular species of monogalactosyl- and digalactosyldiacylglycerols. He used a semisynthetic method starting from compounds isolated from natural sources. The hydroxyl hydrogens of the galactose residues of the substrates were substituted with *O*-(1-methoxyethyl) groups and the acyl groups were then removed with sodium methoxide. Acylation was then carried out with the desired fatty acyl chloride, and the protecting groups were removed with boric acid. Heisig and Heinz (1972) also used a similar method for preparing galactosylmonoacylglycerols.

The first synthetic diglycosyldiacylglycerol was prepared by Wehrli and Pomeranz (1969) using a condensation reaction between racemic isopropylideneglycerol and acetobromocellobiose. The isopropylidene group was removed by acid hydrolysis. Acylation with fatty acids and deacetylation with hydrazine yielded β-D-glucopyranosyl(1→4)-D-glucosyldiacylglycerol. Another diglucosyldiacylglycerol (derived from kojibiose, α-D-glucopyranosyl(1→2)-D-glucose) was prepared by van Boeckel and van Boom (1985). They used a route involving condensation of perbenzylated glucosylbromide with the α-glucosyldiacylglycerol derivative.

The important plant glycolipid, digalactosyldiacylglycerol (α-*D*-galactopyranosyl(1→6)-β-D-galactopyranosyl (1→3)-1,2-di-*O*-acylglycerol) was synthesised using a route beginning with the condensation of acetobromogalactose with 1,2-di-*O*-benzyl-*sn*-glycerol as shown in Figure 5.13. The β-galactosyldiacylglycerol was also prepared. Other diglycosyldiacylglycerols, such as those important in bacteria and for serological activity, have been synthesised (see Gigg and Gigg, 1990, for more details).

The plant sulfolipid (sulfoquinovosyldiacylglycerol) was synthesised (Gigg, 1978; Gigg et al., 1980) by a route shown in Figure 5.14. A glucosyl chloride derivative was condensed with 1,2-di-*O*-(but-2-enyl)-*sn*-glycerol to give α-glucoside, which was converted to its acetonide. The toluene-β-sulfonate was converted to the thioacetate, which was then hydrolysed and oxidised (with iodine) to give the disulfide. The isopropylidene group was removed by acid hydrolysis and the alcohol product was acetylated with hexadecanoyl chloride. The resultant ester was oxidized with 3-chloroperbenzoic acid and subsequent debenzylation yielded sulfoquinovosyldiacylglycerol. A comprehensive recent review on sulfoquinovosyldiacylglycerol is that by Harwood and Okanenko (2003).

Methods for the chemical synthesis of 6-*sn*-phosphatidylglucosyldiacylglycerol (found in streptococci), diglycosyldialkylglycerols, triglycosyldiacylglycerols (occurring in plants, bacteria and gastric secretions) (see Kates,

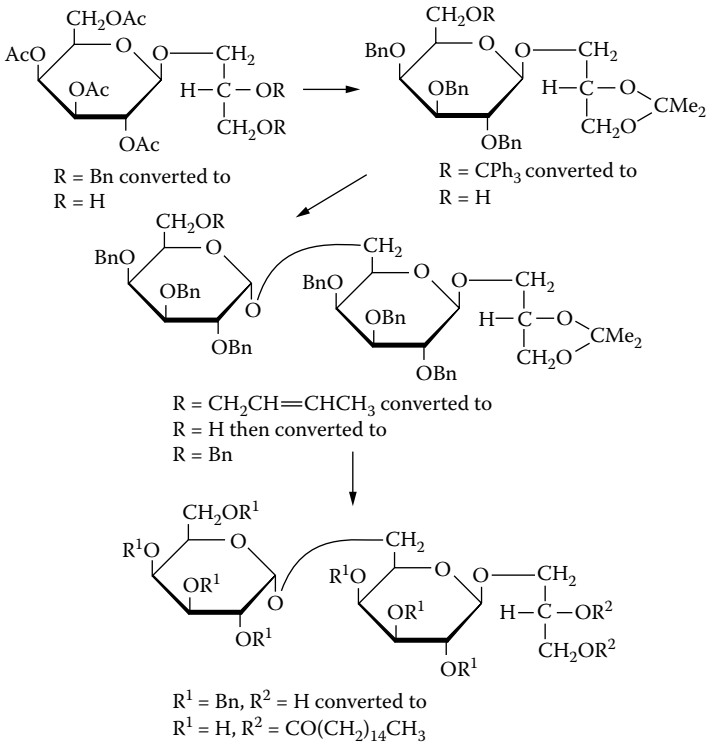


FIGURE 5.13 Synthesis of digalactosyldiacylglycerol. Ac = CH_3CO, Bn = CH_2Ph. See Gent and Gigg (1975).

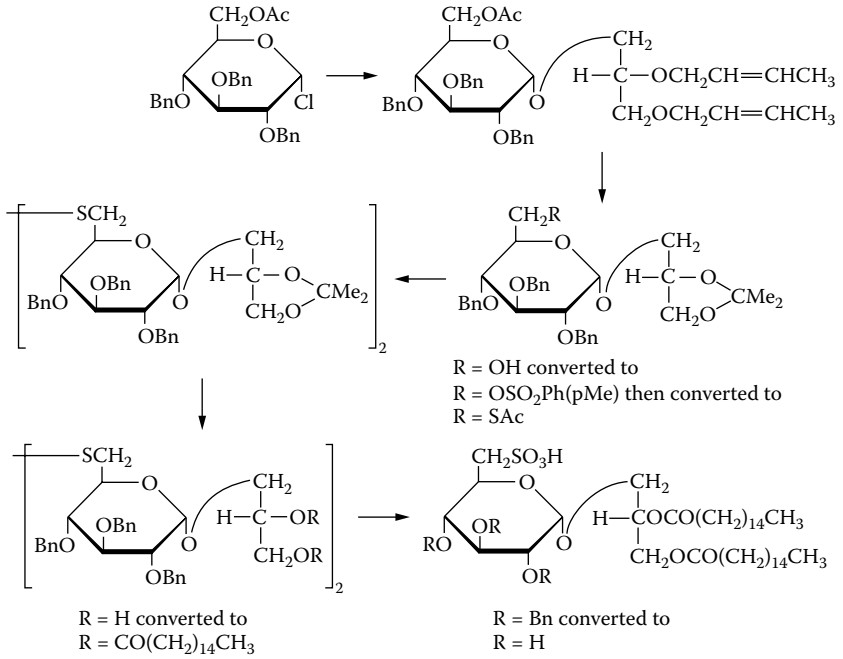


FIGURE 5.14 Synthesis of sulfoquinovosyldiacylglycerol. Ac = CH_3CO, Bn = CH_2Ph. Adapted from Gigg and Gigg, (1990).

1990a) and triglycosyl-*O*-phytanylglycerol (found in halobacteria) (see Kates, 1990b) are discussed by Gigg and Gigg (1990). Heinz (1996) has made a very thorough review of the structure, isolation and analysis of glycosylglycerides. The latter includes structural analytical methods such as mass and nuclear magnetic resonance (NMR) spectrometry.

References

Bashkatova, A.I. et al. (1971a). Glycosyldiglycerides. 1. Synthesis of mannopyranosyldiglyceride and glucopyranosyldiglyceride. *Zh. Org. Khim.,* **7**, 1644–1652.

Bashkatova, A.I. et al. (1971b). Diglycosyldiglyceride synthesis. *Zh. Org. Khim.,* **7**, 2627.

Batrakov, S.G. et al. (1976). Synthesis of monoglycosyl diglycerides and their derivatives. *Bull. Acad. Sci. USSR, Chem.,* **25**, 626–632.

Brundish, D.E. and Baddiley, J. (1968). Synthesis of glucosylglycerols and diglucosylglycerols, and their identification in small amounts. *Carbohydr. Res.,* **8**, 308–316.

Brundish, D.E. et al. (1967). The structure and possible function of the glycolipid from *Staphyloccus lactis* 13. *Biochem. J.,* **105**, 885–889.

Charlson, A.J. et al. (1957). The configuration of glycosidic linkages in oligosaccharides. IV. Further degradations of reducing disaccharides to 2-*O*-glycosyl-glycerol. *Can. J.Chem.,* **35**, 365–373.

De Stefanis, V.A. and Ponte, J.G. (1969). Simple method for isolation of crude mono-and digalactosyl diglycerides from wheat flour. *Biochim. Biophys. Acta,* **176**, 198–201.

Gent, P.A. and Gigg, R. (1975). Synthesis of 1,2-di-*O*-hexadecanoyl-3-*O*-(β-D-galactopyranosyl)-L-glycerol (a 'galactosyl diglyceride') and 1,2-di-*O*-octadecanoyl-3-*O*-(6-*O*-octadecanoyl-β-D-galactopyranosyl)-L-glycerol. *J. Chem. Soc., Perkin Trans.,* **4**, 364–370.

Gigg, R. (1978). Studies on the synthesis of sulphur-containing glycolipids ("sulfoglycolipids"). *Am. Chem. Soc. Symp. Ser.,* **77**, 44–66.

Gigg, J. and Gigg, R. (1990). Synthesis of glycoglycerolipids. In *Handbook of Lipid Research*, vol.6, *Glycolipids, Phosphoglycolipids and Sulfoglycolipids,* Ed., M. Kates, Plenum, New York, pp. 489–506.

Gigg, R. et al. (1980). Synthesis of 3-*O*-(6-deoxy-6-sulpho-α-d-glucopyranosyl)-1,2-di-*O*-hexadecanoyl-l-glycerol, sulphoquinovosyl diglyceride. *J. Chem. Soc., Perkin Trans.,* **1**, 2490–2493.

Harwood, J.L. (1980). Plant acyl lipids: structure, distribution and analysis. In *Biochemistry of Plants,* vol. 4, Eds., P.K. Stumpf and E.E. Conn, Academic Press, New York, pp. 1–55.

Harwood, J.L. and Okanenko, A.A. (2003). Sulphoquinovosyldiacylglycerol — the sulpholipid of higher plants. In *Sulphur in Plants,* Eds., Y.P. Abrol and A. Ahmad, Kluwer, Dordrecht, pp. 189–220.

Heinz, E. (1971). Semisynthetic galactolipids of plant origin. *Biochim. Biophys. Acta,* **231**, 567–544.

Heinz, E. (1996). Plant glycolipids: structure, isolation and analysis. In *Advances in Lipid Methodology,* Ed. W.W. Christie, vol.3. The Oily Press, Dundee, U.K., pp. 211–332.

Heisig, O.M.R.A. and Heinz, E. (1972). Semisynthetic lysogalactolipids of plant origin. *Phytochemistry,* **11**, 815–818.

Hitchcock, C. and Nichols, B.W. (1971). *Plant Lipid Biochemistry,* Academic Press, London.

Kates, M. Ed. (1990a). *Handbook of Lipid Research,* vol. 6, *Glycolipids, Phosphoglycolipids and Sulfoglycolipids,* Plenum, New York.

Kates, M. (1990b). Glyco-, phosphoglyco- and sulfoglycoglycerolipids of bacteria. In *Handbook of Lipid Research,* vol. 6,

Glycolipids, Phosphoglycolipids and Sulfoglycolipids, Ed., M. Kates, Plenum, New York, pp. 1–122.

Khan, M. and Williams, J.P. (1977). Improved thin-layer chromatographic method for the separation of major phospholipids and glycolipids from plant lipid extracts and phosphatidyl glycerol and bis(monoacylglyceryl) phosphate from animal lipid extracts. *J. Chromatogr.,* **140**, 179–185.

Mannock, D.A. et al. (1987). An improved procedure for the preparation of 1,2-di-*O*-acyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerols. *Chem. Phys. Lipids,* **43**, 113–127.

Miyano, M. and Benson, A.A. (1962). The plant sulfolipid, VI: configuration of the glycerol moiety. *J. Am. Chem. Soc.,* **84**, 59–62.

Murray, R.K. and Narasimhan, R. (1990). Glycoglycerolipids of animal tissues. In *Handbook of Lipid Research,* vol. 6, *Glycolipids, Phosphoglycolipids and Sulfoglycolipids* (Ed., M. Kates), Plenum, New York, pp. 321–361.

O'Brien, J.S. and Benson, A.A. (1964). Isolation and fatty acid composition of the plant sulfolipid and galactolipids. *J. Lipid Res.,* **5**, 432–436.

Sastry, P.S. (1974). Glycosyl glycerides. *Adv. Lipid Res.,* **12**, 251–310.

Sastry, P.S. and Kates, M. (1964). Lipid components of leaves. V. Galactolipids, cerebrosides, and lecithin of runner-bean leaves. *Biochemistry,* **3**, 1271–1280.

Shvets, V.I. et al. (1973). Synthesis of glycosyl diglycerides. *Chem. Phys. Lipids,* **10**, 267–285.

Van Boeckel, C.A.A. and van Boom, J.H. (1985). Synthesis of phosphatidyl-α-glucosyl glycerol containing a dioleoyl diglyceride moiety-application of the tetraisopropyldisiloxane-1,3-diyl (tips) protecting group in sugar chemistry, 3. *Tetrahedron,* **41**, 4545–4555.

Van Boeckel, C.A.A. et al. (1985). Synthesis of phosphatidyl-β-glucosyl glycerol containing a dioleoyl diglyceride moiety-application of the tetraisopropyldisiloxane-1,3-diyl (tips) protecting group in sugar chemistry, 4. *Tetrahedron,* **41**, 4557–4565.

Van der Veen, J. et al. (1967). Comparison of column chromatographic methods for the quantitative determination of mono- and digalactosyldiglycerides in fresh alfalfa. *Lipids,* **2**, 406-410.

Wehrli, H.P. and Pomeranz, Y. (1969). Synthesis of galactosyl glycerides and related lipids. *Chem. Phys. Lipids*, **3**, 357–370.

Wickerberg, B. (1958). Synthesis of 1-glycerol D-galactopyranosides. *Acta Chem. Scand.,* **12**, 1187–1201.

5.9 Bulk separation procedures

Syntheses of molecules related to fatty acids have been reported earlier in this chapter. For the most part, these are based on procedures developed and carried out by organic chemists. They have their place in the research laboratory, but there are many other demands for fatty materials that are met by purification or concentration of compounds already present in natural fats. This applies over a range of scales for the research laboratory and for commercial operations and the most commonly employed procedures are described in the following sections. On an industrial scale there has to be a balance between the purity of the product and its cost. As a simple example, refined high-oleic sunflower oil with 80 to 90% of oleic acid may be used in place of triolein. A similar situation exists with phospholipids. A crude mixture (lecithin) may suffice for some purposes, but materials of increasing purity (and cost) frequently display interesting and valuable properties. Procedures, therefore, have been developed to isolate individual phospholipid classes and then to manipulate the fatty acids within these.

5.9.1 Urea fractionation

Urea normally crystallises in tetragonal form, but in the presence of certain aliphatic molecules it forms hexagonal prisms within which some of the aliphatic compound is trapped. These prisms are built up from urea: six molecules form a unit cell 11.1×10^{-10} m long and 8.2×10^{-10} m in diameter containing a channel in which an open chain molecule may be held so long as it is fulfils certain dimensional qualifications. It must not be too short or it will not be held in the channel, and it must not be too wide if it is to fit into the free space, estimated at around 6×10^{-10} m diameter. Many straight chain acids and alkyl esters satisfy these conditions and readily form complexes with urea. These are also called adducts, inclusion compounds, or clathrates. Urea fractionation cannot be applied to triacylglycerols.

Saturated acids form stable complexes more readily than do unsaturated acids, and oleic acid forms inclusion compounds more readily than do the polyunsaturated acids. In practice, urea and mixed acids are dissolved in hot methanol or urea and methyl esters are dissolved in a hot methanol–ethanol mixture. The solution is crystallised at temperatures between 0 and 4°C. After separation, the adduct and mother liquor each furnish acids or esters when mixed with water and extracted with ether or petroleum ether in the usual way.

This procedure is used to separate mixtures of fatty acids according to their degree of unsaturation or to separate straight-chain from branched-chain acids. It has been applied recently, in particular, to separate conjugated linoleic acid isomers and to concentrate the highly unsaturated omega-3 acids in fish oils. Some examples are cited below.

Urea fractionation is simple, does not damage PUFA, can be repeated one or more times to enhance the enrichment of a particular acid, and can be affected on a gram, kg, or tonne scale. It is considered by many to be environmentally benign, but can require large volumes of solvents and a considerable aqueous reject.

Hayes (2002a and b) has discussed the physical processes involved in urea fractionation and has given some examples of its use. He lists the main selectivities in descending order of importance as:

- Increasing discrimination against inclusion as the number of double bonds per molecule increases.
- Preference for molecules of longer chain length.
- Preference for *trans* rather than *cis* double bonds.
- Sensitivity for double bond position.

He further reviews the use of this separation procedure on a large scale suggesting that the urea (and solvent) can be recovered for re-use or used directly as fertiliser.

Urea fractionation was one step in the enrichment of γ-linolenic acid and of stearidonic acid from blackcurrant seed oil carried out on the tonne scale. This included an interesting separation of the α- and γ-isomers of linolenic acid. Under appropriate conditions γ-linolenic acid and stearidonic acid concentrated in the mother liquor fractions while α-linolenic acid was mainly in the adduct (Wille et al., 1988, 1991).

Commercially produced CLA contains two major isomers (9*c*11*t* and 10*t*12*c*) and attempts have been made to separate these by urea fractionation with the 9,11 isomer enriched in the adduct and the 10t12c isomer enriched in the mother liquor (Ma et al., 2002).

In attempts to isolate pure or enriched fractions of highly unsaturated PUFA (EPA and/or DHA) from fish oils urea fractionation on a large scale is frequently employed as the first step to produce concentrates that can be further purified by other procedures, such as molecular distillation or selective enzymatic reaction. Urea fractionation is not only effective for this purpose, it is relatively simple to carry out on kg or tonne scale under mild conditions that do not damage the highly unsaturated acids. For example, the EPA and DHA levels of squid ethyl esters, submitted to urea fractionation, were raised from 12 to 28% and from 15 to 36%, respectively (Hwang et al., 2001). This leads to a significant reduction in the mass of material that has to be submitted to the later processes. At the same time there is a marked reduction in the levels of 20:1 and 22:1 acids that is particularly helpful if the acids are to be subject to molecular distillation to isolate EPA and/or DHA. A second example involves the concentration of PUFA from mackerel processing waste (Zuta et al., 2003).

As a more esoteric example, urea crystallisation was used to prepare a concentrate of pinolenic acid (all-*cis*-5,9,12-18:3) from Korean pine nut oil to study the effect of this acid on LDL-receptor activity. Conditions are reported through which the concentration of pinolenic acid rose from 14% in the oil to 45% in the mother liquor after urea fractionation (Lee 2004).

5.9.2 Distillation under reduced pressure

Distillation of unsaturated methyl or ethyl esters is not generally advised because they may be exposed to high temperatures for a long time. Under these conditions double bond isomerisation (both positional and configurational) and/or cyclisation may occur even though oxidation can be minimised by working under vacuum. Exposure to heat is reduced in short path or molecular distillation and this finds many applications (see below for examples). Reduced pressure distillation depends on boiling point and can be used to separate acids or alkyl esters according to their chain length but not by their degree of unsaturation. On a commercial scale C_8 and C_{10} acids can be isolated from hydrolysed lauric oils and the acids from fish oils may be distilled to give separate cuts rich in C_{14}, C_{16}, C_{18}, C_{20}, and C_{22} acids.

Since the effectiveness of distillation depends on differences of boiling point, chain length is more important than degree of unsaturation. It is possible to separate the esters of C_{12} to C_{22} acids from each other and distillation is commonly used for this purpose. On the other hand, stearate, oleate, linoleate, and linolenate are not usefully separable by distillation.

Distillation of fatty acids is important in industry where it is used primarily to separate saturated fatty acids such as (1) 16:0 and 18:0 from hydrogenated tallow or hydrogenated seed oils; (2) 18:0, 20:0, 22:0, and 24:0 from hydrogenated fish oil or hydrogenated high-erucic rapeseed oil; and (3) 8:0, 10:0, 12:0, and 14:0 from lauric oils. The temperatures range from 160 to 230°C at pressures below 20 mmHg in specially constructed stills (up to 2m in diameter) that run continuously. Some boiling points are collected together in Table 5.3.

TABLE 5.3 Names and boiling points of some alkanoic acids and their methyl esters

Acid Chain Length	Acid Systematic Name	Methyl Ester Trivial Name	Mol wt	BP (°C)[a]	BP (°C)[a]
4	butanoic	butyric	88.1	164	103
6	hexanoic	caproic	116.2	206	151
8	octanoic	caprylic	144.2	240	195
10	decanoic	capric	172.3	271	228
12	dodecanoic	lauric	200.3	130[1]	262
14	tetradecanoic	myristic	228.4	149[1]	114[1]
16	hexadecanoic	palmitic	256.4	167[1]	136[1]
18	octadecanoic	stearic	284.5	184[1]	156[1]
20	eicosanoic	arachidic	312.5	204[1]	188[2]
22	docosanoic	behenic	340.6	–	206[2]
24	tetracosanoic	lignoceric	368.6	–	222[2]

[a] BP at 760 mm Hg or at 1 or 2 mm Hg as indicated by the superscript.

Source: Gunstone, F.D., Harwood, J.L., and Padley, F.E. (1994) *The Lipid Handbook*, 2nd ed., Chapman & Hall, London.

The major uses of distillation at laboratory to industrial scale include:

- Separation of saturated acids from C_6 to C_{22} (and occasionally of monounsaturated acids) using appropriate starting materials (lauric oils, C_{16}/C_{18} seed oils or animal fats, fish oils). This is the major route to saturated acids on a commercial scale. These are obtained at purities up to 99% though lower grades are available (cheaper) and find many uses.
- Purification of glycerol from aqueous solutions.
- Isolation of dimer acid concentrates.
- Tall oil furnishes fractions rich in fatty acids and rich in resin acids (Hubers and Fritz, 1988).
- Isolation of products from enzymatic processes.
- Separation of volatile materials — both desirable and undesirable — such as tocopherols, squalene, sterols, and persistent organic pollutants.
- Concentration of monoacylglycerols from mixed glycerolysis products.

Molecular distillation is used to separate squalene, diacylglycerol ethers, and concentrates of n-3 acids from fish oils on a scale of 200k/24 hours (Anon 2004).

The level of organic environmental pollutants, such as PAH and halogenated organic compounds in fish oils, can be reduced by short path distillation. This procedure is improved by addition of 3 to 6% of an ester mixture that co-distills with the pollutants. The procedure can be applied to fish oils for human consumption or to those that are to be fed to farmed fish thereby reducing their levels in the final marketed product (Breivik and Thorstad 2005).

The products of enzymatic hydrolysis, alcoholysis, or interesterification frequently contain free acids, alkyl esters, free alcohols, and glycerol esters. To recover the desired fraction it may be useful to remove one or more volatile fractions by molecular distillation. Depending on its nature the desired material is sometimes present in a distillate and sometimes in the residue. (See Breivik et al. [1997], Xu et al. [2001, 2002], Yang et al. [2004], Haraldsson et al., [2006].)

Molecular distillation can be used to separate C_{20} and C_{22} acids/esters and is frequently a stage in the isolation of concentrated 20:5 and 22:6 after these have been separated from less unsaturated acids/esters by urea fractionation, e.g., the level of EPA was raised from 9 to 15% and that of DHA from 13 to 25% (Hwang et al., 2001, Liang and Hwang, 2000).

The best samples of commercial CLA contain only two CLA isomers (9*c*11*t* and 10*t*12*c*), but for some purposes it has been desirable to separate these or, at least, to obtain concentrates. This has been achieved through enzyme-catalysed (*Candida rugosa* lipase) esterification with lauric acid. The 9*c*11*t* isomer is the more reactive and concentrates in the ester fraction while the 10*t*12*c* isomer remains mainly as unreacted acid. The esters, free acids, and unreacted lauryl alcohol are separated by molecular distillation (Nagao, 2002).

Soybean deodoriser distillate is an important by-product containing valuable sterols and tocopherols among other less valuable components. The isolation and recovery of the sterols and tocopherols can be achieved in a number of ways that generally include molecular distillation to recover and separate the sterols (sometimes as esters) and tocopherols. Appropriate fractions contain tocopherols (up to 76%), sterols (96%), and sterol esters (98%) (Shimada et al., 2000, Watanabe, 2004).

Many oleochemical processes yield glycerol (in the form of a dilute aqueous solution also containing salts and other impurities) as a commercially important by-product. Evaporation and distillation are important processes in converting this so-called "sweetwater" to glycerol at concentrations of 88%, 95%, and 99.5%.

When heated (to around 230°C for 4 to 8 hours) with a catalyst, such as montmorillonite (4%), unsaturated acids from tall oil or other sources are converted to dimers and trimers that can be separated by distillation. The dimer fraction (~ 80% dimer and 20% trimer) is a mixture of C_{36} dibasic acids used mainly as polyamides.

Glycerolysis is employed on a commercial scale to convert triacylglycerols to mixtures of monoacylglycerols and diacylglycerols by reaction with glycerol in the presence of a basic catalyst. Concentrates of monoacylglycerol (90 to 95%) are produced by molecular distillation and are widely used as emulsifiers.

5.9.3 Crystallisation, fractionation, and hydrophilisation

Crystallisation from an appropriate solvent is a classical method for the isolation and purification of solids, but since most of the interesting fatty acids are liquid this method has to be modified. With only a slight change in procedure and equipment, liquid acids can be crystallised conveniently at temperatures down to –78ºC using solid carbon dioxide as refrigerant. Alternatively, the conventional procedure can be applied to the higher melting salts. Lead salts crystallised from acetic acid permit the separation of saturated from unsaturated acids and lithium salts crystallised from acetone provide a separation of saturated and monoene acids from polyunsaturated fatty acids. More details of these classical procedures are given in the second edition of *The Lipid Handbook* (Gunstone et al., 1994).

The separation of natural fats by crystallisation of the complex mixtures of triacylglycerols present is generally referred to as fractionation. This is now an industrial process of considerable importance applied particularly to palm oil, but also to palm kernel oil, milk fat, and to animal body fats. It has been discussed in detail in Section 4.4.

Crystallisation is frequently a step in the production of pure synthetic acids and triacylglycerols discussed in the earlier sections of this chapter.

Hydrophilisation is a process for obtaining concentrates of oleic acid from tallow or palm fatty acids. After

crystallisation of the mixed acids at about 20°C, the crystals are treated with a wetting agent and form an aqueous suspension that can be separated from the liquid fraction by centrifugation. The latter is mainly oleic acid (70 to 75%) along with palmitoleic, linoleic, and 10% or less of saturated acids. Hydrophilisation is sometimes described as the Henkel or Lipofrac process (Fritz 1988).

5.9.4 Enzymatic enhancement

Under appropriate conditions lipases promote not only the hydrolysis of esters but also their formation from acids and alcohols or from other esters serving as a source of acids and alcohols. Because of specificities shown by certain lipases they can distinguish between acids according to their chain length or, more important in the present discussion, according to the position of the double bonds with respect to the acyl function. Reactions are slower with acids or esters that have a double bond close to the carboxyl or ester function. This has been exploited in preparing concentrates of γ-linolenic acid (6,9,12-18:3), EPA (5,8,11,14,17-20:3), and DHA (4,7,10,13,16,19-22:6) all of which have a double bond closer to the carboxyl group than the common Δ-9 acids, such as oleic and linoleic. Of particular value in this context are the lipases from *Candida cylindricea, C. rugosa, C. antarctica, Rhizopus miehei, R. delemar, Pseudomonas fluorescens,* and *Geotrichum candidum,* which distinguish unsaturated acids according to double bond position.

These possibilities are illustrated in Figure 5.15 showing how it is possible to prepare EPA and DHA each of at least 95% purity.

CLA has been identified at low levels in milk fat (3 to 6 mg/g of total fat), butter fat (12 to 14), cheeses (2 to 20), and in lamb and beef meat (4 to 5). Several isomers may be present and the major component (the 9*c*11*t* isomer) is designated rumenic acid (linked to its formation in the rumen of the cow). This acid is believed to be a metabolic product resulting from linoleic acid by two linked pathways: isomerisation of linoleic acid (9*c*12*c*-18:2) and Δ9-desaturation of vaccenic acid (11*t*-18:1). The 7*t*9*c* and 10*t*12*c* dienes are also present at lower levels along with many other isomers. CLA preparations can be made in larger volumes and higher concentrations by alkali isomerisation of linoleic-rich vegetable oils, such as safflower. Early preparations were mixtures of the 9*c*11*t* and 10*t*12*c* dienes and other isomers formed by further isomerisation. Now that the reaction is better understood and better controlled in terms of alkali selected, choice of solvent, and restriction of reaction temperature, the product contains these two isomers in similar proportions as virtually the only CLA present along with unreacted palmitic, stearic, and oleic acids from the starting material. These two CLA isomers show different physiological properties and procedures have been devised to concentrate them individually in

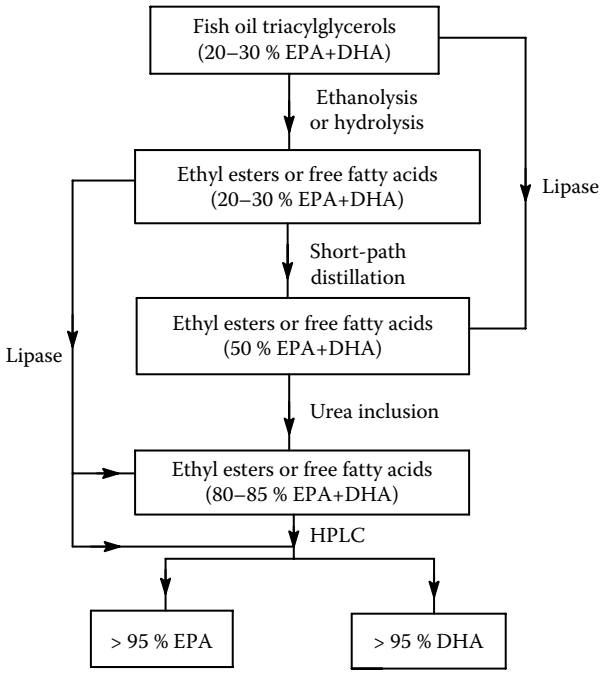


FIGURE 5.15 Possible routes to EPA and DHA from fish oil triacylglycerols by enzymatic enhancement and traditional separation techniques. (Adapted from Haraldsson, G.G., Hjaltason, B., in Modifying Lipids for Use in Food, Gunstone, F.D. (Ed.), Woodhead Publishing, Cambridge, England, 2006, pp. 347–352.)

separate fractions. This is best achieved using enzymes (such as that from the fungus *Geotrichum candidum*), which distinguish between these two isomers with the 9c11t isomer being the more reactive. The products of the isomerisation process are free acids that are generally converted to triacylglycerols before being used in human or animal diets. This can be done enzymatically with lipases, such as those from *Mucor miehei* or *Candida antarctica,*, since esterification then proceeds under mild conditions without modification of the double bond systems in the CLA. Further examples and references are cited in Section 5.4.4.

5.9.5 Chemical methods

Chemical methods of isolating fatty acids according to their level of unsaturation depends on reactions that distinguish one acid or one group of acids from others present in a mixture and which, after appropriate separation, can be reversed to give the isolated acid(s).

The reaction of olefinic acids with silver ions might be considered to fall into this category though the complex is never isolated. Olefinic compounds also react with mercuric acetate to give sadducts that are easily converted to methoxy bromomercuric compounds – CH(HgBr)CH(OMe) — by reaction with NaBr-MeOH. It is possible by chromatographic procedures to separate saturated, from monoene (mono adduct) from polyene acids (di-adduct, tri-adduct, etc). The olefinic compounds can be regenerated by reaction with methanolic HCl (Section 8.5).

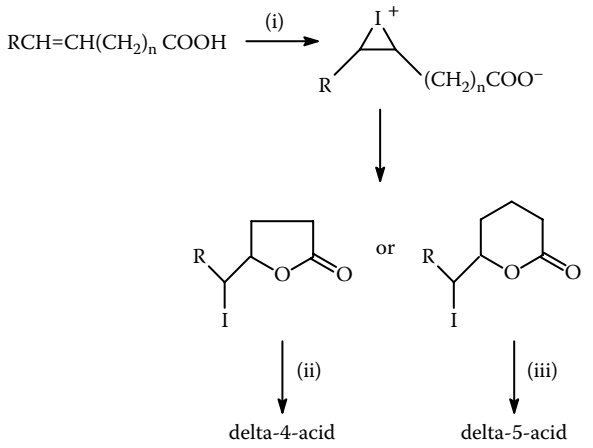


FIGURE 5.16 Iodolactonisation of acids with unsaturation at Δ4 (as in DHA) or Δ5 (as in EPA or AA). (i) I_2, KI, $KHCO_3$; EtOH at 25°C or tetrahydrofuran at –2 to +6°C; (ii) and (iii) TMSCl and NaI in CH_3CN.

Appropriate olefinic acids react with $KHCO_3$, I_2, and KI to give iodolactones (Gaidey et al., 1991). Acids with a Δ4 double bond (such as DHA) form an iodo γ-lactone and acids with a Δ5 double bond (such as AA or EPA) form an iodo δ-lactone. This scheme is formulated in Figure 5.16. There are several interesting features about these reactions:

- The iodolactones are neutral molecules and are easily separated from unreacted acids. This provides the basis of a method for separating and isolating Δ4 and Δ5 acids from acids that do not have unsaturation at these positions.
- The original unsaturated acids can be recovered from the iodolactones by reaction with trimethylsilyl iodide (or Me_3SiCl and NaI).
- The iodolactonisation reaction is dependent on solvent, reaction temperature, and the ratio of iodine to iodide. For example, with ethanol as solvent and a reaction temperature of 25°, DHA gives a maximum yield of iodo-γ-lactone in 10 min, but EPA requires 90 min to produce the iodo-δ-lactone.

References

Anon., (2004) Squalene, ethers and n-3 fatty acids from fish oil, *Lipid Technol.,* **16**, 26.

Breivik, H., et al., (1997) Preparation of highly purified concentrates of eicosapentaenoic acid and docosahexaenoic acid, *J. Am. Oil Chem. Soc.,* **74**, 1425–1429.

Breivik, H. and Thorstad, O. (2005) Removal of organic environmental pollutants from fish oil by short-path distillation, *Lipid Technology*, **17**, 55–58.

Fritz, E. (1988) Separation of fatty acids, in *Fatty Acids in Industry,* R.W. Johnson and E. Fritz, Eds., Marcel Dekker, New York, pp. 73–84.

Gaiday, N.V., et al., Separation of natural polyunsaturated fatty acids by means of iodolactonization, *J. Am. Oil Chem. Soc.,* **68**, 230–233, 1991.

Gunstone, F.D. (1994) Crystallisation, in *The Lipid Handbook*, Gunstone, F.D., Harwood, J.L., and Padley, F.B., Eds., 2nd ed., Chapman and Hall, London, pp. 225–226.

Haraldsson, G.G. and Hjaltason, B. (2006) PUFA Production from Marine Sources, in *Modifying Lipids for Use in Food*, Gunstone, F.D., Ed., Woodhead Publishing, Cambridge, England, 2006, 347–352.

Hayes, D.G. (2002a) Urea inclusion compound formation, *Inform*, **13**, 781–783.

Hayes, D.G. (2002b) Free fatty acid fractionation via urea inclusion compounds, *Inform*, **13**, 832–834.

Huibers, D.T.A. and Fritz, E. (1988) Distillation of fatty acids, in *Fatty Acids in Industry,* R.W. Johnson and E. Fritz, Eds., Marcel Dekker, New York, pp. 85–112.

Hwang, L-S. and Liang, J-H. (2001) Fractionation of urea-pretreated squid visceral oil ethyl esters, *J. Am. Oil Chem. Soc.,* **78**, 473–476.

Lee, J-W. et al., (2004) Selective increase in pinolenic acid (all-*cis*-5,9,12-18:3) in Korean pine nut oil by crystallisation and its effect on LDL-receptor activity, *Lipids*, **39**, 383–387.

Liang, J-H. and Hwang, L.S. (2000) Fractionation of squid visceral oil ethyl esters by short-path distillation, *J. Am. Oil Chem. Soc.,* **77**, 773–777.

Ma, D.W.L. et al. (2002) Countercurrent approach to the enrichment of Δ9*c*11*t*- and Δ10*t*12*c*-18:2 isomers by urea complexation, *J. Am. Oil Chem. Soc*., **79**, 755–758.

Nagao, T. (2002) Fractionation and enrichment of CLA isomers by selective esterification with *Candida rugosa* lipase, *J. Am. Oil Chem. Soc.,* **79**, 303–308.

Shimada, Y. et al., (2000) Facile purification of tocopherols from soybean oil deodorizer distillate in high yield using lipase, *J. Am. Oil Chem. Soc.,* **77**, 1009–1013.

Watanabe, Y. (2004) Purification of tocopherols and phytosterols by a two-step *in situ* enzymatic reaction, *J. Am. Oil Chem. Soc.,* **81**, 339–345.

Wille, H-J. and Traitler, H. (1988) Continuous process for the concentration of polyunsaturated fatty acids, *Fat Sci. Technol.,* **90**, 476–481.

Wille, H-J. et al., (1991) Preparation of stearidonic acid concentrate, *Fat Sci. Technol.,* **93**, 363–368.

Xu, X. et al., Purification of specific structured lipids by distillation: effects on acyl migration, *J. Am. Oil Chem. Soc.,* **78**, 715–718, 2001.

Xu, X. et al., (2002) Purification and deodorisation of structured lipids by short path distillation, *Eur. J. Lipid Sci. Technol.,* **104**, 745–755.

Yang, T. et al., (2004) Diacylglycerols from butterfat: production by glycerolysis and short-path distillation and analysis of physical properties, *J. Am. Oil Chem. Soc.,* **81**, 979–987.

Zuta, C.P. et al. (2003) Concentrating PUFA from mackerel processing waste, *J. Am. Oil Chem. Soc.,* **80**, 933–936.

6

ANALYSIS

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Lipid analysis comprises a vast range of methods, approaches and analyses that serve totally different purposes. Oils and fats are analysed on the shop floor by a plant operator titrating their free fatty acid content so that he/she can calculate how much lye to use in the chemical refining process. A homemaker may analyse the contents of a partially used bottle of walnut oil organoleptically for rancidity to decide whether or not to throw it away. An analyst in a clinical laboratory uses instrumental methods to determine fatty acid compositions and may even distinguish between various fatty acid isomers. A plant breeder uses an automated, high throughput system of analysis to screen individual seeds in a nondestructive manner to decide which seeds to plant next season.

Several of these analytical methods have been developed and validated by a close cooperation between the parties that are directly involved. These methods have been published by international or national bodies that issue standards or by national learned societies. The reader is referred to these publications for details about their execution, but in being referred, he/she faces an embarrassment of choice.

Rather than repeat details of selected methods, we have considered it more useful to the reader to list official methods and then to provide a commentary on some aspects relating to lipid analysis (Sections 6.1 to 6.3).

Accordingly, the first of these sections (Section 6.1) lists a selection of available, official methods and aims at putting them somewhat in perspective. The second section discusses the various possibilities facing an industrial operator so that hopefully, he/she can make a sensible choice from what is offered. The third section (Section 6.3) discusses several methods in some detail to highlight their relevance and facilitate the understanding of old, but often surprisingly relevant literature; it also describes a simple method to determine the oil loss during bleaching that, for some unknown reason, was never developed into an official method.

6.1 Introduction

The importance of the analysis of oils, fats, and related products is well illustrated by the fact that the American Oil Chemists' Society (AOCS) originates from the Society of Cotton Products Analysts (Blank, 1942). The reason why analysis is so important is that these products are traded and that trading contracts may contain penalties if a certain property does not meet a certain, specified limit. Then buyer and seller must apply the same standards and use the same analytical methods.

Accordingly, in several countries, such as for instance France and The Netherlands, Official Methods are issued by the respective national Bureaus of Standards. In this respect there is extensive international collaboration, as illustrated, for example, by the fact that some French norms (AFNOR) and Dutch norms (NEN) are identical to ISO (International Standards Organisation) norms and/or have been published in English as well. AOCS Official Methods may also mention that they are identical to ISO norms. In fact, organisations like FOSFA (Federation of Oils, Seeds and Fats Associations) rely more and more on ISO methods, now that the International Union of Pure and Applied Chemistry (IUPAC) has stopped issuing agreed methods. Accordingly, the first column in Table 6.1 provides the ISO method numbers even when the method is still under development. In other countries like Germany and the U.S., the learned societies in the

field of edible oils and fats have taken the initiative to develop, validate, and distribute such Official Methods; they also organise proficiency contests, etc., not just for their members but as a service to society.

Not surprisingly, there isn't a lot of bureaucracy involved before an analytical method becomes an Official Method. Moreover, chemists concerned in this "beatification" process may be more worried about the reproducibility and the repeatability of the method than about its pertinence (for definitions, see Section 6.2). This has led, for instance, to the situation whereby a method to determine the Oil Stability Index (OSI) became official in 1997 (AOCS Official Method Cd 12b), although it was already known (Dijkstra et al., 1995; Dijkstra et al., 1996) that, although it is quite reproducible, the method does not predict shelf life (Lacoste et al., 1999). On the other hand, subsequent authors (Broadbent and Pike, 2003) claim that the OSI correlates so well with a sensory induction period to warrant the use of the OSI in industrial applications. However, they stored their samples at 60°C and what happens at that temperature may not be the same as what happens during storage at ambient temperatures.

Methods may also seem to promise more than they actually deliver:

- The oil content of oil seeds, for instance, is a parameter that is of vital interest to the crusher. Oil content is determined by grinding the seeds and extracting the ground seeds in a prescribed manner. However, the method does not say anything about the extent to which seed cells are opened up in the industrial crushing plant and, therefore, does not predict potential oil yield. The amount of oil extracted and especially its phosphatide content also vary widely according to the solvent used (Desnuelle et al., 1951).
- Quite correctly, the French method refers to the extract obtained with hexane, as the "so-called oil content"[1] and this is only one example of the fact that the analytical chemists who write specifications are fully aware of their limitations. Laboratory technicians carrying out the analyses will also be aware of this, but higher up in the hierarchy, problems may arise if people interpret "oil content" at face value.
- Similarly, weight loss on heating a sample at atmospheric pressure or *in vacuo* is not the same as its water content. Calling this weight loss "moisture and volatiles" may be nearer the truth, but even so, heating the sample may lead to chemical dehydration and/or thermal degradation reactions and there is no guarantee that all free water will leave the sample under test conditions.
- Acidity is not the same as free fatty acid content; phosphatides present in crude oil are also titrated with alkali so that water degumming an oil apparently causes its FFA to decrease.
- Alkalinity is not the same as soap content because there may be additional alkaline compounds present.
- Besides, calcium and magnesium soaps are not determined by the normal titration method (Sen Gupta, 1988).
- Phosphorus may originate from phosphatides, but also from residual phosphoric acid used as a degumming acid.
- A palmitic acid content as determined by GLC analysis of FAME is not the same as the palmitic acid content of the sample, since the GLC analysis does not take dimeric, polymeric, and oxidised compounds into account. For crude oils, the difference will be only marginal, but for oils that have been used in deep fat frying the "increase in palmitic acid content" can be quite considerable. On paper it is; in reality it does not exist.
- The iodine value is a very useful parameter to follow the progress of a hydrogenation reaction, but what about conjugated double bonds?

The above list is far from exhaustive and the instances given are only examples of the need to be aware of the limitations of analytical methods. However, the limitations of a method are always clearly indicated and within its limitations each method can still be quite useful. Far more serious situations arise when there is no Official Method as is, for example, the case for the determination of oil retention by bleaching earth. Again, knowing how much oil he/she stands to lose when bleaching oil constitutes vital information for the refiner.

However, extracting spent earth with a solvent in a Soxhlet — and this is what most people do in the absence of an Official Method — underestimates the oil loss since this extraction doesn't extract oil that has been polymerised (Morton, 1995). Treating the spent earth in a muffle furnace (see Section 6.3) is a far more accurate way of providing the data required to calculate the yield loss on bleaching (Dijkstra, 1993), but this is not an Official Method.

In fact, there are quite a few parameters in lipid technology for which there is no Official Method to quantify them; neutral, triglyceride oil in soapstock is one of them, but this is not the time or place to examine this in detail. Readers can do so for themselves and then, hopefully, do something about it.

The standards and methods listed in Table 6.1 are a selection of the methods published by the issuing bodies concerned. For source materials, meal and oils and fats alone, the AOCS has published almost 250 different Official Methods and this figure excludes so-called "surplus methods," methods that often have become obsolete

[1] Extrait à hexane, dit "teneur en huile."

TABLE 6.1 Numbers (and latest year of issue) of Official Analytical Methods or norms issued in various countries

Subject matter	ISO[a]	AFNOR[b]	NEN[c]	AOCS[d]	DGF[e]
General					
Nomenclature, oilseeds, oils, fats	5507 (02)	V00-300			C-I 1 (99)
Execution and evaluation of ring test					A-II 1 (03)E
Application of repeatability limit and reproducibility limit			6303 (88)		A-II 2a (97)E
Detection limits, significance tests					A-II 2b (97)
Validation of analytical methods					A-II 3
Hazardous materials					A-III 1 (97)
Vegetable oil source materials					
Sampling	542 (95)	V03-900		Aa 1 etc.[f]	B-I 1 (87)
Sample preparation	664 (90)	V03-902			B-I 2 (87)
Impurities	658 (02)	V03-904		Am 4 (02)	B-I 4 (87)
Moisture and volatile matter	665 (00)	V03-909		Aa 3 etc.[f]	B-I 5a (87)
Oil and water content (pulse NMR)	10565 (98)	V03-916		Ak 4 (99)	B-I 5a (89)
Oil and water content (NIR)				Am 1 (01)	B-I 5b
Oil content (reference method)	659 (98)	V03-905		Am 2 (93)	
Oil content (continuous wave NMR)	5511 (92)	V03-907		Ak 3 (00)	
Acidity of oil in oilseeds	729 (88)	V03-906		Aa 6 etc. [f]	
Glucosinolate in rapeseed (HPLC)	9167-1 (92)	V03-918-1		Ak 1 (97)	B-I 7a (97)
Glucosinolate (x-ray fluorescence)	9167-2 (94)	V03-918-2			B-1 7 (97)
Glucosinolate HPLC rapid method		V03-918-3			
Chlorophyll (spectrophotometrically)	10519	V03-911		Ak 2 (92)	
Bulk density					B-I 8 (89)
Pressure loss on air flow through grain	4174	V03-743			
Oilseed residues					
Sampling	5500 (86)	V03-920		Ba 1 (97)	B-II 1 (87)
Preparation meal test sample	5502 (92)	V03-926			B-II 2 (87)
Moisture and volatile matter	771 (77)	V03-921		Ba 2	B-II 3 (87)
Residual oil content (petroleum ether)				Ba 3 (97)	B-II 4a (03)
Residual oil (petroleum ether/hexane)	734-1 (98)	V03-924-1			B-II 4b (87)
Residual oil content (rapid extraction)	734-2 (98)	V03-924-2			
Oil and water content (pulse NMR)	10632 (00)			Ak 5 (01)	B-II 4c
Total ash content	749 (77)	V03-922		Ba 5a (97)	B-II 5 (89)
Hydrochloric acid insoluble ash	735 (77)	V03-923		Ba 5b (97)	
Protein content (ammonia titration)		V18-100		Ba 4d (03)	B-II 6 (89)
Crude fibre in meal		V03-040		Ba 6 (97)[f]	B-II 7 (87)
Urease activity in soya products	5506 (88)	V03-942		Ba 9 (97)	
Free residual hexane	9289 (95)	V03-930		Ba 13 (97)	B-II 8 (89)
Total residual hexane (GLC)	8892 (87)	V03-927		Ba 14 (97)	B-II 8a (87)
Screen test				Bc 7 (97)	B-II 9 (89)
Oils and fats					
Sampling	5555 (02)	T60-280	6300 (98)	C 1 (00)	
Insoluble impurities	663 (00)	T60-202		Ca 3a (97)	C-III 11a (84)
Sediment	15301 (01)			Ca 3d (02)	
Preparation of test sample	661 (05)	T60-200			
Unsaponifiable (diethyl ether)	3596 (00)	T60-205-1	6328 (84)	Ca 6b (01)	C-III 1a (77)
Unsaponifiable (pet ether/hexane)	18609 (01)	T60-205-2		Ca 6a (97)	C-III 1b (77)
Moisture and volatiles	662 (98)	T60-201		Ca 2f (97)	C-III 12 ((97)
Moisture (distillation)	934 (80)	T60-218	6323 (87)	Ca 2a (87)	C-III 13 (97)
Moisture (Karl Fischer)	8534 (96)	T60-225		Ca 2e (97)	C-III 13a (97)
Free fatty acids/acid value	660 (96)	T60-204		Ca 5d (03) Cd 3d (03)	C-III 4 (97)
Polar compounds in deep frying fats	8420 (02)	T60-248		Cd 20 (01)	C-III 3b (84)
Polymerised triglycerides (HPSEC)	16931 (02)	T60-247		Cd 22 (00)	C-III 3c (02)
Butyric acid (GLC)				Ca 5c (97)	C-III 8 (97)
Residual hexane	9832 (02)	T60-253	6354 (91)	Ca 3b (02)	
Residual hexane (low values)		T60-257			
Volatile org. contaminants by GC-MS	15303 (01)			Ca 3c (01)	

(Continued)

TABLE 6.1 Continued

Subject matter	ISO[a]	AFNOR[b]	NEN[c]	AOCS[d]	DGF[e]
Low-boiling halogenated hydrocarbons	16035 (05)				
Polycyclic aromatic hydrocarbons (GC-MS)	15753 (05)				C-III 17b
Benzo(a)pyrene (RP HPLC)	15302 (98)	T60-255		Cd 21 (97)	C-III 17c
Ash content	6884 (85)	T60-290	6327 (77)	Ca 11 (03)	C-III 10 (97)
Polythene	6656 (02)	T60-240	6355 (84)	Ca 16 (02)	
Soaps/alkalinity	10539 (02)	T60-217	6330 (77)	Cc17 (97)	C-III 15 (97)
Phosphorus (colorimetric)	10540-1 (03)	T60-228	6349 (91)	Ca 12a (9)	C-III 16a (03)
Phosphorus (graphite furnace AAS)	10540-2 (03)			Ca 12b (02)	
Phosphorus (ICP)	10540-3 (03)			Ca 20 (01)	
Trace metals (ICP)	21033			Ca 17 (01)	C-III 18
Trace metals (graphite furnace AAS)	8294 (99)	T60-251		Ca 18 (03)	
Lead (graphite furnace AAS)	12193 (04)	T60-252		Ca 18c (03)	
Cadmium (graphite furnace AAS)	15774 (00)			Ca 18d (01)	
Slip melting point	6321 (02)	T60-226	6313 (77)	Cc 3b (02)	C-IV 3a (03)
(Amendment for palm oil)	6321 A1				
Titre	935 (88)	T60-208	6316 (77)	Cc 12 (97)	C-IV 3 (81)
Cloud point			6315 (76)	Cc 6 (97)	
Mass per volume (pycnometer)	6883 (00)	T60-214	6311 (93)	Cc 10c (02)	C-IV 2c (02)
Cold test				Cc 11 (03)	C-IV 3d (02)E
Dropping point				Cc 18 (01)	
Solid fat content (pulse NMR)	8292 (95)	T60-250		Cd 16 (99)	C-IV 3g (03)E
Refractive index	6320 (00)	T60-212		Cc 7 (02)	C-IV 5 (02)
Flash point (Pensky–Martens)	15267 (98)	T60-256		Cc 9c (97)	C-IV 8 (02)E
Smoke point (open cup)				Cc 9a (97)	C-IV 9 (02)E
Colour (Lovibond)	15305 (98)	T60-259	6308 (76)	Cc 13e (02)	C-IV 4b (98)
Colour (Iodine scale)			6309 (76)		C-IV 4a (98)
Colour (Gardner)			6310 (76)		C-IV 4c (02)
Colour (spectrophotometric)		T60-224		Cc 13c (97)	
PUFA with *cis,cis*-1,4-diene	7847 (87)			Cd 15 (97)	
Iodine value (Wijs)	3961 (99)	T60-203		Cd 1 (93)	C-V 11d (02)E
Saponification value	3657 (03)	T60-206	6337 (82)	Cd 3 (02)	C-V 3 (02)E
Ester value			6339 (76)		C-V 4 (53)
Hydroxyl value		T60-213	6342 (80)	Cd 13 (97)	C-V 17a (98)E
Polybromide reaction for trienes			6369 (76)		C-V 16 (57)
1-Monoglycerides and free glycerol	7366 (87)	T60-245	6338 (91)	Cd 11 (03)	C-VI 5 (02)
			6340 (91)		
Monoglycerides (GLC)				Cd 11b (03)	C-VI 5a (02)
Antioxidants (TLC)	5558 (82)	T60-235			C-VI 9 (00)E
Antioxidants (HPLC)				Ce 6 (97)	
BHA and BHT (GLC)	6463 (82)	T60-237			
Gallates (spectrophotometric)	6464 (83)				
Individual and total sterols (GLC)	12228 (99)	T60-258			
Total sterols (enzymatic)		T60-243	6350 (77)		
Sterol composition (GLC)	6799	T60-232			
Sterol composition (HPLC plus GLC)		T60-254			
Tocopherols and tocotrienols (HPLC)	9936 (04)			Ce 8 (97)	
Stigmadienes (HPLC)	15788 (019)			Cd 26 (03)	C-VI 8b (99)E
Total *trans* isomers (IR)			6336 (81)		C-IV 11 (98)
Isolated *trans* content (FTIR)	13884 (03)			Cd 14d (99)	C-IV 11a
Trans isomers (capillary GLC)	15304 (02)			Ce 1f (02)	
For olive oil				Ch 2a (02)	
Preparation of FAME (BF_3)	5509 (00)	T60-233		Ce 2 (97)	C-VI 11a (98)
FAME (alkaline ester interchange)					C-VI 11d (98)
Fatty acid composition (GLC)	5508 (90)	T60-234		Ce1 (97)	C-VI 10a (00)
(capillary GLC)				Ce 1e (01)	
Fatty acid composition 2-position (GLC)	6800 (98)	T60-241			
Peroxide value (chloroform)		T60-220		Cd 8 (03)	C-VI 6a (02)E
Peroxide value (isooctane)	3960 (01)			Cd 8b (03)	
p-Anisidine value	6885 (98)	T60-246		Cd 18 (97)	C-VI 6e (84)

(Continued)

TABLE 6.1 Continued

Subject matter	ISO[a]	AFNOR[b]	NEN[c]	AOCS[d]	DGF[e]
Oxidative stability (Rancimat®)	6886 (96)	T60-219		Cd 12b (97)	C-VI 6f
CBE in chocolate (qualitative)	23275-1				
CBE in chocolate (quantitative)	23275-2				

[a] http://www/iso.org
[b] http://www.afnor.fr
[c] http://normen.nen.nl
[d] http://onlinemethods.aocs.org
[e] http://www.dgfett.de
[f] The Official Methods and Recommended Practices of the AOCS provide these methods per vegetable source material.
Abbreviations: CBE, cocoa butter equivalents; FTIR, Fourier transform infrared; HPSEC, high-performance size exclusion chromatography; RP HPLC, reversed phase high performance liquid chromatography.

because a more recent analytical method was found to be more accurate, cheaper, or faster.[2] The selection of these methods is somewhat arbitrary, but intends to provide an overview of the most commonly used methods. Methods that reflect national idiosyncrasies, such as the "Break Test (crude soya bean oil)" and the test to prove the presence of sesame seed oil have also been omitted, now that margarine in various European countries no longer has to distinguish itself from butter by the incorporation of 2% sesame seed oil that could then be detected by the Baudouin test involving shaking an oil sample that had been washed with strong aqueous hydrochloric acid with an alcoholic solution of furfural (Boekenoogen, 1948). Italy was the last country to abolish this regulation. Subjective methods involving taste panels have also been omitted. In general, the list comprises methods that an industrial laboratory should know about and might want to use.

If the various issuing bodies each provide their own method for a certain property, these methods, as listed in Table 6.1, are similar; some may be identical, but there may also be slight variations. These variations may refer to sample weight, the composition of solvent mixtures, duration of treatment, temperature, etc. For practical purposes, they should be regarded as equivalent, but when a contract prescribes a specific method, that is the method that should be used.

A common feature of the tables of contents provided by all the various issuing bodies for their methods is that the order in which the various analytical methods have been listed is to some extent historically determined, but otherwise arbitrary. Each issuing body has its own order. Consequently, Table 6.1 is also organised in a somewhat arbitrary manner, except that different methods for the determination of the same property have been grouped together, e.g., phosphorus (colorimetric), phosphorus (ICP: inductively coupled plasma). To indicate to what extent a method will be applicable in a given laboratory, instruments to be used such as GLC, HPLC, ICP, etc. have been listed between brackets behind the subject matter. This does not mean that no dedicated equipment is required if nothing has been listed, but that, in general, these requirements will form less of a financial hurdle.

[2] This also holds for the "Detection of sulfur by the coin test" (AOCS Official Method Ca 8a). The author was taught to prove the presence of sulfur by his late father, also a chemist, who referred to the method as the "hepar-method" (hepar = liver) because the silver sulfide on the silver coin has a colour reminiscent of boiled liver.

References

AOCS (2003a) Official Methods and Recommended Practices of the AOCS, 5th ed., 2nd printing, AOCS Press, Champaign, IL.

Blank, E.W. (1942) Chronological list of important dates in the history of the fats and waxes, *Oil & Soap*, **19**, 110–113.

Boekenoogen, H.A. (1948*),* De Scheikunde der Oliën en Vetten, A. Oosthoek's Uitgevers Mij., Utrecht.

Broadbent, C.J. and Pike, O.A. (2003) Oil Stability Index correlated with sensory determination of oxidative stability in canola oil, *J. Am. Oil Chem. Soc*., **80**, 59–63.

Desnuelle, P. et al. (1951) Traitement thermique des graines oléagineuses et teneur en lécithine des huiles, *Oléagineux*, **6**, 264–267.

Deutsche Gesellschaft für Fettwissenschaft (2004) DGF-Einheitsmethoden.

Dijkstra, A.J. (1993) Degumming, refining, washing and drying fats and oils, in *Proceedings of the World Conference on Oilseed Technology and Utilization*, Applewhite, T.H., Ed., American Oil Chemists' Society, Champaign, IL, 138–151.

Dijkstra, A.J. et al. (1995) Interpreting oil stability data, in *Oils-Fats-Lipids 1995, Proceedings of the 21st ISF Congress*, Castenmiller, W.A.M., Ed., P.J. Barnes & Associates, Bridgwater, U.K., 629–637.

Dijkstra, A.J. et al. (1996) Interpreting the oil stability index, *Oléagineux Corps Gras Lipides/OCL*, **3**, 378–386.

Lacoste, F. et al. (1999) Comparison of Rancimat stability test and ambient storage of edible oil, *Paper presented at the 23rd ISF World Congress*, Brighton, U.K.

Morton, R.B. (1995) Oil content of filter cakes, *Paper presented at the 86th AOCS Annual Meeting and Expo, San Antonio, TX*.

Sen Gupta, A.K. (1988) Micellar structures and their implication in the chemistry and technology of fats and other lipids, *Fat Sci. Technol*., **90**, 251–256.

6.2 Requirements stemming from quality control and process investigation

Before discussing ways and means to meet regular analytical requirements, a reminder of the definition of a few analytical concepts may be opportune:

- *Accuracy:* the accuracy of a measurement is given by its bias, which is the systematic difference between the true value and the measured value. Such a bias may, for instance, originate from an inappropriate choice of indicator during a titration that visualises the endpoint too early (or too late). Incomplete cell opening when determining the oil content of a seed by solvent extraction will also lead to a bias.
- *Reproducibility:* the degree to which a measurement can be repeated within the same analytical laboratory. The reproducibility of an analytical determination is commonly quantified by its standard deviation, a measure of the spread of the individual data points around their average.
- *Repeatability:* the extent to which the same analytical method yields the same results when applied by different analytical laboratories. This means that the reproducibility of a measurement is always better (smaller standard deviation) than its repeatability. The latter can also be quantified by a standard deviation, although mathematically, this is not correct since the individual data points are distributed around individual averages that have a different bias with respect to the true value and, thus, do not constitute a normal distribution themselves.

6.2.1 In-house vs. external analyses

Table 6.1 in Section 6.1 lists a large number of analytical methods, but this list is only a selection of the large number of methods available. Many of them are rarely used and most of them are hardly ever needed by a plant manager operating a plant and selling its products. He can limit the methods to be used to those that permit him to make sure that his products are within specification and those that enable him to quantify and, subsequently, optimise the performance of his plant or plants. However, he must decide first of all whether he should have the analyses carried out in-house, ask an external laboratory to carry them out, or opt for a combination of both approaches.

There may be occasions when analyses are called for that require sophisticated instrumentation. If these occasions are incidental, it is most convenient to subcontract these analyses to a specialised outside laboratory. Phosphatide composition by ^{31}P NMR can be an example of such an analysis. On the other hand, the determination of residual phosphorus by inductively coupled plasma (ICP) spectroscopy (Dijkstra and Meert, 1982) or even IPC-MS (Wiedemann et al., 2004), which also requires a sophisticated instrument, is preferably carried out in-house for the simple reason that the analytical data are required rapidly since they form the basis of urgent decisions and subcontracting may take too long.

On the other hand, if the property concerned can be determined by NIR (near infrared spectroscopy), a service called "Quality Trait Analysis" (QTA) has recently been established (Anon., 2005), which allows a crusher to relay the NIR-probe signal via the Internet to a central QTA® processor that sends the estimated values back within minutes. This service obviates the need for analytically skilled personnel and the development and maintenance of the database used to arrive at the NIR-based estimates. It only requires investment in NIR equipment and training of plant personnel in how to use it and relay the data.

The system has the potential disadvantage that NIR-based estimates cannot be used as evidence during disputes; these stipulate classical, often wet methods. Of course, the QTA® service uses these methods to arrive at its database, but it may not be in a position to act as an expert witness in such disputes because of conflict of interests. Moreover, although the use of the database by several different crushers saves money, the QTA® service has to make a profit to survive. Accordingly, a situation may well arise whereby relatively small, single site crushers make use of an external QTA® service, whereas large, multisite operators prefer an in-house service that may operate along similar lines. They may also operate a central processor and a single database serving several users throughout the company.

In-house services have the advantage that an additional analysis hardly causes additional costs, whereas the operator relying on an external QTA® service probably has to pay a standard amount for each additional analysis. Accordingly, a crusher (especially if MBA controlled) may want to save real money by decreasing the number of samples being analysed and, in doing so, lose control over his process and product; this may well be more costly in the long run.

6.2.2 Labour intensive vs. automated analyses

The analytical methods required for product quality control follow from the sales specifications of the various products. Thus, if urease activity is specified, the crusher has to make sure that he can measure it. Occasionally, the analytical method is also specified. This was, for example, the case when an external company recuperating oil from spent bleaching earth by extraction with hexane, specified that the oil content of the earth had to be determined by Soxhlet extraction with hexane.

If an oil colour is specified, the refiner must provide the data required. If the colour is specified in Lovibond

units — and this is invariably the case — he has a number of options:

- He can purchase a cheap, manual instrument that was developed for the brewing industry (Stillman, 1955) and rely upon the skill of his laboratory assistants in comparing the colour of a set of built-in glasses with the oil colour to come up with proper readings.
- He can also purchase a more expensive automated instrument that immediately provides readings in these units and readings that are more reproducible because they eliminate operator influence.
- He can stay away from Lovibond and purchase a spectrometer (UV-visible) and calculate on the basis of the visible light transmission spectra to which Lovibond-values the oil colour would correspond (Maes et al., 1997). Since a spectrometer can serve several other purposes, such as the determination of chlorophyll content, which equipment to purchase should be an obvious choice.

Investment in the simplest analytical equipment that does the specified job is, of course, easy to justify by being unavoidable. However, it can be difficult to justify less simple and, thus, more expensive equipment. Labour saving in comparison with the simple equipment is becoming a very sound reason as well as the actual analyses it is to perform. In practice however, once the equipment has been purchased and commissioned, it can often be used for other, unforeseen analyses and, thus, yield unexpected positive results that would in hindsight have easily justified its purchase.

Laboratory equipment should not be regarded like plant equipment as far as investment decisions are concerned. Plant equipment should be in almost constant use, but most laboratory equipment is only used sporadically. Nobody wants to peer down a microscope all day, but on the occasions that only a microscopic examination can provide the answer, its presence is essential.

Not all equipment in a plant laboratory needs to be highly sophisticated and require highly skilled staff to keep it running. Drying ovens, for instance, were never very sophisticated and that is still the case today; they just do what they have to do. Analytical balances, on the other hand, are regularly improved and instead of purchasing a rather elementary model, a plant laboratory might be better served by a model that can be linked to a laboratory information management system (LIMS). Perhaps it does not have to be linked up straightaway, but it would be a pity if this were impossible when such a system were to be installed at a later date.

The FFA (free (nonesterified) fatty acid) content of an oil is specified for both crude and refined oils. It is commonly determined by titration and this method of analysis can be carried out on the shop floor. Recently, an FTIR (Fourier transform infrared) method has been developed (Verleyen et al., 2001) that is accurate and reproducible, but has the disadvantage of requiring an initial calibration that is oil-type specific. Subsequently, another FTIR method was developed (Al-Alawi et al., 2004b) that has the advantage that it is specific for fatty acids by measuring them as soaps extracted from the oil by a basic methanolic solution. This specificity is also an advantage over the titration method that just measures acidity.

The FTIR method is fast and can be automated so that 60 samples can be measured per hour and, above all, it is far more reproducible, having a standard deviation that is about half the standard deviation of the titration method (Al-Alawi et al., 2004a). Using this method requires the purchase of an FTIR spectrophotometer, but then, this is a multipurpose instrument that can also be used for other determinations. Moreover, since the labour requirement of this FTIR method is almost negligible, the availability of the instrument will encourage duplicate measurements to be made to verify possible rogue values and outliers. Consequently, its sheer availability may lead to more soundly based decisionmaking.

The FTIR instrument can also be used for the simultaneous determination of *trans*-content and the iodine value (Sedman et al., 1998; Sedman et al., 2000) or just the iodine value (Cox et al., 2000). Soap can also be determined by FTIR spectroscopy with an accuracy that is comparable to that of AOCS Method Cc17-95 and an improved repeatability. Because the reproducibility of instrumental methods is usually quite close to its repeatability, the reproducibility of the FTIR-based method will be an even greater improvement compared with the titration method. Finally, water can be determined with high accuracy (in the order of ±10 ppm) by extracting the water from the oil with acetonitrile and measuring the extract against dry acetonitrile (Al-Alawi et al., 2005). It can be concluded that even for relatively small laboratories the investment in an FTIR instrument looks likely to be fully justified.

Gas-liquid chromatography (GLC) is a similar, widely applicable analytical technique. Its main use is for the determination of fatty acid compositions, but it can also be used for triglyceride analysis according to carbon number, the determination of the sterol composition, which can be useful for the detection of adulterations and many more incidental applications. Moreover, determining fatty acid compositions makes it possible to calculate *trans* content, saponification value, and iodine value. An instrument that can operate with capillary columns should be the object of choice.

Pulse-NMR as an analytical technique, which is also referred to as "time-domain NMR" (Todt et al., 2001), has quite a number of applications, but some of them require dedicated equipment to ensure sufficient sample homogeneity. The technique was developed for the measurement of the solid fat content (SFC) of fat blends (Van den Enden et al., 1978, 1982; Duynhoven et al.,

1999). The ISO 8292 standard and the AOCS Official method Cd 16b-93 describe what thermal treatment to give to the sample before measuring its SFC (see also Section 7.2.5). When using the method for the endpoint control of a hydrogenation batch (Rutledge et al., 1988) a different thermal treatment is used to save time.

The equipment most frequently used for this application operates with a Larmor frequency of 20 MHz and a magnetic field strength of 0.47 T. It can accommodate sample tubes with a diameter of up to 25 mm, but also provides accurate data with sample tubes of 18 and even 10 mm diameter. Consequently, this piece of equipment can also be used in the confectionery industry to determine the oil/fat and moisture in chocolate, cocoa powder and milk powder. In addition, it can be used to determine the droplet size distribution in both water-in-oil emulsions like margarine and butter and oil-in-water emulsions like mayonnaise, dressings, and soft cheese. For routine SFC measurements that require the sample to follow a prescribed temperature profile, a laboratory robot that transfers the sample tubes from one thermostat to another and finally to the measuring cell, makes the thermal history more reproducible, saves labour, and avoids human errors. It can also be easily integrated into a LIMS (laboratory information management system).

The instrument used for this kind of determination has been programmed to average the results of three consecutive measurements and present this average as the reading. In principle, the repeatability of this reading could be improved by calculating the average of more than three consecutive measurements, but in practice, the magnet is kept thermostatically at a temperature (e.g*.* 40°C) that causes crystals present in the sample to melt. Accordingly, the sample gets less and less representative with time so that the time span available for representative measurements is limited. Thus, three consecutive measurements is a compromise between representability and repeatability.

For less homogenous products like oilseeds, nuts, and especially olives, a larger sample tube diameter is required to ensure sample homogeneity and a model, therefore, must be selected that can accommodate tube diameters up to 52 mm; it operates with a Larmor frequency of 7.5 MHz and a magnetic field strength of 0.17 T in accordance with ISO 10565, ISO 10632, and AOCS Ak 4-99. Ak 5-01 and permits simultaneous measurement of oil content and moisture content in oilseeds and their meal.

In comparison with NIR reflectance methods, the pulse-NMR method's advantage is that it analyses the entire sample instead of just investigating its surface and determines the property concerned in a direct manner leading to full proportionality between the NMR signal and the property. NIR reflectance methods are indirect and arrive at estimates based upon multivariant correlations.

Several Official Methods mention the use of HPLC, reversed phase HPLC or gel permeation HPLC. Like GLC, the equipment is indispensable for the property or parameter to be determined, so if a method prescribing it is to be used, the equipment has to be purchased. Again, a versatile and multipurpose instrument is recommended and if some measurements look like becoming routine, autoinjection and connection to a LIMS merit consideration.

Investment in equipment to measure the OSI cannot be avoided if this property has been incorporated in sales specifications. Since the OSI does not predict oil shelf life (Lacoste et al., 1999), its inclusion in a specification preferably should be resisted. This saves money, analysis time and interminable discussions.

References

Al-Alawi, A. et al. (2004a) A new FTIR method for the analysis of low levels of FFA in edible oils, *Paper presented at the 3rd EuroFedLipid Congress, Edinburgh*, p. 40 of the Abstracts.

Al-Alawi, A. et al. (2004b) New FTIR method for the determination of FFA in oils, *J. Am. Oil Chem. Soc.*, **81**, 441–446.

Al-Alawi, A. et al. (2005) A new Fourier transform infrared method for the determination of moisture in edible oils, *Appl. Spectrosc.*, **59**, 1295–1299.

Anon. (2005) A user-friendly process monitoring system, *INFORM*, **16**, 654; see also www.qta.com.

Cox, R. et al. (2000) Determination of iodine value with a Fourier transform-near-infrared based global calibration using disposable vials: an international collaborative study, *J. Am. Oil Chem. Soc.*, **77**, 1229–1234.

Dijkstra, A.J. and Meert, D. (1982) Determination of trace elements in oils by plasma emission spectroscopy, *J. Am. Oil Chem. Soc.*, **59**, 199–204.

Duynhoven, J. van et al. (1999) Solid fat determination by NMR, *INFORM*, **10**, 479–484.

Enden, J.C. van den et al. (1978) A method for the determination of the solid phase content of fats using pulse Nuclear Magnetic Resonance, *Fette Seifen Anstrichm.*, **80**, 180–186.

Enden, J.C. van den et al. (1982) Determination of the solid fat content of hard confectionary butters, *J. Am. Oil Chem. Soc.*, **59**, 433–439.

Lacoste, F. et al. (1999) Comparison of Rancimat stability test and ambient storage of edible oil, *Paper presented at the 23rd ISF World Congress,* Brighton, U.K.

Maes, P.J. et al. (1997) Converting spectra into color indices, *INFORM*, **8**, 1245–1252.

Rutledge, D.N. et al. (1988) Contribution à l'étude d'une méthode de contrôle rapide de la qualité des margarines par RMN-IBR, *Rev. Franç. Corps Gras*, **35**, 157–162.

Sedman, J. et al. (2000) Simultaneous determination of iodine value and *trans* content of fats and oils by single-bounce horizontal attenuated tot reflectance Fourier transform infrared spectroscopy, *J. Am. Oil Chem. Soc.*, **77**, 399–403.

Sedman, J. et al. (1998) Industrial validation of Fourier transform infrared *trans* and iodine value analyses of fats and oils, *J. Am. Oil Chem. Soc.*, **75**, 33–39.

Stillman, R.C. (1955) Bleach and color methods, *J. Am. Oil Chem. Soc.*, **32**, 587–593.

Todt, H. et al. (2001) Quality control with time-domain NMR, *Eur. J. Lipid Sci. Technol.*, **103**, 835–840.

Verleyen, T. et al. (2001) Influence of triacylglycerol characteristics on the determination of free fatty acids in vegetable oils by Fourier transform infrared spectroscopy, *J. Am. Oil Chem. Soc.*, **78**, 981–984.

Wiedemann, S.C.C. et al. (2004) Direct analysis of Ca, P, and Fe in oleochemicals by inductively coupled plasma MS, *J. Am. Oil Chem. Soc.*, **81**, 437–440.

6.3 Some selected analytical methods

Because the edible oils and fats industry has a long and respectable history, the analytical methods developed within the industry reflect this time span. Some methods date from when analytical chemistry was limited to gravimetric and volumetric measurements. Examples of purely gravimetric methods are the measurements of oil content in oilseeds, meal, etc., of their water content, the determination of the unsaponifiable content of oils and of the oil content of spent bleaching earth. A combination of gravimetric and volumetric methods is used to determine the FFA content of oils, their saponification value, hydroxyl value, peroxide value, iodine value, and the determination of the water content of substrates by azeotropic distillation or by Karl Fischer's method.

As will be shown below, several "values" that were formerly determined by these volumetric methods can now be determined more quickly and often more accurately by instrumental methods. That is to say, the instrumental method provides the data with which the "value", such as the iodine value, can be calculated. This calculation demands an understanding of the basic principles of the old method. Accordingly, some of these methods will be explained here; more detailed descriptions can be found in the respective Official Methods.

In general, the gravimetric and/or volumetric methods are well proven. The equipment used in these methods is straightforward and comprises an analytical balance and standard glassware. Accordingly, the initial outlay is quite low, but the methods tend to take a long time and to be labour intensive. Besides, the repeatability of these methods may be unacceptably poor.

Because of these disadvantages, several of these ancient methods have been replaced by modern, often instrumental methods, such as the FTIR-based method for the determination of FFA content (Al-Alawi et al., 2005b), soap and water (Al-Alawi et al., 2005a). In addition, novel methods are constantly being developed to measure properties that were rarely measured in the past. Take, for instance, the fatty acid composition. The average molecular weight of the fatty acids has been known for a long time, since it follows from the saponification value as obtained by titration, but this value does not tell us anything about the fatty acid composition.

The determination of this fatty acid composition comprises as the first step the determination of the saturated fatty acid content by oxidising the unsaturated fatty acids in a controlled manner, followed by a separation based on the insolubility of the magnesium soaps of saturated fatty acids. If no linolenic acid is present, using the iodine value (IV) and the saturated fatty acid content allows us to calculate the oleic acid and the linoleic acid contents in terms of weight percentage, because the two unknowns left (oleic acid content and linoleic acid content) can be calculated from the iodine value with the sum of the three fatty acids content being 100.

Instead of determining the saturated fatty acid content, the rhodanic acid (thiocyanic acid) value could have been determined. Whereas both double bonds in linoleic acid add an iodine molecule during the determination of the iodine value, only one of them adds a rhodanic acid molecule. Accordingly, the difference between the iodine value and the rhodanic acid value is indicative of the linoleic acid content and as shown by Boekenoogen (1948, p. 160), the two methods yield quite close results.

Rhodanic acid also adds to linolenic acid and, since apparently two methylene interrupted bonds are too close to accommodate two rhodanic acid molecules, it was felt that linolenic acid would be able to accommodate two of them. However, if the first one were to be added to the central double bond in the linolenic acid, the two outer ones would be too close for further addition. This would mean the addition of a single molecule, just as for linoleic acid. In practice (Kass et al., 1940), linolenic acid is found to add on average 1.83 mole equivalents of rhodanic acid, indicating some addition to the central bond.

So, in the past, when the analytical tools were more limited, ingenuity was called for. When UV-spectrophotometers became available, this provided more direct information since linoleic acid could be determined by conjugating the acid. But until the advent of chromatographic separation methods, the number of components that could be determined was limited to the number of independent values that were available.

6.3.1 Acidity

For an oil refiner, the acidity of his oil is an important parameter since it affects the yield loss on refining. The acidity of oil is commonly expressed as either the acid number or the FFA content. The acid number is defined as the amount [mg] of KOH (potassium hydroxide) needed to neutralise 1 g of oil; being a ratio of two weights (mg/g), it has no dimension. The FFA content is defined as a mass percentage of these acids while assuming them to be oleic acid with a relative molecular mass of 282 (weight % expressed as oleic acid).

So an oil with an FFA of 1.0 % contains 0.01 g oleic acid per g of oil, which amount of oleic acid equals $0.01:282 = 3.546 \times 10^{-5}$ mol $= 3.546 \times 10^{-2}$ mmol. Given the relative molecular mass of KOH of 56.11, the amount of potassium hydroxide required to neutralise this amount of oleic acid

equals $3.546 \times 56.11 \times 10^{-2} = 1.9897$. For practical purposes, a ratio of 2 can be assumed between the acid number and the FFA content of oil, provided the latter is expressed as oleic acid.

6.3.2 Saponification value

Because the FFA is expressed as oleic acid, the theoretical loss on refining has to be adjusted for the average relative molecular mass of the constituent fatty acids. This average can be calculated from the saponification value of the oil, which has been defined as the amount (in mg) of KOH required to neutralise all fatty acids resulting from complete saponification of a 1 g oil sample. This means that lauric oils with an abundance of medium chain fatty acids contain more fatty acids per unit of mass than for instance soybean oil and, thus, have a higher saponification value.

Triolein, for instance, has a relative molecular mass of 884 and the KOH equivalent mass of the three oleic acids in this species (its saponification value or SV) equals:

$$\mathrm{SV} = (1000 : 884) \times 3 \times 56.11 = 190.42 \qquad (6.1)$$

Working back to the average relative molecular mass of the fatty acids first of all requires the relative molecular mass of the oil (MW_{oil}) to be calculated according to:

$$MW_{oil} = \frac{3 \times 56.11}{SV} \times 1000 \qquad (6.2)$$

which allows the average relative molecular mass of the fatty acids (MW_{FA}) to be calculated according to:

$$\mathrm{MW_{FA}} = (\mathrm{MW_{oil}} - 38) : 3 \qquad (6.3)$$

In the above equation, the term 38 is the difference in relative molecular mass between a triglyceride and its constituent fatty acids. The average relative molecular mass of the fatty acids (MW_{FA}) can then be used to calculate the actual fatty acid content of an oil sample with a given FFA content expressed as oleic acid by multiplying this FFA content with the MW_{FA} value and dividing by 282, which is the relative molecular mass of oleic acid. Often literature values for the MW_{FA} are used as listed in Table 6.2.

The saponification value can also be calculated from the fatty acid composition as determined by GLC. The results are expressed in weight percent of FAME (fatty acid methyl esters). Consequently, the first step in the calculation entails the calculation of the molar composition by dividing each weight percent by the corresponding relative molecular mass of the FAME and normalising to a total of unity. The molar fractions obtained are then multiplied by the corresponding relative molecular mass of the fatty acid and these products are then added to give the average relative molecular mass of the fatty acids (MW_{FA}) from which the saponification value can be calculated.

TABLE 6.2 Literature values for the average relative molecular mass of constituent fatty acids (MW_{FA}), relative molecular mass of the oil (MW_{oil}) and saponification values (SV) for various oils

Oil type	MW_{FA}	MW_{oil}	SV
Canola oil	284	890	189
Coconut oil	212	674	248
Corn oil	283	887	190
Cottonseed oil	274	860	196
Fish oil (anchovy)	260	818	206
Lard	278	872	193
Milk fat	245	893	188
Palm oil	271	851	198
Palm olein	272	854	197
Palm stearin	265	833	202
Palm kernel oil	222	704	239
Rapeseed oil (HEAR)	315	983	171
Soybean oil	280	878	192
Sunflower seed oil	280	878	192
Tallow	274	860	196

HEAR, high erucic acid rapeseed

If the chain lengths of the fatty acids do not differ very much, the above method of calculation can be simplified by omitting the first step. Using the weight percent data as if they represented molar fractions multiplied by 100 will not introduce noticeable errors for oils like soya bean oil, canola and sunflower seed oil. For lauric oils and fish oils, the first step is preferably maintained.

Acid oils resulting from soap stock acidulation contain both FFA and glycerides. The former can be quantified by the acid value and the saponification value is indicative of the average relative molecular mass of the fatty acid moieties, both free and bound, present in the sample. The difference between the saponification value and the acid value is referred to as the ester value and is indicative of the ester bonds still present in the acid oil sample. It is also expressed as the amount (in mg) of KOH required to saponify these ester bonds present in a 1 g oil sample.

6.3.3 Iodine value

The iodine value (IV) is indicative of the degree of unsaturation of a triglyceride (triacylglycerol) oil. It is defined as the amount of iodine (in g) added to 100 g of oil; the IV, therefore, has no dimension. Accordingly, triolein with a relative molecular mass of 884 and three double bonds per molecule has an iodine value of $(100:884) \times 3 \times 2 \times 126.9 = 86.13$. Less unsaturated oils, such as the lauric oils, have much lower iodine values (9 to 18) and vegetable oils with a high linoleic acid content, such as sunflower seed oil, corn oil, and soybean oil, have higher values (120 to 132). Very high values are displayed by linseed oil (185) because of its high linolenic acid content and fish oils containing fatty acids with five or even six double bonds.

Iodine only adds quantitatively to non-conjugated double bonds and when the oil is oxidised, there may be some substitution. IV values should be interpreted with some

caution, but for following the overall progress of a hydrogenation reaction for example, the IV is a most useful parameter indeed. It can also be calculated from the fatty acid composition as determined by GLC.

Like the calculation of the saponification value from GLC data, the calculation of the IV also entails the calculation of the molar fatty acid composition as a first step. The molar fractions are then multiplied by the number of double bonds in the fatty acid and the products, thus obtained, are added. If this total (sum of double bonds) is called S_{DB}, the IV can be calculated according to:

$$IV = S_{DB}\frac{884}{MW_{oil}}86.13 \qquad (6.4)$$

Again, the first step can be omitted for oils with mainly C18 fatty acids. For these oils, the weight fractions of the fatty acids can be immediately multiplied by their number of double bonds, followed by totalling the products obtained, and multiplying this total with the factor 86.13 (the IV of triolein) immediately yields the IV. For fish oils, this simplification would be an oversimplification.

6.3.4 Fatty acid composition

The determination of the fatty acid composition of oil by GLC invariably uses the FAME. Various methods for obtaining a representative sample of FAME have been described, for example, by BF_3-catalysed esterification of free fatty acids. In practice, the method by Jáky (1971) is most convenient.

Oil (0.2 g) is dissolved in diethyl ether (2 ml) and some (2 ml) methanolic solution of KOH (3%) is added. After shaking and a reaction time of some 3 min, pentane (10 to 15 ml) and water (2 to 3 ml) are added and shaken. The upper layer is decanted and washed twice with water and dried by adding some sodium sulfate. The pentane solution is then ready for injection. The only glassware required is a couple of standard test tubes.

Normally, the gas chromatogram peak area can be used to calculate the content of a certain fatty acid; calibration is only required for very demanding analyses and for verification. Nevertheless, FAME gas chromatograms must be interpreted with care since they only refer to standard fatty acids and exclude oxidised, oligomerised, etc., breakdown products. Concluding that the palmitic acid content of a used frying fat equals the chromatogram reading may seriously overestimate its content. The increase in palmitic acid that coincides with a decrease in linolenic acid in oil from damaged soybeans (Robertson et al., 1973) is also indicative of the presence of non-eluting material. Using an internal standard (e.g., C17:0 as provided by triheptadecanoate to ensure that it undergoes the same FAME preparation procedure) overcomes this problem.

6.3.5 Titre

The titre of a fatty material is defined as the solidification point (in °C) of its water-insoluble fatty acids. If the fatty material is an oil or fat, it has to be saponified before its titre can be determined. This determination requires a special, but simple apparatus comprising a beaker, a wide-necked flask, and a test tube fitted with a manual stirrer and a thermometer. Although the test itself may have become obsolete, awareness of its existence is recommended since it facilitates understanding the literature. For example, a patent describing the frac-tionation of fatty acids (Myers and Muckerheide, 1942) indicates the quality of the stearic acid by quoting its low iodine value and illustrates the absence of saturated fatty acids in the oleic acid fraction by quoting its titre of 1.0°C.

6.3.6 Hydroxyl value

The hydroxyl value of a fat is defined as "the amount (in mg) of KOH required to neutralise the amount of acetic acid capable of combining by acetylation with 1 g of oil." This definition is based on the way the hydroxyl value is determined; an oil sample is acetylated with a known excess of acetic acid anhydride. This excess is then allowed to hydrolyse and the amount of acetic acid formed during both the acetylation and the hydrolysis reactions is then determined by titration; the hydroxyl value follows from the difference between this measurement and a blank.

During the acetylation reaction, free hydroxyl groups in hydroxy acids and in partial glycerides are acetylated without distinction; presumably, any free hydroxyl groups in lysophosphatides present are also acetylated. This means that the hydroxy acid content has to be corrected for these partial glycerides and/or lysocompounds when this content is arrived at on the basis of the hydroxyl value.

The monoglyceride monostearate has a relative molecular mass of 358 so 1 g contains (1 000:358) × 2 = 5.5866 mmol free hydroxyl groups; this amount corresponds to 5.5866 × 56.11 = 313.5 mg KOH. Accordingly the hydroxyl value of monostearate is 313.5. The hydroxyl value of the diglyceride distearate is much lower since this compound has only one free hydroxyl group per molecule and a much higher relative molecular mass than monostearate so that there are fewer mmol per gram. Its hydroxyl value equals 56.11 × (1 000:624) = 89.9, which is just over a quarter of the value of monostearate.

Although the hydroxyl value is primarily used for the characterization of oils with hydroxy acids, such as castor oil, it can also be used to give an impression of the partial glyceride content. In fact, partial hydrolysis of an oil leads to free fatty acids and an equivalent amount of free hydroxyl groups in partial glycerides and, since both the acid value and the hydroxyl value are expressed as an

amount (in mg) of KOH per gram of oil, it follows that the acid value and the hydroxyl value of this partially hydrolysed oil should be quite close.

6.3.7 Oil content of spent bleaching earth

As pointed out in Section 3.7.5 and shown in Table 3.10 (Morton, 1995), Soxhlet extraction of spent bleaching earth as recommended by Boring (1995) only partially removes residual oil from the earth. Oxidised and polymerised oil is not extracted. Consequently, Soxhlet extraction only provides an answer to how much oil can be recuperated by solvent extraction of the spent earth, but does not allow the yield loss of the bleaching step to be worked out.

For the determination of the yield loss of the bleaching step, a procedure involving the determination of the water content and the weight loss during calcination in a muffle furnace is recommended (Dijkstra, 1993). The fresh bleaching earth is thereby assumed to consist of a calcination residue (R), water (W), and other, nonaqueous volatile constituents (V) that are evaporated in the muffle furnace. The spent bleaching earth also contains these components and in addition a certain amount of oil (O). By using the subscripts *f* and *s* for the fresh earth and the spent earth, respectively, and introducing *M* to denote the mass of the samples, the mass (M_f) of the sample fresh earth can be written as:

$$M_f = R_f + W_f + V_f$$

so that

$$V_f = M_f - R_f - W_f \tag{6.5}$$

Similarly, the mass (M_s) of the sample spent earth can be written as:

$$M_s = R_s + W_s + V_s + O_s \tag{6.6}$$

Since the nonaqueous volatiles V will be a fixed fraction of the calcination residue R:

$$\frac{V_s}{R_s} = \frac{V_f}{R_f} \quad \text{or} \quad V_s = V_f \frac{R_s}{R_f} \tag{6.7}$$

the amount of oil in the spent earth can then be expressed as:

$$O_s = M_s - R_s - W_s - V_f \frac{R_s}{R_f}$$

or

$$O_s = M_s - R_s - W_s - (M_f - R_f - W_f)\frac{R_s}{R_f} \tag{6.8}$$

The amount M_f' of fresh bleaching earth used to arrive at the amount M_s of spent earth then equals:

$$M_f' = \frac{R_s}{R_f} M_f \tag{6.9}$$

so that the oil loss (OL) can be expressed as a percentage of the amount of fresh earth according to:

$$OL = \frac{R_f(M_s - R_s - W_s) - R_s(M_f - R_f - W_f)}{R_s M_f} 100\% \tag{6.10}$$

References

Al-Alawi, A. et al. (2005a) A new Fourier transform infrared method for the determination of moisture in edible oils, *Appl. Spectrosc.*, **59**, 1295–1299.

Al-Alawi, A. et al. (2005b) A new FTIR method for the analysis of low levels of FFA in refined edible oils, *Spectrosc. Lett.*, **38**, 389–403.

Boekenoogen, H.A. (1948) De Scheikunde der Oliën en Vetten, A. Oosthoek's Uitgevers Mij., Utrecht.

Boring, S.A. (1995) Soybean processing quality control, in *Practical Handbook of Soybean Processing and Utilization*, Erickson, D.R., Ed., AOCS Press, Champaign, IL, 483–503.

Dijkstra, A.J. (1993) Degumming, refining, washing and drying fats and oils, in *Proceedings of the World Conference on Oilseed Technology and Utilization*, Applewhite, T.H., (Ed.), American Oil Chemists' Society, Champaign, IL, 138–151.

Jáky, M. (1971) Über den Reaktionsmechanismus der Bildung von Fettsäure-methylestern, *Fette Seifen Anstrichm.*, **73**, 216–220.

Kass, J.P. et al. (1940) The thiocyanogen value of linolenic acid, *Oil & Soap*, **17**, 118–119.

Morton, R.B. (1995) Oil content of filter cakes, *Paper presented at the 86th AOCS Annual Meeting & Expo*, *San Antonio, TX.*

Myers, L.D. and Muckerheide, V.J. (1942), Method of separating fatty acids, US Patent 2,293,676, assigned to Emery Industries Inc.

Robertson, J.A. et al. (1973) Chemical evaluation of oil from field- and storage-damaged soybeans, *J. Am. Oil Chem. Soc.*, **50**, 443–445.

6.4 Chromatographic analysis of lipids

6.4.1 Introduction

The study of lipids has assumed considerable importance in recent years with the recognition that they are involved in many vital biological processes in animals, plants, and microorganisms. Methods for the analysis of lipids, therefore, are essential for many research, clinical, and quality control applications, and chromatographic techniques are especially important. Indeed, lipid analysts were at the forefront in the development of gas chromatography (GC) and thin-layer chromatography (TLC), and these techniques provided the springboard

for the explosive growth of knowledge in the fields of lipid chemistry and biochemistry that has occurred over the past 30 years. High-performance liquid chromatography (HPLC) was taken up less quickly, but many separations of lipids by means of this technique have now been described that cannot be rivaled by other methods. When such techniques are coupled to mass spectrometry and related techniques, substantial additional information is obtainable. Indeed mass spectrometry without a chromatographic interface is being used increasingly. It is, however, worth noting how much can still be done with relatively simple equipment.

The analysis of lipids presents many problems, mainly because of their diverse nature. Some are almost hydrocarbon-like in their physical properties, while others are slightly soluble in water and there is a spectrum of lipids with properties between these extremes. Also, lipids lack spectroscopic chromophores that permit straightforward identification or quantification. Lipid analysts must first decide exactly what information is needed from a sample, and then decide which of a wide range of techniques available is most suited to the problem. Such techniques have been reviewed in much greater detail than is possible here (Christie, 2003). In the analysis of lipids, the use of organic solvents and potentially toxic reagents is essential and appropriate safety precautions must be taken at all times.

6.4.2 Chromatographic modes of separation

6.4.2.1 Adsorption chromatography

Silica gel is by far the most widely used adsorbent in low-pressure column chromatography and in TLC, and now has innumerable uses in HPLC, but especially for classes of lipids separated according to the number and nature of their functional groups. The adsorptive properties of silica gel are due to silanol groups, i.e., hydroxyl groups linked to silicon, which are attached to the surface and can be free or hydrogen-bonded. In addition, there is water of hydration, which exists first in a strongly bound layer and then in one or more loosely bound layers on the surface. In the early days of the technique, different brands of silica gel tended to vary greatly in their properties, but modern precoated TLC plates and HPLC adsorbents from reputable suppliers can be relatively uniform and consistent in their properties.

In the most common application of this separation mode, in which components are separated according to the number and type of polar functional groups (ester, phosphate, hydroxyl and amine groups, for example), isocratic elution with a mobile phase of constant composition may be practical for restricted types of lipid class, and this is the usual mode for TLC applications. On the other hand, gradient elution in which the polarity of the mobile phase is increased at a controlled rate affords greater versatility for a wide range of lipid types. Detection of lipids separated by adsorption chromatography (and other liquid chromatography techniques) presents particular problems, and these are discussed below (Section 6.4.5).

6.4.2.2 Gas-liquid partition chromatography

GC with capillary or wall-coated, open-tubular columns may be used for any lipid that can be volatilized. The columns consist of narrow bore tubing (0.1 to 0.3 mm in internal diameter and 25 or 50 m in length commonly) of fused silica, the inner wall of which is coated with the liquid phase. Packed columns, i.e., up to 5 mm wide and containing inert support materials coated with stationary phase, are little used for routine analysis, but may be required for some specialist applications, e.g., combined mass and radioactivity measurements. The function of the column is to allow partitioning of the constituents of a sample between the stationary and mobile phases and this is aided by having the liquid phase as a thin film with a large surface area accessible to the flow of the gas phase. As the sample passes down the column, the molecules of each component partition between the liquid and gas phases according to their individual equilibrium constants, and so travel at different rates and are separated.

The efficiency of a given system is dependent on a number of factors, including the nature and flow-rate of the carrier gas, column dimensions, liquid-phase thickness, and column temperature, and these must be optimized to maximize the resolution. Hydrogen is the most efficient carrier gas, but helium may be preferred for safety reasons. Nitrogen is sometimes used for cost and safety reasons, but is much less efficient.

Gas chromatography with flame-ionization detection has become the definitive technique for analysis of the fatty acid components of lipids, when separation is both by chain-length and by degree of unsaturation. The detector is robust and linear over several orders of magnitude in sample size, so accurate and reproducible quantification is possible with a high degree of automation. While the technique can be utilized for the separation of any lipid molecule capable of volatilization, or which can be converted to nonpolar volatile derivatives, up to and including triacylglycerols, separation worsens and quantification becomes more problematic as the molecular weights of the analytes increase.

6.4.2.3 Reversed-phase liquid-partition chromatography

In "reversed-phase" chromatography, the separation is based on the selective interactions of solute molecules with a relatively nonpolar liquid stationary phase and a relatively polar liquid mobile phase. The mechanism of this interaction has been reviewed in relation to lipid separations by Nikolova-Damyanova (1997). HPLC in the reversed-phase mode has been much used by lipid analysts for the separation of molecular species of lipids within a single lipid class, i.e., they are separated principally according to the sum of the chain-lengths of the fatty acyl or alkyl moieties, together with an appreciable dependence

on the number and configuration of any double bonds. Analogous TLC methods are available, but have been little used because of technical difficulties.

Although there are many nonpolar stationary phases in use in reversed-phase HPLC, the most widely employed and most important for lipid analysis are those consisting of relatively long-chain hydrocarbons, bonded chemically to the surface of spherical silica (with particle sizes of 3 to 10 μm). Of these, by far the most widely used stationary phase consists of octadecylsilyl ("C_{18}" or "ODS") groups. For most lipid separations, either acetonitrile or methanol is a major component of the mobile phase, accompanied by a modifier solvent chosen according to the nature of the lipid. Under optimum conditions, separations are according to the number of carbons in the aliphatic chains of the lipids and by the number of double bonds, each double bond reducing the retention time by the equivalent of about two methylene groups.

6.4.2.4 Ion-exchange chromatography

In essence, the process of ion exchange can be considered as a competition between the solute ions and counter ions present in the mobile phase for fixed sites of opposite charge on a support. The quality and extent of a given separation can be manipulated by varying the nature and concentration of the counter ion or by varying the pH of the mobile phase. Silica-based HPLC phases with a chemically bonded primary amine group (anion exchanger) or sulfonic acid (a strong cation exchanger) as the active agent have been applied to phospho- and glycolipid analyses with some success and afford distinctive separations. Earlier, the cellulose-based ion-exchange media, diethylaminoethyl (DEAE)- and triethylaminoethyl (TEAE)-cellulose (anion exchangers) and carboxymethyl (CM)-cellulose (cation exchanger), were much used by lipid analysts in low-pressure column applications for the separation of polar complex lipids. Although this methodology works well in semi-preparative applications, it now appears to be much less used than formerly.

6.4.2.5 Silver ion chromatography

One specialized type of chromatography of particular importance in lipid analysis is a form of complexation chromatography in which silver ions are associated in some manner with the stationary phase. The technique is termed silver ion or argentation chromatography. The principle of the method is that silver ions interact reversibly with the pi electrons of double bonds (*cis* more strongly than *trans*) to form transient polar complexes; the greater the number of double bonds in a molecule, the stronger the complex formation and the longer it is retained. Silica gel impregnated with silver nitrate has been used in TLC applications and even in HPLC columns, but a more practical HPLC technique consists in binding silver ions to an ion exchange phase, e.g., phenylsulfonate residues bonded chemically to a silica matrix. These afford much better and reproducible resolution. The technique has been reviewed comprehensively by Nikolova-Damyanova (1992). The main value is for the separation of simple fatty acid fractions, depending on the number, position and geometry of double bonds, from complex mixtures for further analysis, e.g., for *trans* fatty acids. It is also very useful for molecular species of simple lipids, such as triacylglycerols, where it complements separations by reversed-phase HPLC.

6.4.3 Lipid extraction, storage, and sample handling

6.4.3.1 Introduction and preliminaries

As a first step, it is necessary to extract the lipids from their tissue matrices and free them of any nonlipid contaminants before analysis can be attempted. Ideally, this should be done immediately after removal of the tissue from the living organism, but if this is not possible, the tissue should be stored in such a way that it does not deteriorate significantly. For example, samples should be frozen as rapidly as possible, ideally with dry ice or liquid nitrogen, and stored in sealed glass containers at –20°C (or lower) in an atmosphere of nitrogen. Subsequent extraction should be undertaken without thawing.

During extraction, as in many other aspects of analysis, it is possible to inadvertently introduce contaminants or to bring about some unwanted change in the composition of the lipids. Autoxidation of double bonds in fatty acids, for example, is particularly troublesome and care must be taken at all steps in the analysis of lipids, not just during storage and extraction, to eliminate the problem. Natural tissue antioxidants, such as the tocopherols, afford some protection to lipid extracts, but it is usually advisable to add further synthetic antioxidants, such as 2,6-di-tert-butyl-p-cresol (BHT) to storage solvents at a level of 50 to 100 mg/l. The presence of large amounts of unesterified fatty acids, diacylglycerols, phosphatidic acid, or lysophospholipids in lipid extracts must be an indication that some permanent damage to the tissues and, hence, to the lipids has occurred. Similarly, lipoxygenases can cause artefactual formation of oxygenated fatty acids, compounding the effects of autoxidation. Such changes can make a crucial difference to the concentrations of some important lipid metabolites, such as free fatty acid and 1,2-diacylglycerol concentrations of tissues. For example, very low free fatty acid concentrations were observed when heart tissue was frozen rapidly and pulverized at dry ice temperatures before extraction (Kramer and Hulan, 1978) in comparison to more conventional approaches. All containers made of plastic materials should be avoided for storage purposes, as plasticisers will leach out and contaminate extracts. Extraction procedures in general have been reviewed in some detail elsewhere (Christie, 1993b).

Lipid extracts or lipid standards are still susceptible to autoxidation, though not to enzymic attack. They should be dissolved in a small volume of a relatively nonpolar solvent, such as hexane, and stored at -20°C in glass (never plastic) containers, with screw-caps lined with Teflon™ (PTFE), from which air is excluded by flushing with a stream of nitrogen and in the presence of antioxidants.

6.4.3.2 Extraction methods

Lipids occur in tissues in a variety of physical forms. For example, simple lipids are often part of large aggregates in storage tissues, from which they are extracted with relative ease by solvents such as hexane or diethyl ether. On the other hand, complex lipids are usually constituents of membranes (where they occur in a close association with such compounds as proteins and polysaccharides) and they are not extracted so readily. In this instance, the solvents must not only dissolve the lipids readily, but also overcome the interactions between the lipids and the tissue matrix.

Most lipid analysts use chloroform-methanol (2:1 by volume), with the endogenous water in the tissue as a ternary component of the system, to extract lipids from animal, plant, and bacterial tissues. The tissue can be homogenized in the presence of both solvents, but better results may be obtained if the tissue is first extracted with methanol alone before the chloroform is added to the mixture. With difficult samples, more than one extraction may be needed and, with lyophilized tissues, it may be necessary to rehydrate prior to carrying out the extraction. The homogenization and extraction should be performed in equipment in which the drive to the blades is from above, so that the solvent does not come into contact with any lubricated bearings. For safety reasons, there is some interest in isopropanol-hexane (3:2 by volume) as an extractant because its toxicity is relatively low, while water-saturated butanol has been recommended for some difficult samples.

Lipid extracts from tissues, obtained in this way, will contain significant amounts of nonlipid contaminants, such as sugars, amino acids, urea, and salts, which must be removed before the lipids are analysed. Most analysts use a simple washing procedure, in which a chloroform-methanol (2:1 by volume) extract is shaken and equilibrated with one-fourth its volume of saline solution (Folch, Lees and Stanley, 1957). The mixture partitions into two layers, of which the lower phase is composed of chloroform–methanol–water in the proportions 85:14:1 (by volume) and contains virtually all of the lipids, while the upper phase consists of the same solvents in the proportions of 3:49:48, and contains much of the nonlipid contaminants. It is important that the proportions of chloroform, methanol, and water in the combined phases should be as close as possible to 8:4:3 (by volume), otherwise selective losses of lipids may occur.

The "Folch" procedure is the standard by which other extraction methods are judged. However, it is time-consuming and uses large volumes of solvents. In contrast, a method described by Bligh and Dyer (1959), which was devised originally for the extraction of phospholipids from fish muscle tissue in a relatively economical manner, is often recommended for large samples with a high proportion of endogenous water. Quantitative recovery of triacylglycerols is not always achieved when these are major components of a tissue and a second extraction with chloroform alone is then recommended. With green plant tissues, it is necessary to extract the lipids first with isopropanol, in order to deactivate the enzymes, especially lipases, before partitioning with chloroform, methanol, and saline solution as in the Folch method. Special procedures are also required for extraction of such highly polar lipids as polyphosphoinositides and gangliosides (Christie, 1993b; 2003).

6.4.4 Fatty acid analysis

6.4.4.1 Introduction

Gas chromatography (GC) with flame-ionization detection is undoubtedly the technique that would be chosen in most circumstances for determination of fatty acid compositions, after conversion to simple ester derivatives. Here, the technique is discussed in terms of capillary columns only, as packed columns are now virtually obsolete, other than for combined mass and radioactivity measurements, where greater sample loads are necessary. The technology is robust and mature, and high precision is possible. Individual fatty acids can usually be identified by GC with reasonable certainty from their relative retention times, especially if the analysis is carried out with a variety of stationary phases, and taking into account the large body of knowledge that now exists on the compositions of specific tissues or organisms. On the other hand, when definitive confirmation of fatty acid structures is required, chemical-degradative and spectroscopic procedures are available. The only technique to compare with GC for the analysis of fatty acid derivatives is HPLC in the reversed-phase mode with UV-absorbing derivatives, although both the capital and running costs of this technique are appreciably higher. HPLC has advantages for the isolation of specific components on a small scale for structural analysis or for radioactivity measurements (Christie, 2003).

6.4.4.2 Preparation of fatty acid derivatives for chromatography

Before fatty acid components of lipids are analysed, it is usually necessary to prepare nonpolar derivatives of various kinds, but usually the methyl esters. Because of the high sensitivity of GC procedures, small amounts of material (usually less than 1 mg and certainly less than 10 mg) are all that is required. Methods for preparation of

the methyl ester derivatives have been reviewed (Christie, 1993a). Other derivatives are preferred for mass spectrometric identification.

Free fatty acids are esterified and O-acyl lipids transesterified by heating them with a large excess of anhydrous methanol in the presence of an acidic catalyst, such as 5% (w/v) anhydrous hydrogen chloride in methanol. It is most often prepared by bubbling hydrogen chloride gas into dry methanol, but a simpler procedure is to add acetyl chloride (5 ml) slowly to cooled dry methanol (50 ml). It is usual to heat the lipid sample in the reagent under reflux for about 2 hours, but equally effective esterification is obtained if the reaction mixture is heated in a stoppered tube at 50°C overnight. Boron trifluoride in methanol (12 to 14% w/v) has also been much used as a transesterification catalyst and, in particular as a rapid means of esterifying free fatty acids, and it is recommended in some internationally approved methods. However, there are a number of well-documented disadvantages, especially as the reagent ages. A solution of 1 to 2% (v/v) concentrated sulfuric acid in methanol transesterifies lipids in the same manner and at much the same rate and the reagent can readily be prepared fresh whenever it is required.

If any of the acidic reagents is used carelessly, for example, if they are permitted to super-heat in air or are stored too long, they can cause some decomposition of polyunsaturated fatty acids. Acidic reagents will also release aldehydes from plasmalogens, converting them to dimethyl acetals.

O-acyl lipids are transesterified very rapidly in anhydrous methanol in the presence of a basic catalyst. Free fatty acids are not esterified, however, and care must be taken to exclude water from the reaction medium to prevent their formation by hydrolysis of lipids. 0.5M sodium methoxide in anhydrous methanol, prepared simply by dissolving fresh clean sodium in dry methanol, is the most popular reagent, but potassium methoxide (or occasionally the hydroxide) has also been used as catalyst. The reagent is stable for some months at refrigeration temperature, especially if oxygen-free methanol is used in its preparation. The reaction with lipids is very rapid; phosphoglycerides, for example, are completely transesterified in a few minutes at room temperature.

Diazomethane was used as a rapid means of specific methylation of free fatty acids in the past, but is now banned in many countries as both it and the reagents required for its preparation are toxic and potentially carcinogenic.

It should be noted that nonpolar lipids, such as cholesterol esters or triacylglycerols, are not soluble in reagents composed predominantly of methanol and will not react in a reasonable time, unless a further solvent is added to effect solution. Toluene is usually recommended for the purpose.

Special care may be required if samples are believed to contain fatty acids with cyclopropene, cyclopropane, and epoxyl groups, as they are disrupted by acidic conditions. While short-chain acids, as in milk fat or coconut oil, are completely esterified by all of the procedures described above, quantitative recovery of the esters from the reaction medium can be very difficult because of their high volatility and partial solubility in water. The best methods are those in which there are no aqueous extraction or solvent removal steps and in which the reagents are not heated; these criteria are met by the alkaline transesterification procedure of Christopherson and Glass (1969). Butyl rather than methyl esters may be preferred in this instance. Sphingolipids, which contain fatty acids linked by N-acyl bonds, are not easily transesterified under acidic or basic conditions, but methylation can be achieved with methanol containing concentrated hydrochloric acid (5:1 v/v) under reflux for 5 hours.

6.4.4.3 GC analysis of fatty acid derivatives

The liquid phases in use for the GC analysis of methyl ester derivatives are almost exclusively polar polyesters, with which unsaturated components elute after the analogous saturated fatty acids. They permit clear separations of esters of the same chain-length and with zero or up to six double bonds. Polyesters can be classified according to their degree of polarity and, in current practice, only two main types need be considered, i.e., those of low to medium polarity, such as those of the Carbowax™ type (polyethylene glycol — under various trade names), and those of high polarity, such as CP-Sil 88™ (Chrompack, Middleburg, The Netherlands), BPX70™ (SGE Melbourne, Australia), or SP-2340™ (Sigma-Aldrich, Poole, U.K.). Nonpolar phases may be required in mass spectrometry because of their low bleed characteristics, but afford poorer resolution (unsaturated elute before analogous saturated fatty acids in this instance).

Fused silica capillary columns are robust and, with modern cross-linked and chemically bonded phases, will last for 2 or more years, while affording high resolution and low adsorptivity (quantitative recovery). Excellent results can be obtained with columns of 10 or 25 m in length with most samples, and longer columns are only rarely required. For analytical purposes, those columns used most often have internal diameters of 0.2 to 0.3 mm.

Changing the polarity of a polyester phase does not change the order of elution of components within a given chain-length group, but it can affect the elution order relative to components of other chain lengths. With the phases of low to medium polarity, all the unsaturated C_{18} fatty acids, i.e., with one to four double bonds, emerge from the column before any of the C_{20} components. Therefore, columns of fused silica coated with phases of the Carbowax type can be recommended for the analysis of the common fatty acids of animal and plant origin, as they give satisfactory resolution of all the important polyunsaturated fatty acids with no interference by components differing in chain-length by two carbon atoms, at least until the C_{22} to C_{24} regions. Thus, a 25-metre column (0.25

mm i.d.) coated with Carbowax 20M™ is suitable for all routine analyses of clinical, seed oil, and fish oil samples.

The nature of the injection system for capillary GC is obviously important, and the easiest to use is one of the split/splitless type. It is capable of high accuracy in fatty acid analysis and it should probably be the first choice for this purpose, especially as it is more easily coupled to an automatic injection facility than are those of the on-column type. It can be assumed that all modern GC instruments will be supplied with a temperature-programming facility.

A separation of methyl esters of fatty acids from erythrocyte lipids on a Carbowax 20M™ column is illustrated in Figure 6.1. Each of the main chain-length groups is reasonably well resolved. For example, three minor 16:1 isomers are seen and they are distinct from the trace amounts of C_{17} fatty acids. Similarly the important C_{18} components are clearly separated and they are in a different region of the chromatogram from the C_{20} unsaturated constituents. With the last, the only serious overlap problem is with 20:3(n-3), which co-chromatographs with 20:4(n-6). Finally, all the biologically important C_{22} fatty acids are cleanly resolved, although care is necessary to distinguish them from the 24:0 and 24:1 fatty acids. In this instance, the methyl esters were prepared by acid-catalysed-methylation so that vinyl-ether bound chains of plasmalogens were converted to dimethyl acetals, which elute just ahead of the corresponding fatty acid derivatives.

With columns of the highest polarity (e.g., CP-Sil 88™, BPX70™, or SP-2340™), excellent resolution is obtained, especially of positional or geometrical isomers. Their principal disadvantage is that there is some overlap of fatty acids of different chain lengths and the nature and extent of the problem can be rather sensitive to column temperature or the temperature gradient (see below). The inherent resolution is such that there may be few problems of actual overlap of major components, but a multiplicity of peaks can be revealed, so compounding the identification problems. Columns of the more polar phases are essential for analysis of lipids containing *trans* fatty acids or for conjugated linoleic acid isomers. Then, 100 metre columns are often recommended and, indeed, may be essential. In Europe, CP-Sil 88™ seems to be preferred, while the SP-2340™ stationary phase is favoured in the U.S. (Ratnayake, 1998). However, more accurate results are obtained if such GC techniques are used after a preliminary separation by silver ion chromatography.

Standards are available to permit identification of the common range of fatty acids likely to be encountered in samples, and analysts soon acquire an intuitive understanding of the relationship between the retention times of peaks on a GC trace and their identity. Secondary external reference standards consisting of a natural fatty acid mixture of known composition can also be useful; for example, cod liver oil has been used in this way to identify the fatty acids of other marine species (Ackman and Burgher, 1965). Another useful means of identification is to use equivalent chain-lengths (ECLs) (Miwa et al., 1960). These are obtained by reference to the straight line obtained by plotting the logarithms of the retention times of a homologous series of straight-chain

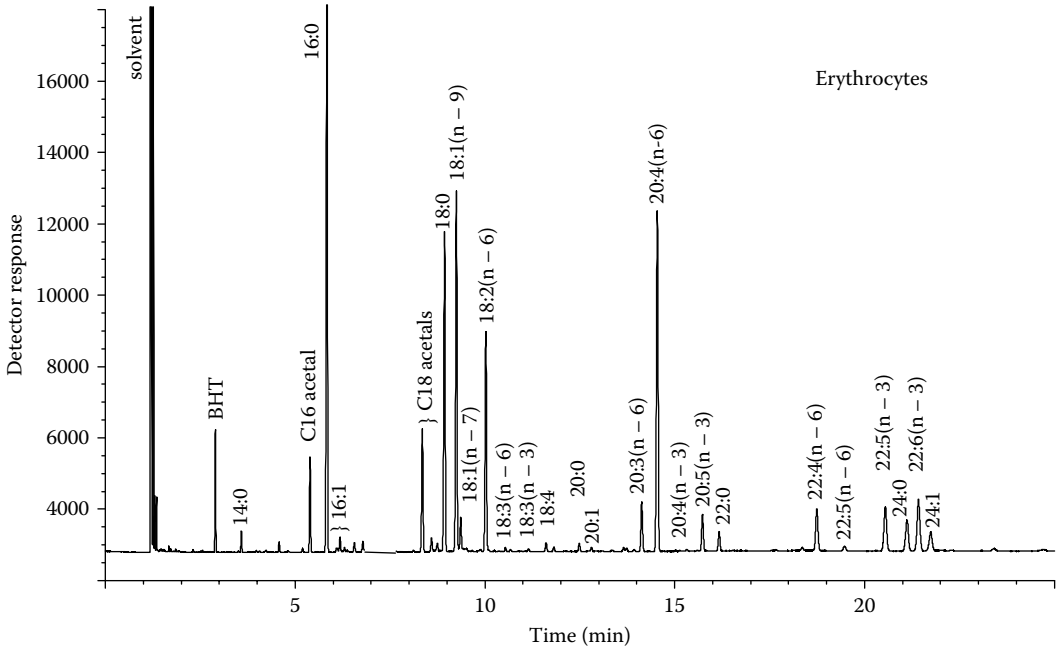


FIGURE 6.1 Separation of fatty acids of human erythrocytes as methyl esters by GC on a column (25 m × 0.25 mm × 0.2 μm), coated with CP-Wax 52CB™ (Chrompack BV, The Netherlands). The oven temperature was held at 170°C for 3 min, then was raised by 4°C/min to 220°C. Hydrogen was the carrier gas at a flow rate of 1 ml/min. (From Christie, W.W. (2003) *Lipid Analysis*, 3rd ed., Oily Press, Bridgwater, U.K. With permission.)

saturated fatty acid methyl esters against the number of carbon atoms in the aliphatic chain of component under isothermal operating conditions. The retention times of the unknown acids are measured under the same conditions and the ECL values are read directly from the graph, for comparison with data obtained with a range of standards or with those published in the literature.

With modern flame ionisation detectors, the areas under the peaks on the GC traces are within limits linearly proportional to the amount (by weight) of material eluting from the columns. Manual and electronic methods are available, but there is no doubt that the latter are best (Christie, 2003). Problems of measuring peak areas arise mainly when components are not completely separated, and there is no way of overcoming this difficulty entirely. When overlapping peaks have distinct maxima, computer analysis of peak shapes may improve the accuracy of the estimation. Where one component is visible only as a minor shoulder or broadening of a major peak, no manual or computer method is likely to give very precise results for the individual components, although electronic integration can at least give an accurate measure of the total amount of material present in a multiple peak. With all column types, it is necessary to check at frequent regular intervals whether losses are occurring by running standard mixtures of accurately known composition through the columns. Such checks also give an estimate of the precision and reproducibility of the method of measurement. Small correction factors may be applied, when high precision is required, to compensate for the fact that the carboxyl carbon atom in each ester is not ionized appreciably during combustion (Ackman, 1972). If analysts find further correction factors to be necessary, it is probable that some aspect of the chromatographic technique, instrument settings, or column installation has not been carried out correctly. In general, the proper approach to the generation of results of high accuracy is to optimize the equipment parameters and operational technique (sample preparation and injection) so that the true answer is obtained with a primary standard, rather than to employ empirical correction factors to correct for faulty practice (Craske, 1993). It is often of value to add an internal standard, usually an odd-chain fatty acid that does not occur in the sample, as an aid to quantification. This is especially useful if the total amount of each fatty acid, as opposed to the relative proportion, is required.

6.4.4.4 Definitive identification of fatty acid structure by mass spectrometry

GC analysis alone can be an excellent guide to the identity of fatty acids, but there are times when this may not be sufficient. Then, it may be necessary to isolate particular components by silver ion or reversed-phase chromatography (Nikolova-Damyanova, 1992; 1997) for structural analysis by chemical degradative techniques, reviewed by Sébédio (1995), or by spectroscopic means. ^{1}H- and ^{13}C-nuclear magnetic resonance spectroscopy are extremely powerful techniques for structural analysis of fatty acids, but may require more of a pure fatty acid component than is easily attainable (Gunstone, 1992; 1993). On the other hand, mass spectrometry coupled to GC requires very little material and prefractionation is only occasionally necessary (Christie, 1997). Methyl esters are not particularly good derivatives for mass spectrometry and a better approach is to prepare derivatives designed for mass spectrometry, such as the pyrrolidides, picolinyl esters or 4,4′-dimethyloxazolines (Christie, 1997; 1998).

Methyl esters are by far the most widely used derivatives for the analysis of fatty acids in general, as they are simple in structure and have good chromatographic properties. In addition, there is a wealth of data on relative retention times and how they vary with the type and position of various structural features. Location of methyl branch points or the position and type of oxygen atom (hydroxyl, epoxyl, keto) in the chain is usually possible by GC-MS, especially when standards are available for comparison. On the other hand, the most common methyl-branched fatty acids, i.e., with iso- or anteiso-methyl branches, can only be recognized from subtle changes in the intensity of some minor ions (Apon and Nicolaides, 1975). Ring structures, other than cyclopropane rings, attached to the aliphatic chain can usually be recognized, although it is never easy to define the positions with certainty.

It is usually considered that methyl ester derivatives are not suitable for locating double bonds or other centres of unsaturation, and this is certainly true for monoenoic fatty acids and most dienes, but polyenoic fatty acids often give distinctive mass spectrometric fingerprints with specific ions related to double bond position. This has not always been recognized. As examples, Figure 6.2 shows the mass spectra of methyl esters of three octadecatrienoic fatty acids (molecular weight 292). It is apparent that each spectrum is different and distinctive. The best known of these are methyl γ- and α-linolenate, and Holman and Rahm (1971) showed that the mass spectrum of each contained several ions that were indicative of double bond position. In particular, the spectrum of methyl 6,9,12-octadecatrienoate contains an ion at m/z = 150, presumed to be formed from the terminal part of the molecule by cleavage between carbons 7 and 8. The analogous ion in the mass spectrum of methyl 9,12,15-octadecatrienoate is at m/z = 108, and is formed following cleavage between carbons 10 and 11. It is now recognized that ions at m/z = 150 and 108 may be in general characteristic for methyl esters of polyunsaturated fatty acids of the (n-6) and (n-3) families, respectively (Fellenberg et al., 1987). On the other hand, while they are useful guides, it is not certain that they are always present or unique to the mass spectra of such fatty acids.

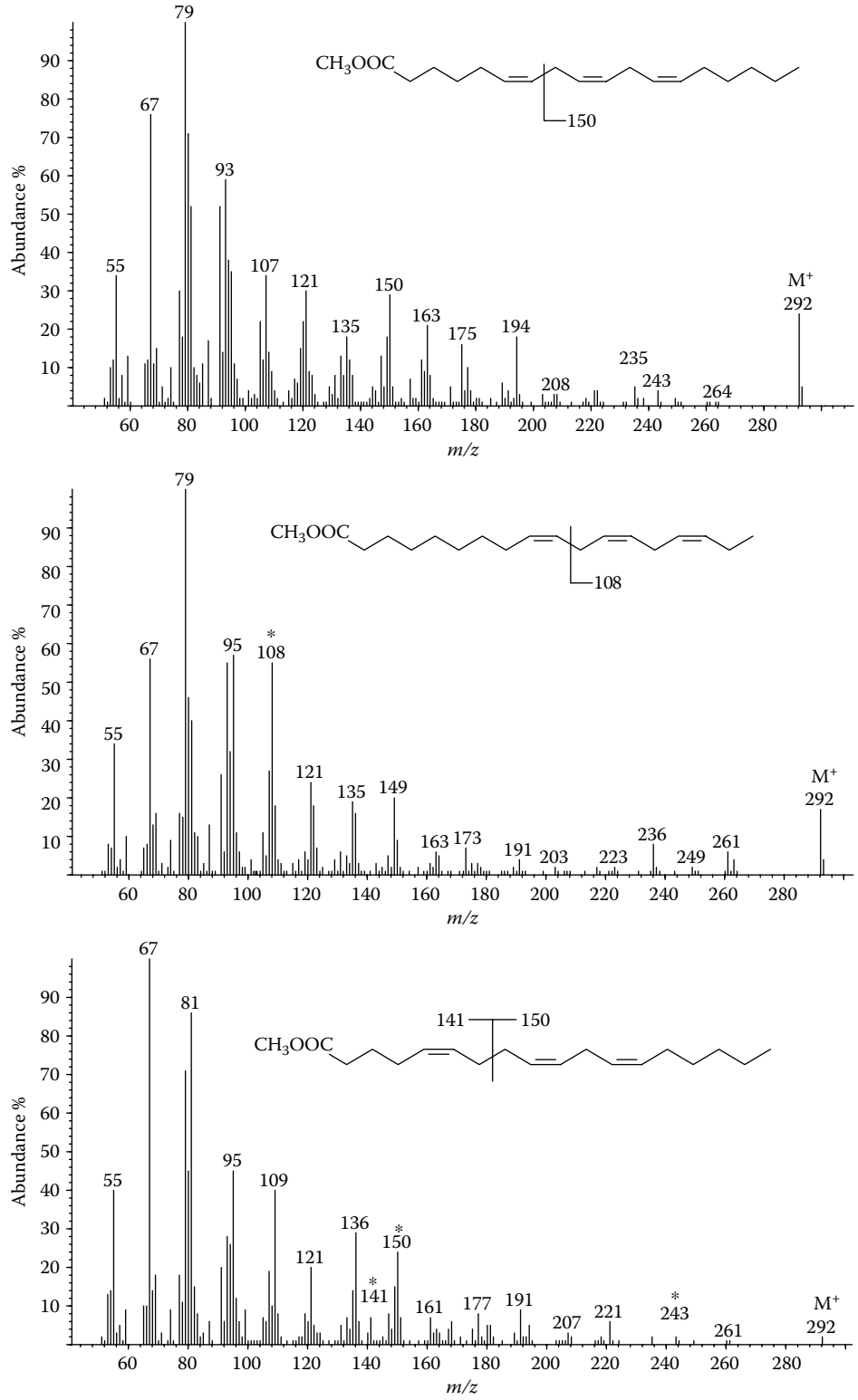


FIGURE 6.2 Mass spectra of methyl 6,9,12-octadecatrienoate or γ-linolenate (top), 9,12,15-octadecatrienoate or α-linolenate (middle), and 5,9,12-octadecatrienoate (bottom).

cis,cis,cis-Octadeca-5,9,12-trienoic acid is a common constituent of many conifer species, especially pines. The mass spectrum of its methyl ester has the characteristic ion at m/z = 150 for the n-6 family of fatty acids. In addition, it has a small ion at m/z = 141 that is presumably the corresponding fragment from the carboxyl end of the molecule. We have found an analogous ion in the mass spectra of many different methyl esters of fatty acids with

a 5,9-double bond structure from sponges, including dienes as well as polyenes (unpublished). In addition, there is a distinctive ion at m/z = 243 or $[M-49]^+$, and this is present in the spectra of other 5,9-dienoic or polyenoic esters. It is hard to see how this could arise other than by sequential loss of a methoxyl ion and water (not necessarily in this order). However, it may also occur in some other spectra.

Many different alternative derivatives have been utilized for identification of fatty acids by mass spectrometry, but the most useful contain nitrogen atoms because the ester/amide moiety rather than the alkyl chain carries the charge when the molecule is ionized. Radical-induced cleavage occurs in a relatively simple way, giving mass spectra that are usually easy to interpret in terms of the positions of functional groups, including double bonds, in the chain. Of the many derivatives that have been tested, pyrrolidides (Andersson, 1978), 4,4-dimethyloxazoline (DMOX) (Spitzer, 1997) and picolinyl ester (Harvey, 1992) derivatives have been widely used and a wealth of information is available on their mass spectrometric properties (see www.lipidlibrary.co.uk). DMOX derivatives are only slightly less volatile than methyl esters on gas chromatography, and their resolution is comparable. On the other hand, picolinyl esters require a temperature of about 50°C higher to elute and usually require specialist high-temperature cross-linked polar GC phases. Pyrrolidides have intermediate properties.

In the mass spectra of these derivatives, a saturated chain is indicated by regular gaps of 14 amu between ions due to cleavage of adjacent methylene groups and functional groups in the chain lead to a change in this pattern. For example, methyl branches are located by a gap of 28 rather than 14 amu (see below). By coincidence, pyrrolidides and DMOX derivatives of a given fatty acid have the same molecular weight, although the structures are very different. With electron-impact ionization, they give very similar mass spectra with often identical types of fragmentation although the relative abundances of the ions may vary and DMOX derivatives tend to give somewhat more intense diagnostic ions. For example, in both, the McLafferty ion is at m/z =113 and this is usually the base peak. In general, exactly the same type of fragmentation is seen (with some important exceptions described below).

The most common application for these derivatives is in the location of double bonds in unsaturated fatty acids. If a double bond is positioned between carbons n and n + 1 then a gap of 12 amu between ions corresponding to fragments containing n-1 and n carbons is usually observed for pyrrolidide and DMOX derivatives. A gap of 26 amu between ions corresponding to fragments containing n – 1 and n + 1 carbons is more reliable for picolinyl esters.

Monoenes (C_{18}) with double bonds between positions 7 and 5 follow these rules, and also give intense allylic ions corresponding to fragments containing n + 1 and n + 2 carbons. When the double bond is closer to the carboxyl group, other characteristic features are evident. Thus, DMOX and pyrrolidides derivatives with double bonds in positions 4, 5, and 6 have characteristic odd-numbered ions at m/z = 139, 153, and 167, respectively, while at pos-ition 3, m/z = 152 is the base peak and at position 2, m/z = 110 is a prominent ion. The mass spectra can become a little more difficult to interpret with increasing degree of unsaturation, and often the DMOX spectra are more informative. Taking α-linolenate as an example, for the picolinyl ester (Figure 6.3), gaps of 26 amu between m/z = 234 and 260, 274 and 300, and 314 and 340 locate the double bonds in positions 9, 12, and 15, respectively. For the DMOX derivative, gaps of 12 amu between m/z = 196 and 208, 236, and 248 and between 276 and 298 locate double bonds in positions 9, 12, and 15, respectively. The mass spectrum of the pyrrolidide derivative is very similar to that of the DMOX in this instance. In polyunsaturated acids it is sometimes useful to look for gaps of 40 amu between ions corresponding to fragments containing the double bond and the alpha-methylene group. When the position of all double bonds are not located unambiguously, the positions of the other double bonds can be inferred with reasonable confidence because most polyunsaturated double bonds are methylene-interrupted (the total number of carbons and degree of unsaturation can be determined from the molecular ion). DMOX derivatives are particularly informative for locating double bond position in conjugated dienes, such as conjugated linoleic acid (CLA). Double bond positions can be confirmed unambiguously by deuterating the double bonds and looking for gaps of 15 amu between adjacent carbons. However, the reaction is best carried out on simplified mixtures as deuterated components with the same number of carbons but different degrees of deuteration (from fatty acids of different degrees of unsaturation) will co-elute.

In general, problems of interpretation tend to arise when functional groups are adjacent to either end of the molecule. When they are close to the carboxyl group, interpretation can be assisted by reference to spectra of similar types of compound. When the functional group is near the terminal part of the molecule, picolinyl esters give the best results usually, while pyrrolidides may also be suitable. However, DMOX derivatives often give confusing results. This can be seen from Figure 6.4, which illustrates spectra of anteiso-methyl-hexadecanoate in the form of various derivatives. The picolinyl ester gives a clear and unambiguous spectrum in which the gap of 28 amu between m/z = 304 and 332 defines the position of the methyl group. The appropriate part of the spectrum of the pyrrolidide has to be magnified for clarity, but again a gap of 28 amu (m/z = 266 to 294) locates the methyl group. However, there is no such gap in the spectrum of the DMOX derivative and it is presumed that a loss of one or more methyl groups from the derivatizing moiety confounds the expected pattern.

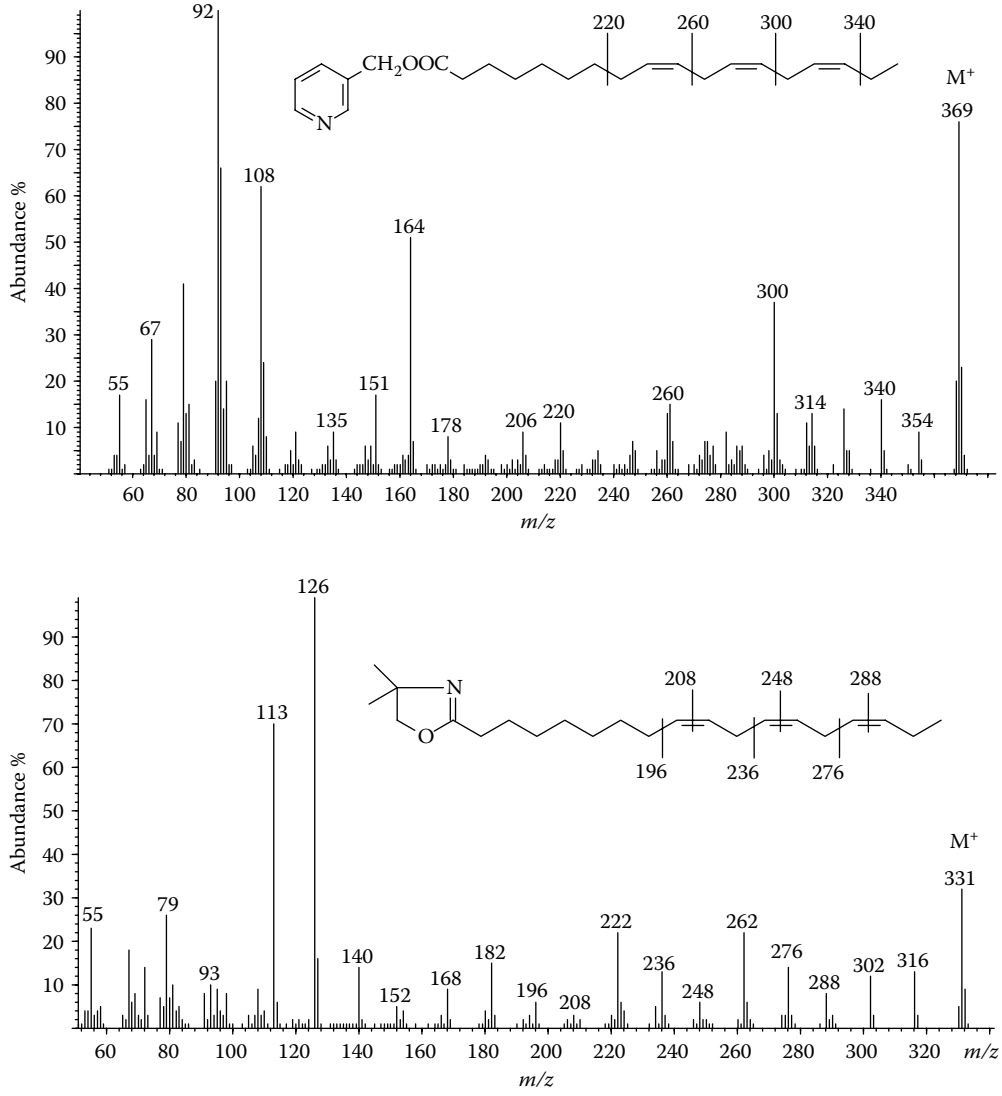


FIGURE 6.3 Mass spectra of 9,12,15-octadecatrienoate or α-linolenate in the form of the picolinyl ester (upper) and dimethyloxazoline derivative (lower).

There are many examples of characterizing the fatty acids in natural mixtures using GC-MS in the literature. However, often the mixtures are complex and it should be noted that complementary techniques, e.g., reversed-phase and silver-ion HPLC, are extremely useful for fractionating samples into simpler mixtures prior to GC-MS analysis to allow a full characterization of all fatty acids (Christie, 1997; 1978). It is always helpful when at least two derivatization techniques are employed as an aid to identification.

6.4.5 Separation and analysis of lipid classes

6.4.5.1 Preliminaries

While it is often possible to analyse a number of different lipid classes in a single analytical procedure, it is often easier technically to isolate small amounts of pure lipid classes after a preliminary fractionation kind has been carried out. Small proprietary solid-phase extraction cartridges of silica gel are convenient for the purpose, nonpolar lipids being recovered by elution with hexane-diethyl ether (1:1 by volume), while the complex lipids (phospholipids and glycolipids) are recovered by elution first with methanol and then with chloroform–methanol–water (3:5:2 by volume) (Bitman et al., 1984). Alternatively, aminopropyl-bonded phase cartridges (Bond-Elut™) have been much used for a more comprehensive fractionation, though particular care is required to recover the acidic lipids quantitatively. Separate fractions containing simple lipids, free fatty acids, glycolipids, zwitterionic phospholipids, and acidic phospholipids can be recovered by a simple elution sequence (Christie et al., 1998) (Table 6.3).

Sphingolipids, and especially the complex glycosphingolipids, are best analysed separately and can be isolated by a similar method to the last (Bodennec et al., 2000). More comprehensive separations of glycosphingolipids

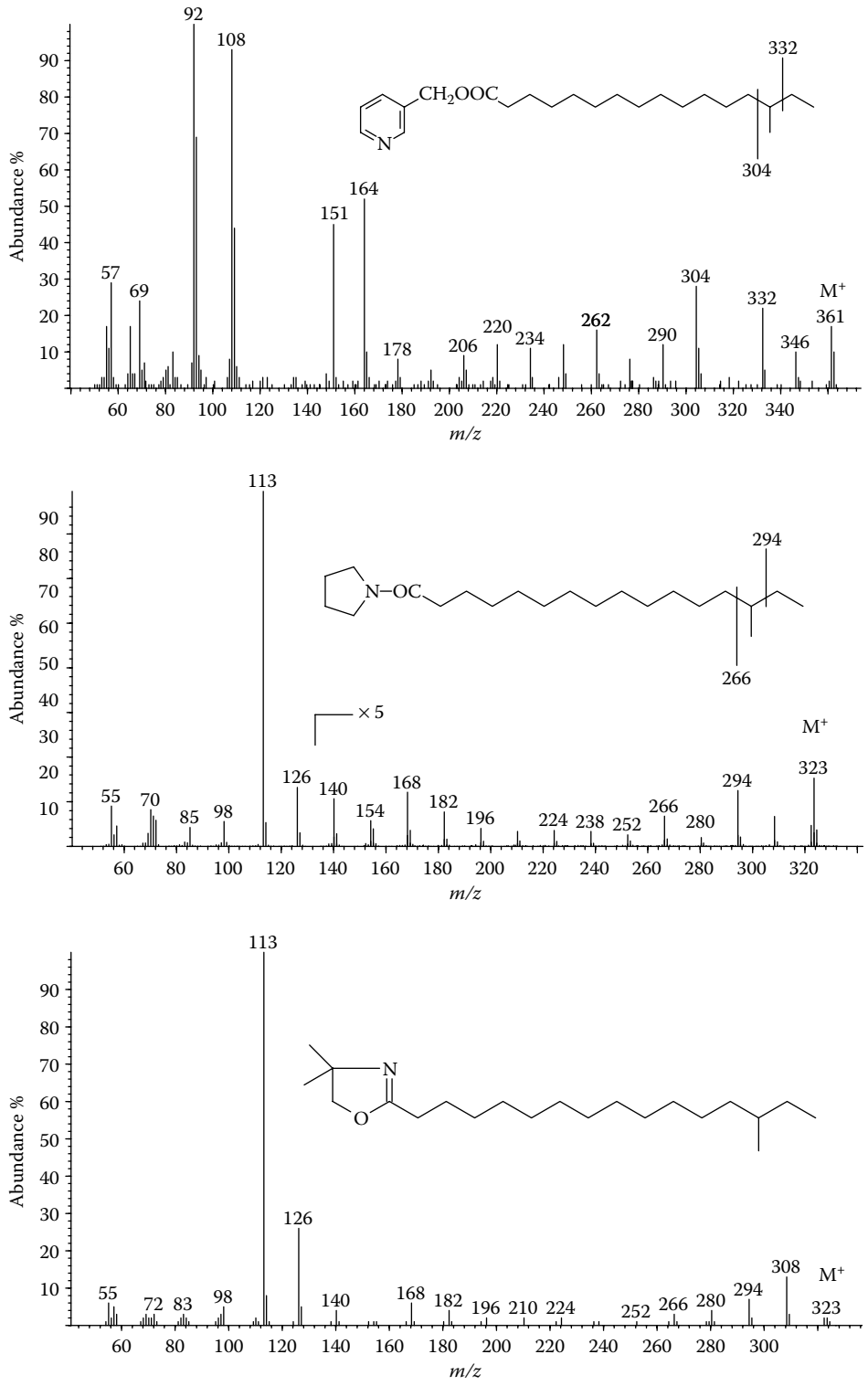


FIGURE 6.4 Mass spectra of 14-methyl-hexadecanoate (anteiso-methyl) as the picolinyl ester (top), pyrrolidide (middle), and dimethyloxazoline derivatives (bottom).

can be obtained by chromatography following derivatization or by larger-scale, ion-exchange chromatography. It is also possible to eliminate any nonsphingolipid components from a concentrate of these compounds by mild alkaline transesterification, by means of which any lipids containing O-acyl fatty acids are converted to methyl esters and water-soluble products, while the glycosphingolipids and sphingomyelin (which contain amide-bound fatty acids) are not affected (Wells and Ditmer, 1965). Hakomori (1983) has reviewed such procedures.

TABLE 6.3 Preliminary separation of lipid classes

An Isolute™ NH_2 (500 mg) (or equivalent) cartridge is conditioned by elution with iso-hexane (2 ml) and the lipid extract (up to 4 mg lipid or 2 mg complex lipids) is applied to the column in the minimum volume of chloroform. All solvents are allowed to run under gravity in the following sequence.

Simple lipids are eluted with diethyl ether (10 ml).

Free fatty acids can be eluted with diethyl ether-acetic acid (98:2, v/v; 5 ml) (optional, as small amounts of glycolipids may co-elute).

Sterol glycosides, and mono- and digalactosyldiacylglycerols are eluted with acetone-pyridine (1:1, 10 ml).

Phosphatidylethanolamine and the choline-containing lipids are eluted with methanol (20 ml).

Acidic phospholipids (phosphatidylglycerol, phosphatidylinositol and phosphatidylserine) together with sulfoquinovosyldiacylglycerol are recovered by elution with chloroform–methanol–28% aqueous ammonia containing 0.05M ammonium acetate (4:1:0.1 by volume; 10 ml). In this instance, the lipids are recovered by adding methanol (2 ml), water (3 ml), and acetic acid (0.12 ml). Two phases form. The upper aqueous phase is removed by means of a Pasteur pipette and discarded; the lower contains the lipids.

The solvents from each fraction are evaporated by means of a rotary evaporator or in a gentle stream of nitrogen at a temperature no greater than 50°C. Lipid components are stored in iso-hexane (with BHT) at 4°C.

6.4.5.2 Detectors for HPLC

Before considering specific methods for separating lipid classes by HPLC, it is advisable to consider the properties of the various detection systems available (Table 6.4.), since these determine to a large extent the nature of the solvent systems that can be used. They have been reviewed by Christie (1992, 2003).

Spectrophotometric detectors in the UV-visible range are probably by far the most widely used detectors for HPLC in general. Much the best response is given with compounds containing conjugated double bond systems and aromatic rings, but such substituent groups are found only rarely in natural lipids. Some seed oils contain fatty acids with conjugated double bond systems and these are frequently present in lipids subjected to chemical or enzymatic hydroperoxidation. In addition to mere detection, second-derivative UV spectroscopy has proved of value for the identification of configurational isomers of conjugated dienes. In some instances, it has proved possible to convert lipids to derivatives that absorb strongly in the UV range. For example, fatty acids have been converted to aromatic esters (e.g., phenacyl or naphthacyl), the sugar moieties of glycolipids have been benzoylated and diacylglycerols derived from phospholipids have been esterified with aromatic acid derivatives for analysis. Good quantification is often possible, as there is then a linear molar response to the derivatizing moiety. Most lipids exhibit a weak absorbance in the range 200 to 210 nm and that is the result of the presence of isolated double bonds predominantly, although other functional groups, such as carbonyl, carboxyl, phosphate, amino, and quaternary ammonium, have some effect; it is sometimes termed "end absorption." There are a number of disadvantages to using UV detection at such wavelengths, however. Many of the solvents of proven value in the chromatography of lipids, such as chloroform, acetone, ethyl acetate, or toluene, absorb strongly between 200 and 220 nm and so cannot be used. Because small differences in the degree of unsaturation of each component can make a big difference to the response, quantification is not at all easy, and saturated lipids might be overlooked. However, direct quantification has been achieved by determining the apparent extinction coefficient for each component in standard mixtures very similar to those to be analysed.

If the samples are likely to be variable in composition or indeed if the composition is not determined, it is necessary to collect the peaks for estimation by appropriate micromethods. Many analysts have followed this approach, using phosphorus analysis for phospholipids, for example. GC analysis of the methyl ester derivatives of fatty acid constituents, prepared from fractions, with an added internal standard permits identification and quantification simultaneously and has wider applicability.

TABLE 6.4 Properties of different detectors

Detector	Advantages	Disadvantages
UV	Relatively inexpensive and widely available	Few natural lipids have suitable chromophores Detection at ~210 nm where isolated double bonds absorb is sensitive to the nature of the fatty acids Gradient elution rarely possible Many solvents not suitable as they absorb at the wavelengths of interest.
RI	Linear response to most lipids	Not very sensitive Gradient elution not possible Sensitive to minor fluctuations in ambient temperature
ELSD	Sensitive response to most lipids Most solvents can be used, and with complex gradients	Response is not rectilinear Requires careful calibration for each lipid class Inorganic ions cannot be used in mobile phase

Refractive index (RI) detectors, which function by monitoring continuously the difference in refractive index between the eluent and the pure mobile phase, are universal in their scope and can be used with any solute for which the refractive index is different from that of the mobile phase. They are at their best with isocratic elution and, unfortunately are very sensitive to fluctuations in ambient temperature. For quantitative analysis of lipids, the consensus appears to be that acceptable results can be obtained by equating detector response directly with the mass of components, on the assumption that differences in the chain-length or degree of unsaturation of the fatty acyl moieties will have little or no effect on the refractive indices of molecular species. Most analysts have used this simple approach. On the other hand, a few systematic studies have demonstrated that careful calibration with pure standards can improve the accuracy. Only for size-exclusion chromatography do RI detectors appear to be especially favoured, although they are also well suited to the isolation of particular lipid components by preparative HPLC.

With evaporative light-scattering detectors, the solvent emerging from the end of the HPLC column is evaporated in a stream of air or nitrogen in a heating chamber; the solute does not evaporate, but is nebulized and passes in the form of minute droplets through a light beam, which is reflected and refracted. The amount of scattered light is measured and this bears a relationship to the amount of material in the eluent. These detectors can be considered as universal in their applicability, in that they will respond to any solute that does not evaporate before passing through the light beam. Most solvents, including ketones, esters, and chlorinated and aromatic compounds, can be used in complex gradients; up to 20% of water and small amounts of ionic species are also permissible. They give excellent results under gradient elution conditions and are simple and rugged in use, with sensitivity better than that of refractive index detection. The detector is destructive in that the sample is lost, but it is possible to insert a stream splitter between the end of the column and the detector to divert much of the sample to a collection device.

Sadly, the evaporative light-scattering detector does not give a rectilinear response to analyte concentrations. In fact, detector response increases sigmoidally with increasing sample concentration in a manner that can be predicted by changes in the size distribution of particles in the aerosol. Thus, at low solute concentrations, the solute particles scatter light to a proportionately lesser extent. As the diameters of the droplets begin to approach the wavelength of light, they no longer affect its passage and the response falls off rapidly (Mourey and Oppenheimer, 1984). Also, different lipid classes give very different responses with evaporative light-scattering detection, although chain-length and degree of unsaturation of the acyl constituents do not have a significant effect. With careful calibration, data obtained in lipid analyses of this kind should be at least as reliable in terms of accuracy and reproducibility as those from most other analytical methods in use with intact lipids.

6.4.5.3 Separation of simple lipid classes

Although TLC is sometimes considered old-fashioned or "low-tech," it is effective and gives excellent separations of simple lipid classes. Many solvent systems have been used for the purpose with silica gel G as adsorbent in a single dimension. Those used most frequently contain hexane, diethyl ether and acetic (or formic) acid in various proportions. For example, with these solvents in the ratio 80:20:2 (by vol.), the separation illustrated schematically in Figure 6.5 is achieved in which most of the common simple lipids are separated — cholesterol esters near the solvent front followed by triacylglycerols, free fatty acids, cholesterol, diacylglycerols (1,3- and 1,2-) and monoacylglycerols. Phospholipids and other complex lipids remain at the origin and are determined as a single class — a useful property of TLC methods.

A 0.1% (w/v) solution of 2′,7′-dichlorofluorescein in 95% methanol is most frequently used for detection and causes lipids to show up as yellow spots under UV light. The plates are sprayed in a uniform manner until just visibly moist. In recent years, a 0.05% solution of primulin dye (a 1:100 dilution of a 5% aqueous stock into acetone–water, 8:2, v/v) appears to be gaining popularity for the purpose (Skipski, 1975). These sprays are nondestructive and the lipids can be recovered from the plates for further analysis. As all lipids contain fatty acids, it is possible to determine the amounts of lipid classes by quantifying the amounts of the fatty acids that they contain. Typically, the fatty acid components of each lipid are converted to methyl esters in the presence of a known amount of the ester of an acid that does not occur naturally in the sample (e.g., an odd-chain compound, such as methyl heptadecanoate (17:0)),

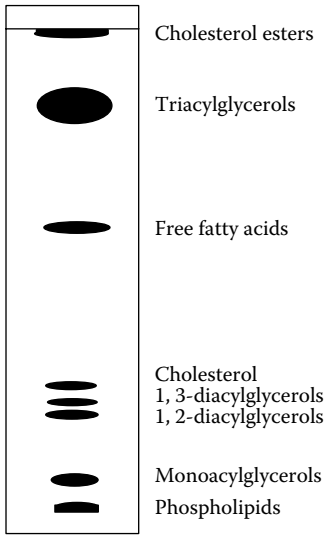


FIGURE 6.5 Schematic TLC separation of simple lipids on silica gel G. Developing solvent: hexane-diethyl ether-formic acid (80:20:2 by vol.).

and this serves as an internal standard. Transesterification can be performed on pure lipids recovered from TLC plates, or it can be carried out in the presence of the adsorbent. By means of gas chromatography, the total amount of the fatty acids relative to that of the standard is obtained by dividing the sum of the areas of the relevant peaks on the recorder trace by that of the internal standard. It has the additional merit that both the fatty acid compositions and the amounts of the lipid classes in a given mixture are determined in a single analysis. However, it is necessary to allow for the weight of nonfatty acid material (e.g., glycerol or cholesterol) in each lipid class by multiplying each result by appropriate arithmetic factors. Problems arise also with complex lipids that contain variable amounts of ether-linked alkyl moieties. To avoid such problems, many analysts simply record the total amount of fatty acids in each lipid class.

Alternatively, the plates may be sprayed with a solution of 50% sulfuric acid, or similar reagent and the lipids made visible as a black deposit of carbon by heating the plates at 180°C for an hour or so. Although such charring procedures have the obvious disadvantage that they completely destroy the lipids, they are very sensitive, and as little as 1 μg of lipid can be detected by this means. Sterols give a red-purple colour in a few minutes with charring reagents before blackening and this is a useful diagnostic guide. Of course, charring procedures cannot be used with commercial plastic-backed plates. Procedures of this kind are often used to quantify components separated by TLC, when the amount of charred material is measured by means of a scanning photodensitometer, which is capable of reasonable precision after suitable calibration. The procedure has a number of disadvantages; the sample is destroyed, the yield of carbon is variable and affected by degree of unsaturation, authentic standards with a similar fatty acid composition to the analytes are necessary for calibration, but are not always available, and constant updating of the calibration is necessary.

While a number of HPLC methods have been described for the separation of simple lipid classes, they do not appear to be in widespread use. One reason is that it is not possible to apply total lipid extracts to columns, as complex lipids would accumulate, causing a build-up of pressure and eventually blocking them. Simple lipid fractions prepared as in Section 6.4.5.1 can be used. Another reason why HPLC is not employed more often is because the detectors available are not ideal for the purpose.

A refractive index (RI) detector was utilized with a column of silica gel and isocratic elution with iso-octane-tetrahydrofuran-formic acid (90:10:0.5 by volume) to separate most of the common simple lipid classes encountered in animal tissue extracts, such as those of liver (Greenspan and Schroeder, 1982). Cholesterol esters, triacylglycerols and cholesterol all gave symmetrical peaks. Although an attempt was made to use the technique quantitatively, the results were not convincing, as negative solvent peaks tended to interfere and some variation in response with fatty acid composition was observed. However, others showed that this elution system can give acceptable accuracy with relatively simple mixtures, such as those obtained in commerce by glycerolysis of seed oils, if an internal standard (ricinoleic acid) is used and a careful calibration is performed (Ritchie and Jee, 1985). The detector response was found to be rectilinear up to as much as 1 mg of glyceride.

Relatively few applications of UV spectrophotometry at 200 to 210 nm in the separation of simple lipids have been described, possibly because this form of detection is of limited value for quantification purposes. Some analysts have used columns of silica gel, but better results appear to have been obtained with cyanopropyl-bonded stationary phases. One potential advantage is that unsaturated terpenoid "lipids," such as retinol, vitamin E, dolichol, ubiquinone, and their esterified forms show up prominently (Palmer et al., 1984).

Evaporative light-scattering detection has been used extensively as part of methods to separate both simple and complex lipids in a single step (see Section 6.5.5 below). However, it has been used also for simple lipids alone. Both silica gel and diol stationary phases have been used for the purpose, usually with a gradient of isopropanol (or with 0.1% acetic acid added) into hexane as the mobile phase. However, some of the more convincing separations have been with bonded nitrile phases. For example, Foglia and Jones (1997) demonstrated a comprehensive separation of cholesterol esters, methyl esters, tri-, di- and monoacylglycerols, cholesterol, and free fatty acids. In this instance, a bonded nitrile column (Phenomenex™) was employed with a gradient of methyl tert-butyl ether and acetic acid in hexane. The authors calibrated the detector carefully for each of the analytes with suitable standards and obtained the typical curvilinear weight-response curves for evaporative light-scattering detection.

6.4.5.4 Separation of phospholipid classes

Preparative-scale procedures involving adsorption and ion-exchange chromatography for isolation of phospholipid fractions have been described (Christie, 2003), but only the analytical separations are described here. Phospholipid fractions prepared as described in section 6.4.5.1 are usually analysed, but total extracts may also be used, as simple lipids tend to migrate with the solvent front, so do not interfere with analyses.

For many years, TLC was by far the most widely used method for the analytical scale separation of individual complex lipid classes, and with little modification and a minimum loss of resolution it can be used for small-scale preparative purposes. Although HPLC has now made considerable inroads, TLC retains many advantages. For example, TLC offers considerable versatility and precision in lipid analysis with relatively low capital costs. In the analysis of complex lipids, it is a simple matter to use specific spray reagents to detect particular functional

groups in lipids separated by TLC, e.g. ninhydrin for free amino groups as in phosphatidylethanolamine and phosphatidylserine, but this is not possible with HPLC. While highly polar lipids, such as the phosphoinositides, are not always well resolved with TLC, they are at least detected; this cannot be guaranteed with HPLC.

Commercial precoated TLC plates, especially, give reproducible results in analyses of complex lipids. Phospholipids and glycolipids separated by TLC can be recovered, after they have been detected by an appropriate nondestructive method, by scraping the adsorbent band into a small chromatographic column or sintered disc funnel and eluting with chloroform-methanol-water (5:5:1, by volume). One-dimensional TLC procedures are preferred for rapid group separations or for small-scale preparative purposes; two-dimensional TLC procedures will resolve the maximum number of distinct components.

Innumerable combinations of mobile-phase solvents have been described for the separation of phospholipids over the years, many of which should be re-evaluated with modern adsorbents. However, methyl acetate–isopropanol–chloroform–methanol–0.25% aqueous potassium chloride (25:25:25:10:9, by volume) enables separation of the important phospholipids in animal tissues, including all the choline-containing phospholipids, phosphatidylserine, phosphatidylinositol and phosphatidylethanolamine can be separated from each other (Vitiello and Zanetta, 1978). Phosphatidic acid and phosphatidylglycerol tend to run together just behind the phosphatidylethanolamine spot.

Similarly, many two-dimensional systems have been devised that are suited to the analysis of animal or plant lipids. The most successful separations are achieved when contrasting solvents are used for development in each direction; for example, a neutral or basic solvent mixture in the first direction may be followed by development with an acidic solvent mixture in the second direction, or the second system may contain acetone to retard the migration of phospholipids relative to glycolipids, with which they might otherwise overlap. As with one-dimensional TLC, many published systems represent minor adjustments only of earlier ones to suit local conditions (e.g., of temperature or humidity). As an example, plant phospholipids and glycosyldiacylglycerols can be separated by first developing the plate in chloroform–methanol–water (75:25:2.5, by volume) in the first direction. After allowing sufficient time for drying, the plate is developed, at right angles to the first development, in chloroform–methanol–acetic acid–water (80:9:12:2, by volume), as illustrated in Figure 6.6. (Rouser et al., 1967).

Complex lipids can be located on TLC plates by either the nonspecific destructive or the nondestructive reagents described in Section 6.4.5.3. If the latter are used, care must be taken to remove polar solvents, such as water or glacial acetic acid, otherwise lipid spots will be obscured.

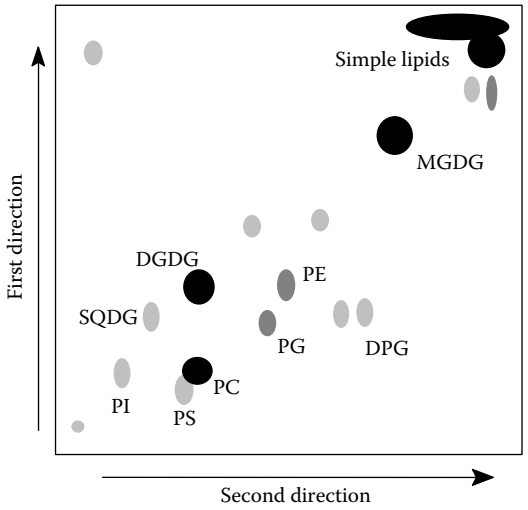


FIGURE 6.6 Schematic two-dimensional TLC separations of complex lipids from plant tissues on silica gel. Solvent systems: first direction, chloroform–methanol–water (75:25:2.5, by volume), and second direction, chloroform–methanol–acetic acid–water (80:9:12:2, by volume). Abbreviations: DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PG, phosphatidylglycerol; MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; SQDG, sulfoquinovosyldiacyglycerol.

This is particularly important if the fatty acid components are required for further analysis and, in this instance, solvents should be evaporated in a vacuum oven at close to room temperature or by blowing nitrogen on to the surface of the plate. Excess acetic acid can be removed by neutralization with an ammonia spray. Such precautions minimize the risk of autoxidation. Phospholipids can be quantified by phosphorus determination, or by analysis of the fatty acid components with an internal standard as discussed in the previous section.

HPLC methods are now used routinely in large numbers of laboratories for the analysis of phospholipids, although there is room still for improvement, especially in the analysis of acidic phospholipids. The topic has been comprehensively reviewed (Christie, 1996). HPLC is much more expensive than TLC in terms of both equipment and running costs, but it can be automated to a considerable degree and gives much cleaner fractions in micro-preparative applications. There is no simple recipe that can be recommended unequivocally, as the method of choice will depend to a large degree on the nature of the equipment available to the analyst. For example, when UV spectrophotometry is the only type of detector available, isocratic elution may be essential. Gradient elution techniques afford possibilities for improved resolution, but an evaporative light-scattering detector is then required. HPLC in the adsorption mode must be used for class separations of phospholipids and, in most published work, silica gel has been the adsorbent although chemically bonded stationary phases are being used increasingly. The evaporative

light-scattering detector can also be employed for analyses of this kind with complicated gradients in the mobile phase to improve resolution and this is discussed in the next section.

Two solvent systems transparent at UV wavelengths in the range of 200 to 210 nm were developed first for phospholipid separations, and these still form the basis of most published methods today, i.e., mixtures of hexane–isopropanol–water (Geurts van Kessel et al., 1977) and acetonitrile–water (sometimes with added methanol) (Jungalwala et al., 1976) (Table 6.5). With the latter system, phosphatidylethanolamine elutes before phosphatidylcholine and then sphingomyelin and, indeed all the choline-containing phospholipids tend to be well resolved. A special virtue is that acidic lipids, like phosphatidylserine and phosphatidylinositol, are eluted with relative ease ahead of phosphatidylethanolamine. With mobile phases based on hexane–isopropanol–water, phosphatidylethanolamine elutes before phosphatidylcholine, but the other choline-containing lipids, such as sphingomyelin and lysophosphatidylcholine, tend to be less well resolved. The acidic lipids, such as phosphatidylinositol, phosphatidylserine, and phosphatidic acid, are separated from each other, but in this instance they emerge between phosphatidylethanolamine and phosphatidylcholine. This system has proved easier to adapt to simultaneous separation of simple lipids and glycolipids than has that based on acetonitrile.

In addition, it is necessary to be aware of the fact that phospholipids are ionic molecules and may require an ionic species in solution if they are to elute as sharp peaks. Sulfuric and phosphoric acids have often been used, but apart from dissolving HPLC equipment they will bring about complete destruction of any plasmalogens present. Similarly, ammonia can have deleterious effects on packing materials by dissolving silica or hydrolysing chemically bonded phases. Organic buffers are often preferred as counter ions in the mobile phase, as it is still possible to use evaporative light-scattering detection.

TABLE 6.5 Phospholipid separations with two different solvent systems

The order of elution of phospholipids in mobile phases based on acetonitrile and propan-2-ol.*

Acetonitrile-based	Propan-2-ol-based
phosphatidic acid	cardiolipin
cardiolipin	phosphatidylethanolamine
phosphatidylinositol	phosphatidylinositol
phosphatidylserine	phosphatidylserine
phosphatidylethanolamine	phosphatidic acid
phosphatidylcholine	phosphatidylcholine
sphingomyelin	sphingomyelin
lysophosphatidylcholine	lysophosphatidylcholine

*Note there may be some modification to the order given (especially of cardiolipin), depending on the nature of other solvents and of any ionic species in the mobile phase.

Isocratic elution methods have the merit of employing simple pumping systems, so reducing the requirements in terms of costly equipment. UV spectrophotometric detectors are by far the most common in laboratories, and they are usually set at 205 nm where isolated double bonds absorb. Of the large number of published procedures for phospholipid analysis of this kind, that of Patton et al. (1982) appears particularly convincing, and has been adopted by many others (Figure 6.7). Their mobile phase is based on hexane–isopropanol–water, but contains a phosphate buffer, ethanol and acetic acid also, while silica gel is the stationary phase. In the

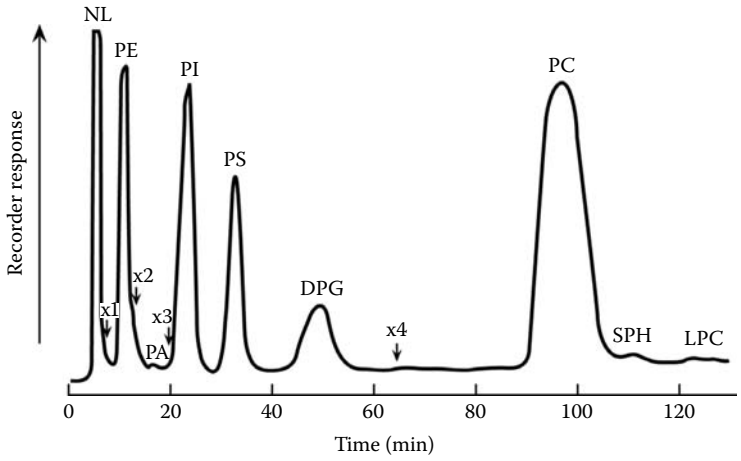


FIGURE 6.7 Isocratic elution of rat liver phospholipids from a column of silica gel with hexane–isopropanol–25 mM phosphate buffer–ethanol–acetic acid (367:490:62:100:0.6 by volume) as mobile phase at a flow rate of 0.5 mL/min for the first 60 min, then of 1 mL/min, and with spectrophotometric detection at 205 nm. X1, X2, X3, and X4 are unknown lipids. Abbreviations: NL, neutral lipids; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PA, phosphatidic acid; PI, phosphatidylinositol; PS, phosphatidylserine; PC, phosphatidylcholine; SPH, sphingomyelin; LPC, lysophosphatidylcholine. (Adapted from Patton, G.M. et al. (1982) *J. Lipid Res.*, 23, 190. With permission.)

original paper, as each component was eluted, it was collected, washed to remove the buffer and determined by phosphorus assay. In addition, the fatty acid composition of each lipid class was obtained with relative ease by gas chromatographic analysis after *trans* methylation, though it should also have been possible to add an internal standard for quantification purposes. Direct quantification from the response of the UV detector has been attempted by a number of analysts, but this is dependent on the degree of unsaturation of each lipid class. Very careful calibration is essential with standards identical to the lipids in the samples, but rectilinear responses can be obtained for each lipid. Such a method may be of value for routine analysis of very similar samples.

6.4.5.5 Simultaneous separation of simple and complex lipid classes

Although it is important to be able to analyse phospholipid classes *per se*, it is even more of a technical challenge to accomplish simultaneous separation of both simple and complex lipid classes in a single chromatographic run. At the extremes of polarity, cholesterol esters are almost hydrocarbon-like while lysophospholipids are partly soluble in water and there is a broad spectrum of lipids with differing properties in between. However, useful separations are possible with the aid of an evaporative light-scattering detector (ELSD) and a ternary gradient pumping system (Christie, 1985). When solvents are selected for the mobile phase, the choice is constrained by the need for sufficient volatility for evaporation in the detector under conditions that do not cause evaporation of the solute. Usually, a gradient is selected that starts with hexane to separate the lipids of low polarity and ends with a solvent containing water to elute the phospholipids; a solvent of medium polarity is then needed to mediate the transfer from one extreme to the other and mixtures based on isopropanol (with added chloroform to improve the selectivity of the separation) give satisfactory results. Finally, a gradient is generated in the reverse direction to remove most of the bound water and to re-equilibrate the column prior to the next analysis.

Plant lipids are a special challenge because of their content of glycolipids, but it has proved possible to resolve all the important glycolipid classes as well as the simple lipids and phospholipids in a single chromatographic run. Instead of silica, a new stationary phase that is proving especially useful is chemically similar to diol and silica gel in its properties and is manufactured by polymerizing and cross-linking vinyl alcohol to silica gel, i.e., PVA Sil™ (YMC Co., Japan). The whole surface is covered and deactivated so that the mobile phase and analytes interact with a uniform layer of hydroxyl groups only. Again, a ternary elution gradient scheme is necessary with isohexane-methyltertbutyl ether (98:2, v/v), isopro-panol–acetonitrile–chloroform–acetic acid (84:8:8:0.025 by volume) and isopropanol–water–triethylamine (50:50:0.2 by volume) as the three components, and simple lipids, glycolipids and phospholipids are eluted sequentially under mild conditions (Christie et al., 1998). The separation is illustrated in Figure 6.8.

When evaporative light-scattering detectors are used directly in quantitative analysis, it is necessary to work out the optimum conditions for the desired separations first and then carry out a calibration with lipid standards that are as close as possible in composition to the

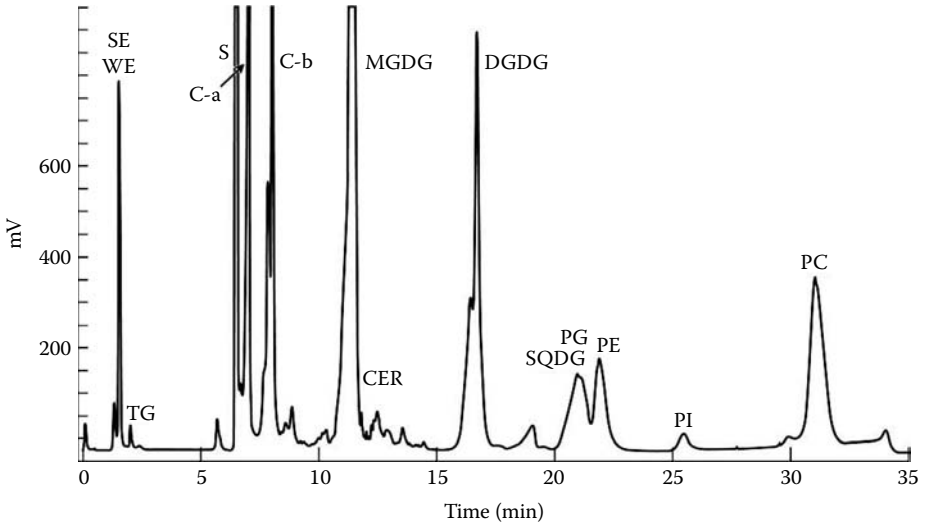


FIGURE 6.8 Separation of lipid classes from leaves of a single plant of *Arabidopsis thaliana* by HPLC on a YMC-PVA-Sil™ column (chromatography conditions as described in the text). Abbreviations: WE, wax esters; SE, sterol esters; TG, triacylglycerol; C-a, chlorophyll a; C-b, chlorophyll b; SG, sterylglycosides; MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; CER, cerebroside; PG, phosphatidylglycerol; SQDG, sulfoquinovosyldiacylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol, PS, phosphatidylserine; PC, phosphatidylcholine.

samples to be analysed. The operational parameters for the instrument, such as gas pressure and flow rate, evaporator temperature and attenuation, must also be standardised rigorously. Of course, very different results are obtained with different commercial instruments. If the elution conditions or detector settings have to be changed later for any reason, a tedious re-calibration may be necessary. The response differs for each lipid class and is rectilinear over a limited concentration range only. A plot of detector response vs. amount of lipid is sigmoidal, but a straight line is obtained when the logarithm of detector response is plotted against the logarithm of the amount. With careful calibration, the data obtained in lipid analyses of this kind appear to be at least as accurate and reproducible as those from any other analytical method in use for lipids. An internal standard, such as phosphatidyl-*N,N*-dimethylethanolamine, can be utilized to improve direct quantification with the ELSD.

Many lipid analysts have used an ELSD and columns of silica gel to separate phospholipid classes in the absence of simple lipids. One practical system for routine analysis uses a diol column and a gradient of hexane–isopropanol–acetic acid to water–isopropanol–acetic acid (Herslöf et al., 1990; Kaufmann, 1995). With this, it is interesting that acidic lipids, such as phosphatidylinositol and phosphatidylserine, elute after phosphatidylcholine. This procedure is under consideration as a standard approved method for "plant lecithins." The PVA-Sil™ column is also being used increasingly for phospholipid separations *per se* (cf. Deschamps et al., 2001).

A great deal has been accomplished by separating natural lipid mixtures according to molecular weight by high-temperature GC, principally in the laboratory of Kuksis (Kuksis et al., 1990; 1993). In brief, the lipids are first digested with phospholipase C, which converts phosphatidylcholine, lysophosphatidylcholine, and sphingomyelin to diacylglycerols, monoacylglycerols, and ceramides, respectively. The hydrolysis products are converted to the trimethylsilyl (TMS) ether (or related) derivatives, while the cholesterol and free fatty acids also react to form TMS ether and ester derivatives, respectively. Tridecanoin is added as an internal standard for quantification purposes, and the mixture is subjected to GC separation over a large temperature range so that as many as possible of the components are separated. The absolute amounts of the various lipid classes are easily determined, while the proportions of the molecular species give an indication of the chain-length distributions of the fatty acid constituents.

Although such methodology could in theory be applied to any tissue, most work has been done on human plasma lipids and related body fluids such as lymph, as rapid screening procedures here can lead to the diagnosis of disorders of lipid metabolism and can assist in monitoring the effects of clinical therapy. For these purposes, a partially resolved lipid profile may often contain sufficient information, provided that enough data are available on normal and diseased states to enable significant comparisons to be made. In historical terms, the development of the methodology has followed closely behind that for the separation of intact triacylglycerols since these inevitably must be resolved if the procedure is to be of value. Capillary columns of fused silica with polarizable stationary phases are preferred. Improvements in computerised data handling have contributed greatly to applications involving routine screening, but careful calibration is necessary, especially for the components of highest molecular weight, the triacylglycerols.

6.4.5.6 Separation of sphingolipid classes

Sphingomyelin is best analysed with other phospholipids. Analyses of the glycosphingolipids are considered here. These lipids are essential participants in a number of vital processes in living tissues, as they perform a definitive function in the immunogenicity and antigenicity of cells, and they are involved in cellular interactions, differentiation and oncogenesis. The nature of the carbohydrate residue is especially important in this regard, but a further distinctive structural feature of sphingolipids, including both the glycosphingolipids and sphingomyelin, is the presence of long-chain aliphatic bases (sphingoid bases) linked by amide bonds to long-chain fatty acids, which are rather different in composition from those found in the glycerolipids; these are also important for biological activity. Because of the complexity of the carbohydrate residue, analysis of such compounds has become a distinct discipline in its own right. TLC has been used most often for the purpose and HP-TLC especially could be considered to have set the standard. It has the advantage that immunological tests can be applied directly to the TLC plate. However, HPLC procedures are becoming more important.

Procedures for TLC separation of neutral glycosphingolipids into classes differing in the number and type of hexose units have been reviewed elsewhere (Schnaar and Needham, 1994; van Echten-Deckert, 2000). Commercial precoated HP-TLC plates, with finer grades of adsorbents, are preferred for most analytical applications. Before use, they should be pre-run in the solvent to be used for the separation and then dried at 125°C for 15 min and cooled and stored in a sealed dry box. Chloroform–methanol–water mixtures varying in proportions from 70:30:4 to 50:40:10 (by volume), depending on the complexity and polarity of the sample, are usually recommended for neutral glycolipids. As a good practical compromise, these solvents in the ratio 60:35:8 can be tried. Ionic species must be added to the mobile phase when gangliosides are present, however. In general, glycosphingolipids with one to four hexose units are clearly separated, as are species differing in the nature of the hexose unit (glucose vs. galactose). Some bands may split into two, according to the presence of normal and 2-hydroxy fatty acyl constituents.

There have been two general approaches to the separation and analysis of glycosphingolipids by means of HPLC, each having its devotees and being suited to particular purposes, i.e., to subject them to chromatography in the native form or to convert them to nonpolar UV-absorbing derivatives, such as the benzoates, prior to fractionation. These have also been reviewed elsewhere (Christie, 2003; McCluer et al., 1989). Improved methods are increasingly being sought for the chromatographic isolation of glycosphingolipids in the native state, in part to reduce the number of steps and to minimize possible losses or alterations during derivatization, but mainly to permit direct investigations of the antigenicity of specific components, or for physical chemical studies of their interactions with cellular membranes. Adsorption chromatography with silica gel has generally been favoured, but bonded phases have found some applications. As with other aspects of HPLC analysis of lipids, the choice of solvents for the mobile phase has frequently been dependent upon the availability of a particular detector in a laboratory.

After conversion to perbenzoyl derivatives, glycosphingolipids are much less polar and can be eluted with nonaqueous mobile phases. More importantly, they can be detected and quantified spectrophotometrically with high sensitivity and specificity by their absorbance at 230 nm. If an appropriate benzoylation method is used, they can be restored to their native state by alkali-catalysed transesterification.

6.4.6 Separation and analysis of molecular species of lipids

6.4.6.1 The nature of the problem

Each lipid class in a tissue exists in nature as a complex mixture of related components in which the composition of the aliphatic residues varies from one molecule to the next. Sometimes, as in cholesterol esters, only the single fatty acid component will change. On the other hand in triacylglycerols, each of the three positions in the molecules may contain a different fatty acid. Both the long-chain base and fatty acid constituents of sphingolipids can be variable and can exist in distinctive combinations. For a complete structural analysis of a lipid, it, therefore, is necessary to separate it into molecular species, i.e., into groups of molecules with single specific alkyl or acyl moieties (fatty acids, alcohols, alkyl ethers, etc.) in all the relevant portions of the molecule. With lipids that contain only one aliphatic residue, this can frequently be accomplished without difficulty. When there are two aliphatic residues, the task is much more difficult, but is not impossible. While triacylglycerols can be subjected to some considerable molecular simplifica-tion, it is not yet feasible technically to obtain single species, especially if differing enantiomeric forms are considered. For example, a triacylglycerol with only five different fatty acid constituents may consist of 75 different molecular species (not including enantiomers).

Under ideal circumstances, the analyst would wish to separate a lipid into individual molecular species in its native form, in order that the biosynthesis or metabolism of every part of the molecule can potentially be studied, or so that the physical properties of each species can be assessed in relation to those of the whole. With polar complex lipids, the analysis can be simplified in a technical sense by converting the compounds to nonpolar forms by removing the polar head group by enzymatic or chemical hydrolysis. The approach of the analyst will depend on the nature and amount of information required, or on the availability of specific equipment.

The chromatographic procedures utilized for the separation of molecular species of glycerolipids resemble in kind those used for the separation of fatty acids (Section 6.4.4), modified according the higher molecular weight of the former. With most lipids, the separations achieved depend on the combined physical properties of all the aliphatic residues in each molecule. Considering triacylglycerols for illustrative purposes, reversed-phase HPLC will separate molecules according to the sum of the chain lengths of the fatty acids, with the retention times being reduced by the equivalent of approximately two carbon atoms for each double bond in the three fatty acid constituents. Silver ion chromatography will separate those molecules containing three saturated fatty acids from those with one monoenoic and two saturated fatty acids, and these are, in turn, separated from further fractions with an increasing degree of unsaturation. Adsorption chromatography can be used to separate molecular species containing three normal fatty acids from those containing two normal fatty acids and one with a polar substituent, such as a hydroxyl group. Often no single method will give the required degree of fractionation, but if two of the above separation modes are used in sequence, a high degree of molecular simplification may be possible. GC and HPLC coupled to mass spectrometry are increasingly proving their value for identification of molecular species.

6.4.6.2 Molecular species of triacylglycerols

The most useful single procedure for separation of molecular species of triacylglycerols is arguably reversed-phase HPLC (Nikolova-Damyanova, 1997). However, silver ion chromatography (HPLC or TLC) followed by re-chromatography of fractions by reversed-phase HPLC or high-temperature GC will always give more information. As mentioned above, when reversed-phase HPLC is applied to intact triacylglycerols, the separation is in ascending order of the total number of carbon atoms in the aliphatic chains of the three fatty acids, with a double bond in any of the fatty acids reducing the retention time to roughly that of a component with two fewer carbon atoms in total. The relative retention time of a given component has been defined in terms of an "equivalent carbon number" (ECN) or "partition number" value, defined as the actual number of carbon atoms in the aliphatic residues

(CN) less twice the number of double bonds (n) per molecule (the carbons of the glycerol moiety are not counted for this purpose), i.e.,

$$ECN = CN - 2n$$

Two components having the same ECN value are said to be "critical pairs." For example, triacylglycerol species containing the fatty acid combinations 16:0-16:0-16:0, 16:0-16:0-18:1, 16:0-18:1-18:1, and 18:1-18:1-18:1 have the same ECN value and tend to elute close together. However, with the best modern equipment, all four components should be separable. The positions of the fatty acids within the triacylglycerol molecules have no effect on the nature of the separations.

The ECN concept was useful in the early days of the technique, when the resolving power was relatively limited. On the other hand, the formula is now only of utility as a rough guide to what may elute in a given area of a chromatogram, since the greatly increased resolving power of modern HPLC phases means that the factor for each double bond is not simply 2.0, but has to be defined much more precisely. Also, this factor can no longer be treated as a constant, as a second double bond in a molecule has a slightly different effect from the first. Accordingly, more complex formulae are necessary to define the order of elution of triacylglycerols from modern reversed-phase columns, which in essence means from octadecylsilyl (ODS) stationary phases, as these have been used almost exclusively for the purpose. Acetonitrile is used as the main component of the mobile phase, but it must be modified by a proportion of an additional solvent, such as chloroform, tetrahydrofuran, (or better methyltert-butyl ether), dichloromethane, isopropanol, or acetone. The choice has often been governed by the availability of a specific type of detector rather than by objective separation criteria. Control of column temperature, usually in the range 20 to 25°C, is desirable for reproducible retention times and optimum quantification.

Various methods of describing the elution characteristics of triacylglycerols in quantitative terms have been published of which the most successful is probably that of Goiffon et al. (1981a,b). They presented a scheme for identifying triacylglycerol species in which the principle of additivity of solution free energies of saturated and unsaturated acyl residues (up to C_{18}) was utilized. The results were presented as a plot of log k (or retention volume) against the number of double bonds in the triacylglycerol. In practical terms, identifications were accomplished most readily by plotting the number of double bonds in the molecule against the logarithm of the retention time for each component relative to that of triolein, expressed as log α. Parallel straight lines were obtained for all the homologous series. For a given triacylglycerol species, log α was the sum of the equivalent values for each of the three constituent fatty acids, the latter being equal to one third of log α of the corresponding simple triacylglycerol. The retention time of any triacylglycerol could be calculated from such data, or from the graphical relationships. Unlike relative retention times, the numerical values can be seen to have some immediate relevance. However, such data are not easily applied to unknowns.

All of such methods present the analyst with lengthy preparatory work, not only in standardizing the chromatographic conditions, but also in running a variety of natural samples and standards to obtain retention data. Graphical methods can be tedious and can obviously only be used if sufficient data points are available. Perrin and Naudet (1985) have simplified the task by tabulating retention data relative to that of triolein for 120 different triacylglycerol species commonly encountered in seed oils. The absolute values listed may not be reproduced directly by other workers, but the data are certainly important for predicting relative orders of elution.

The same detector and quantification problems arise in triacylglycerol analysis as in many other aspects of the HPLC of lipids (see Section 6.4.5.2 above). When UV detection at 200 to 210 nm (for isolated double bonds) must be employed, isopropanol, methyltertbutyl ether, or tetrahydrofuran are the most appropriate modifiers of the mobile phase. Alternatively, propionitrile has been used as the sole solvent, but this is costly and highly toxic. Herslöf (1981) showed that by using a wavelength of 215 to 230 nm, i.e., the region where the carbonyl group exhibits a weak absorbance, but away from that where isolated double bonds absorb, sufficiently sensitive detection and good quantification could be obtained with acetonitrile-tetrahydrofuran mobile phases. There are fewer constraints in the choice of solvents when an ELSD is used, and Figure 6.9 illustrates the separation of a low-erucic rapeseed oil, with a gradient of acetonitrile-acetone (author, unpublished). Of course, careful calibration is required if the technique is to be used quantitatively.

Silver ion complexation chromatography, used in conjunction with TLC, revolutionized the study of triacylglycerol structures during the 1960s (Nikolova-Damyanova, 1992). With relatively simple equipment, it is possible to obtain distinct molecular fractions, separated on the basis of a single well-defined property, i.e., degree of unsaturation. More recently, silver-ion HPLC has come to the fore and affords distinct advantages. When fractions obtained in this way are subsequently separated by reversed-phase TLC or by high-temperature GLC, additional information is obtained on the chain-length distributions of the fatty acid constituents. For example, silver nitrate TLC has proved immensely useful in separating triacylglycerols, containing a normal range of fatty acids with zero to three *cis*-double bonds, into simpler molecular species. A triacylglycerol of this type can contain species with up to nine double bonds in the fatty acid moieties per mole of glycerol. Components migrate in the order:

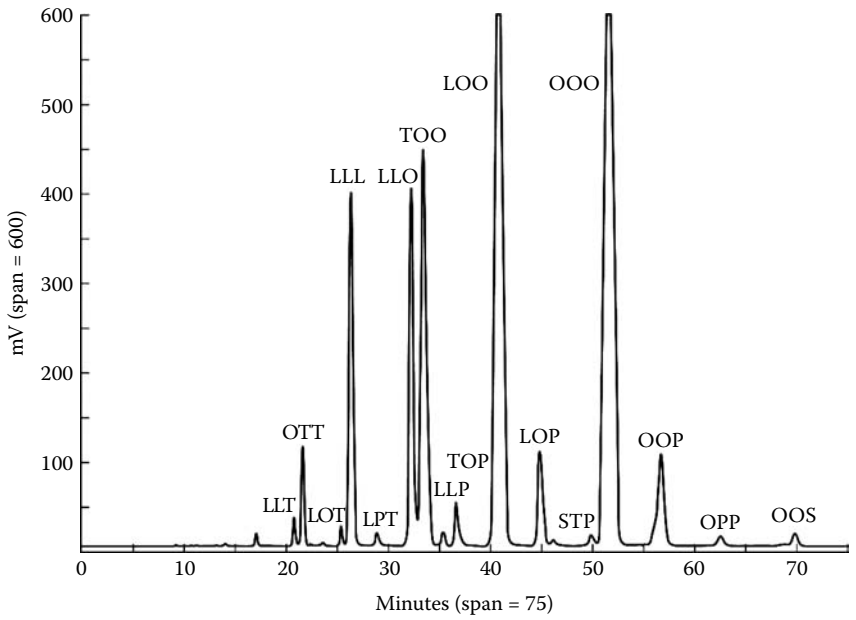


FIGURE 6.9 Reversed-phase HPLC of triacylglycerols from low-erucate rapeseed oil, with a gradient of acetonitrile-acetone, an ODS stationary phase, and evaporative light-scattering detection. Abbreviations: P, palmitate; S, stearate; O, oleate; L, linoleate; T, linolenate.

SSS > SSM > SMM > SSD > MMM > SMD > MMD > SDD > SST > MDD > SMT > MMT > DDD > SDT > MDT > DDT > STT > MTT > DTT > TTT

where S, M, D, and T denote saturated, mono-, di- and trienoic acids, respectively (they do not indicate the positions of the fatty acids on the glycerol moiety). Silver nitrate (up to 10%) is incorporated into the layers and the solvent systems generally employed consist of hexane–diethyl ether, toluene–diethyl ether, or chloroform (alcohol-free)–methanol mixtures. As all the fractions listed above cannot be separated on one plate, it is common practice to separate the least polar fractions first with hexane–diethyl ether (80:20, v/v) or chloro-form–methanol (197:3, v/v) and then to separate the remaining fractions with more polar solvents such as diethyl ether alone or chloroform–methanol (96:4, v/v). Bands are detected under UV light after spraying with 2′,7′-dichlorofluorescein solution, components are recovered and they are identified and determined by gas chromatography of the fatty acid constituents with an added internal standard (the concentration depending on the scale of the separation).

Columns with silver ions attached to cation exchangers (silica based) are used exclusively now for silver ion HPLC of lipids and prepacked columns are available from commercial sources or they are easy to prepare from a commercial ion-exchange HPLC column (Christie, 1987). While chlorinated solvents together with gradients of acetone and/or acetonitrile, which complexes strongly with silver ions displacing unsaturated lipids, were the first practical systems to be described, heptane-acetonitrile is often preferred nowadays. Ultraviolet detectors at wavelengths of about 205 nm can be used with gradients of heptane-acetonitrile, although base-line drift can be a problem and quantification is difficult if not impossible. Evaporative light-scattering detectors are those most often employed, although as in other applications the nonlinearity of the response, and the difficulties of calibration present problems. By including a stream-splitter just before the detector, fractions can be collected, an internal standard added and, following trans-methylation, fractions can be identified by GC of the component fatty acids. The order of elution of triacylglycerol species is very similar if not identical to that by silver ion TLC, and is easy to understand, intuitively, unlike HPLC in the reversed-phase mode. A typical separation is illustrated in Figure 6.10 (Christie, 1988).

Although useful separations of intact triacylglycerols by high-temperature GC can be achieved routinely, the technique can still be fraught with difficulties (Buchgraber et al., 2004). The conditions necessary to elute lipids of such high molecular weight from the columns approach the limits of thermal stability both of the stationary phases and of the compounds themselves. Columns constructed from fused silica are preferred, the length being a compromise between the optimum in terms of resolution with a need to limit the exposure time of the analyte to high temperatures to the minimum; commonly the length is 5 to 25 m with an internal diameter of 0.2 to 0.32 mm. Initially, nonpolar stationary phases only (of the methyl silicone type) were used in high-temperature GC, and cross-linking and chemical bonding improved the properties of the columns appreciably. More polar (or polarizable) bonded phases, consisting of phenylmethyl silicones, came into use later and are increasingly being used.

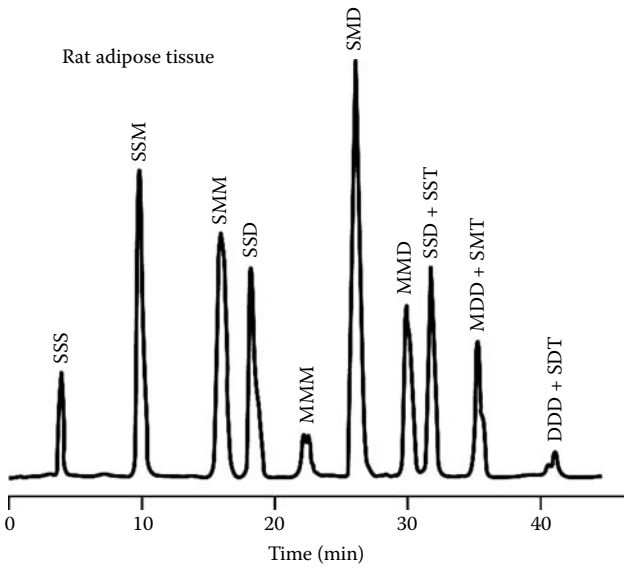


FIGURE 6.10 Separation of triacylglycerols from rat adipose tissue by HPLC on a Nucleosil™ 5SA column in the silver ion form with a gradient of acetone into dichloromethane-dichlorethane (1:1, v/v). Abbreviations: S, saturated; M, monoenoic; D, dienoic; T, trienoic fatty acyl residues. (From Christie, W.W. (1988) *J. Chromatogr.*, 454, 273. With permission.)

When nonpolar phases are used in capillary columns, triacylglycerols are separated according to molecular weight only and there is no useful resolution by degree of unsaturation, although some partial separations may be seen on occasion. Components varying in carbon number from 44 to 56 can be clearly resolved. Some remarkably effective separations of triacylglycerols, both by chain-length and degree of unsaturation, have been achieved on capillary columns coated with more polar (or "polarizable") silicone phases containing a high proportion of phenyl groups, but the recovery of polyunsaturated species is poor.

The linearity and robustness of the flame-ionization detector is a considerable advantage, but the key to a wider acceptance of high-temperature GC of triacylglycerols on capillary columns is the precision that can be attained in quantification. Good injection technique and clean samples can eliminate some of the losses, but there is little that can be done to prevent thermal degradation entirely. By careful optimization of the operating conditions, reproducible if not quantitative recoveries can be attained and careful calibration can give meaningful results, at least for the more saturated species as in cocoa butter (Buchgraber et al., 2003; 2004).

Supercritical fluid chromatography is a hybrid between GC and HPLC, using much of the instrumentation of the former while the mobile phase is in effect a liquefied gas, commonly carbon dioxide. Much effort is being expended in developing the instrumentation and applications, but only the published separations of molecular species of triacylglycerols appear to offer real competition to alternative techniques (reviewed by Blomberg et al., 1998). Here the advantages are that elution temperatures can be employed that are low in comparison to gas chromatography, while flame-ionization detection permits more convenient and accurate quantification than is possible by HPLC. Similar chromatographic columns are used as with gas chromatography. The technique is being used in industry in process control of triacylglycerol composition. It is also possible to use the technique in conjunction with mass spectrometry, as the relative volatility of the mobile phase is an advantage in comparison to conventional HPLC-MS (Laakso and Manninen, 1998).

6.4.6.3 Molecular species of complex glycerolipids

Molecular species of polar lipids, such as glycerophosphatides or glycosyldiacylglycerols, can be separated in native form (see below), but analysis is simplified if the polar head group can be removed. While this is self-evident for GC, it can also be true for HPLC as the polar moiety can interfere and limit the quality of the separation. Also, its removal means that molecular species of each complex lipid are analysed under exactly the same conditions. Gas chromatography on a polarizable stationary phase is probably the most appropriate method for analysis of diacylglycerol derivatives when suitable equipment is available. High resolution is possible and the sensitivity, linearity and convenience of the flame ionization detector means that quantification is a relatively simple task. GC can also be linked to mass spectrometry to simplify identification of the separated components. Reversed-phase HPLC after conversion of diacylglycerols to UV-absorbing derivatives is a useful alternative that permits good resolution and simplifies quantification with increased sensitivity. Also, it can be used as a micro-preparative technique, so that fractions can be collected for more detailed analysis or for radioactivity assays in biological experiments, for example. Some years ago, silver ion TLC would have been the first method most analysts would have adopted, but the convenience and accuracy of modern instrumental methods have tended to displace it. Nonetheless, it should not be forgotten as it permits a great deal of useful information to be obtained with relatively simple equipment.

A variety of enzymes capable of releasing 1,2-diacyl-sn-glycerols from phosphoglycerides or of ceramide from sphingomyelin, and termed phospholipase C, have been isolated from microorganisms, but especially from *Clostridium perfringens* and *Bacillus cereus*. Each organism produces an enzyme with distinctive properties for particular applications and these are available commercially. Bell (1997) has reviewed the methodology and recommends the use of phospholipase C from *B. cereus* for the preparation of diacylglycerols from phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine. An enzyme from *B. thuringiensis* is recommended for phosphatidylinositol. The diacylglycerols produced should be derivatized at once

to TMS ether, acetates, or UV-absorbing derivatives, for example, as these can be stored for long periods in an inert atmosphere at low temperatures without coming to harm. Ceramides can be prepared from sphingomyelin by digestion with phospholipase C in this manner. In addition, diacylglycerols can be released from the glycosyldiacylglycerols of plants by a series of simple chemical reactions, and then analysed by the methods below (Heinz, 1996; Heinze et al., 1984). The procedure involves oxidation with periodic acid in methanol; after decomposition of excess reagent with ethyleneglycol, the oxidized lipid is extracted and treated with 1,1-dimethylhydrazine to release the diacylglycerols by beta-elimination.

With many phospholipid classes (and phosphatidylethanolamine and phosphatidylserine especially), it is advisable to separate alkenylacyl, alkylacyl and diacyl forms (the "diradyl" forms) as the acetate or other derivatives before proceeding to more detailed analysis. The compounds migrate in the order stated in the form of various derivatives and can be adequately resolved on silica gel TLC layers, with a first development to half way up the plate with hexane–diethyl ether (1:1, v/v) followed by a full development in the same direction in toluene (Renkonen and Luukkonen, 1976) (HP-TLC plates may be preferred nowadays). Nakagawa and Horrocks (1983) achieved similar separations with HPLC.

The greater resolving power of capillary columns has been put to good use for the resolution of diacylglycerol species derived from phospholipids, first with apolar and, more recently, with polarizable stationary phases in columns of fused silica. GC-MS will greatly simplify identification of peaks, but in the absence of this facility excellent results can be obtained by comparison with relative retention times of reference standards or on the basis of relative retention times calculated from the additive contributions of the fatty acyl chains. While good separations are possible on nonpolar columns, such as SE-54™, much better results are obtained in separations of unsaturated species on polar phases, such as RTx-2330™ or SP-2380™, with which unsaturated species follow saturated. Indeed, molecular species with positional isomers of double bonds in the fatty acyl residues were found to be separable. As an example, a separation of the diacylglycerol moieties derived from the phosphatidylcholine of rat liver is illustrated in Figure 6.11 (Myher and Kuksis, 1989).

As the molecular weights of diacylglycerol derivatives are much lower than those of triacylglycerols, there appear to be no difficulties with quantification and uncorrected detector responses of TMS ether derivatives should give comparable results to those obtained by other means, provided that all the instrumental parameters have been properly optimized.

Excellent resolution of molecular species of diacylglycerols derived from phospholipids has been achieved by means of reversed-phase HPLC. Although intact phospholipids can also be separated by this technique, the diacylglycerol approach means that there is no requirement for inorganic ions in the mobile phase, while UV-absorbing or fluorescent derivatives of diacylglycerols may be employed, simplifying detection and quantification. In addition, complementary chromatographic techniques can more easily be brought to bear for the further resolution of fractions and individual components can be collected for further analysis (reviewed by Bell, 1996; Christie, 2003). The principle of the separation is the same as that described above (Section 6.4.6.2) for triacylglycerols,

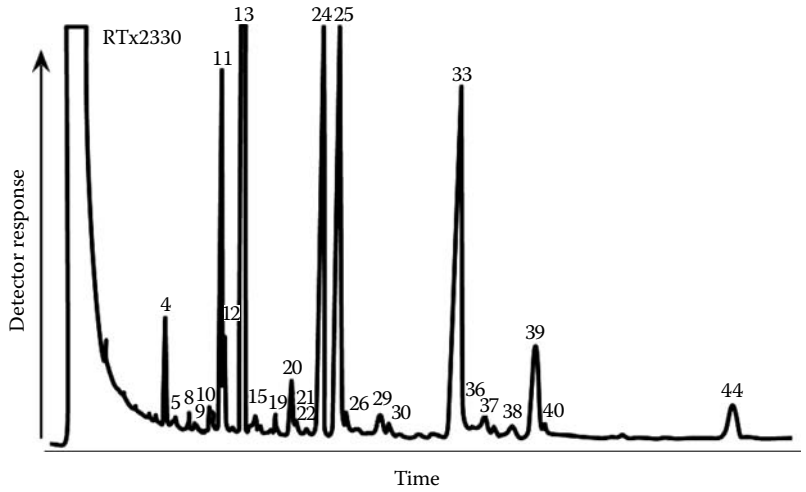


FIGURE 6.11 GC separation of TMS ethers of diacylglycerols derived from the phosphatidylcholine of rat liver on a fused silica capillary column coated with cross-bonded RTx-2330 (Restek, Port Matilda, PA) with hydrogen as carrier gas at an isothermal temperature of 250°C. Peak identifications: 4, 16:0-16:0; 5, 16:0-16:1; 8, 16:0-17:0; 10, 16:0-18:0; 11, 16:0-18:1(n-9); 12, 16:0-18:1(n-7); 13, 16:0-18:2; 19, 17:0-18:2; 20, 18:0-18:1(n-9); 21, 18:0-18:1(n-7); 22, 18:1(n-9)-18:1(n-9); 24, 18:0-18:2; 25, 16:0-20:4, 16:0-20:3, 18:1(n-9)-18:2; 26, 18:1(n-7)-18:2; 29, 18:2-18:2, 16:0-20:5; 30, 17:0-20:4; 33, 18:0-20:4(n-6), 18:0-23(n-6); 36, 18:1(n-9)-20:4; 38, 18:0-20:5; 39, 16:0-22:5, 16:0-22:6; 44, 18:0-22:5, 18:0-22:6. (From Myher, J.J. and Kuksis, A. (1989) *J. Chromatogr.*, **471**, 187. With permission.)

except that only two long-chain fatty acids need be considered, i.e., the separation is in ascending order of the total number of carbon atoms in the aliphatic chains of the two fatty acids, with a double bond in any of the fatty acids reducing the retention time to roughly that of a component with two fewer carbon atoms. Components considered formerly as critical pairs, e.g., dipalmitoyl and palmitoyloleoyl species, are easily separated on modern columns. However, only in exceptional circumstances is it possible to separate isomers in which the positions of the fatty acids on the glycerol moiety differ. The choice of stationary and mobile phases tends to be the same as for triacylglycerols and many of the practical points raised in the section dealing with these lipids are appropriate here also. ODS columns with a high carbon content are usually favoured, but octyl phases are a little different in selectivity and this can be useful in some applications.

Various types of diacylglycerol derivatives have been employed. Acetates are the simplest, but UV detection at low wavelengths must then be used, leading to difficulties in quantification. However, this approach may be useful if it is intended that fractions be subjected to identification directly by MS, or indirectly following collection by GC or GC-MS. Various UV-absorbing and fluorescent derivatives have been utilized and there appears to be no information on which of these is best in chromatographic terms. Benzoates are perhaps the simplest UV-absorbing derivatives, but 3,5-dinitrobenzoates offer greater sensitivity (to picomole levels). Anthroyl and naphthoyl are the most common fluorescent derivatives to have been used and these can increase the sensitivity of detection by a further three orders of magnitude.

With dinitrobenzoyl derivatives of diacylglycerols, impressive separations of molecular species derived from phosphatidylcholine from a variety of tissues have been achieved. For example, with a column of Ultrasphere™ ODS and elution with acetonitrile–isopropanol (4:1 by volume) as the mobile phase, 29 distinct fractions were detected, identified, and quantified (Takamura et al., 1986). When methanol–isopropanol (19:1, v/v) was the mobile phase, only 17 fractions were seen, but some components were resolved that were not separated by the previous system. By collecting fractions containing more than one component from the first eluent, a more comprehensive analysis can be obtained by rerunning with the second eluent. In this way, up to 36 distinct molecular species can be obtained from each lipid class. This method used in sequence with silver ion chromatography might prove to be an even more thorough approach. These methods have the advantages that isocratic elution and, therefore, simpler HPLC equipment can be used, while the detector response is directly proportional to the molar amount of each species.

Molecular species of plant galactolipids (mono- and digalactosyldiacylglycerols and sulfoquinovosyldiacylglycerol) have been analysed in the form of diacylglycerols, following preparation by the chemical procedure described above; the *p*-anisoyl derivatives were then prepared and fractionated on an ODS column with a gradient of 30 to 0% water in acetonitrile as the mobile phase with specific detection and quantification at 250 nm (Kesselmeier and Heinz, 1985).

HPLC in the reversed-phase mode is virtually the only technique that needs be considered for the separation of molecular species of intact polar lipids nowadays. Again, columns of the ODS type with a high carbon loading are used mainly, though octyl phases offer alternative properties for some applications. Mobile phases are based on methanol or acetonitrile with additional modifier solvents (usually including water) and it is essential to add an ionic species to the mobile phase to counter unwanted interactions of the ionic head group with the stationary phase that would otherwise cause peak broadening.

Patton, Fasulo and Robins published a seminal paper on the subject of separations of phospholipid molecular species in 1982. They utilized a column of the ODS type with methanol–water–acetonitrile as the components of the mobile phase with choline chloride added as the ion suppressant. The separation can be considered as bimodal, with those molecular species containing a 16:0 fatty acyl group eluting before those containing 18:0. As with triacylglycerols, the position of the acyl group within the molecule has no effect on separation by reversed-phase chromatography. All of the major components in rat liver phosphatidylcholine are clearly resolved, although inevitably some minor fractions co-elute with the main ones. With plant lipids, the molecular species differ markedly from those found in the corresponding lipids of animal tissues and they tend to be much simpler in composition. Essentially the same elution conditions as those of Patton et al. have now been used in a number of laboratories to effect similar separations of phosphatidylcholine species, always a good recommendation. However, there may be times when an alternative elution scheme is required, either to suit the availability of particular HPLC equipment, especially the detection system, or to change the selectivity of the separation to permit resolution of specific molecular species. Phosphatidylethanolamine and phosphatidylinositol from rat liver were separated into molecular species and quantified under exactly the same conditions employed for phosphatidylcholine and, by increasing the polarity of the mobile phase, phosphatidylserine could be fractionated. Indeed, fractions identical in composition were obtained, although the relative proportions were rather different, as expected.

In order to use evaporative light-scattering detection, molecular species of phosphatidylethanolamine from animal tissues were separated by reversed phase HPLC on an ODS phase with methanol–acetonitrile (7:3, v/v) containing 5 μM triethylamine as the mobile phase (Brouwers et al., 1999). After careful calibration, components were quantified directly from the detector responses of an

evaporative light-scattering detector, although peaks could also be collected for analysis (see previous section).

The galactosyldiacylglycerols of higher plants and algae in the intact form have been successfully separated into molecular species by reversed-phase HPLC by several research groups, and again this seems to be the only technique that need be considered nowadays. The methodology has been reviewed by Heinz (1996). For example, mono- and digalactosyldiacylglycerols from leaves were fractionated by this technique with a gradient of 50 to 0% water in acetonitrile as the mobile phase, and with spectrophotometric detection at 200 nm (Kesselmeier and Heinz, 1985). Response factors were determined for each molecular species so that the technique could be used quantitatively. The procedure developed by Patton et al. (1982) described above has also been adapted for galactolipids by Demandre et al. (1985). In an alternative approach, digalactosyldiacylglycerols from oats were peracetylated with acetic anhydride and pyridine to reduce their polarity prior to analysis by reversed-phase HPLC (Bergqvist and Holmback, 2000), although, perhaps surprisingly, benzoylation does not appear to have been attempted.

6.4.6.4 Molecular species of sphingolipids

In sphingomyelin and the glycosphingolipids, the molecular species contain different combinations of a fatty acid moiety with a long-chain base and reversed-phase HPLC is the preferred method of analysis. In only a few studies have attempts been made to separate intact sphingomyelins into simpler molecular fractions and, as they are relatively saturated lipids, UV detection at low wavelengths is not ideal. An HPLC system that makes use of an evaporative light-scattering detector may afford better results, and Olsson et al. (1992) have described suitable conditions.

Molecular species of sphingomyelin can also be analysed in the form of ceramides after removal of the polar head group (as described in the previous section). It is also possible to generate ceramides from glycosphingolipids by means of ceramide glycanase, which removes the carbohydrate moiety, but I am not aware of it being used for analysis of molecular species of the ceramide as opposed to the oligosaccharide portion of such lipids (though a chemical procedure has been used) (see Christie, 2003). Ceramides are less polar and more volatile than the parent sphingolipids and can be analysed by both high-temperature GC and HPLC methods, which can be linked to mass spectrometry. Methods for the analysis of ceramides by HPLC (Cremesti and Fischl, 2000; Gaudin et al., 2002) and GC (Raith et al., 2000) were recently reviewed.

There are advantages in being able to resolve underivatized glycosphingolipid species, as these can then be subjected to immunogenicity tests. Monoglycosylceramides obtained from the intestines of Japanese quail were subjected to HPLC on phase comprising a very high proportion of ODS groups relative to the inert support (20% by weight) and eluted with methanol UV detection at 210 nm (Hirabayashi et al., 1986). A large number of components were resolved and most were provisionally identified by their relative retention times as containing homologous series of saturated hydroxy and nonhydroxy fatty acids linked to sphingosine. These predictions were confirmed for the most abundant components by fast atom bombardment MS, a technique that is extremely useful for the structural identification of glycolipids. A further series of peaks was identified as containing phytosphingosine linked to nonhydroxy saturated fatty acids.

Gangliosides are more difficult to analyse by HPLC because of their high polarity, but good results can be achieved by a suitable choice of mobile phase (Sonnino et al., 1985).

As with the separation of classes of glycolipids (see Section 6.4.5.6), benzoylation has proved useful for the analysis of molecular species compositions as it enables sensitive and quantitative determination by UV detectors. In general, much better separations are achieved than is possible with the underivatized glycolipids, and with less polar mobile phases. Among the first applications of derivatization of sphingolipids for separations of molecular species was one of perbenzoylated glucosylceramide from a patient with Gaucher's disease. This was fractionated on a column of an ODS phase, with methanol as the mobile phase and UV detection at 254 nm (Suzuki et al., 1976). Nine fractions were obtained, differing in their fatty acid compositions, but apparently not in the compositions of the long-chain bases. Perbenzoylation has also proved a useful approach to resolution of molecular species of highly polar monosialogangliosides and glycolipid sulfates.

6.4.7 Mass spectrometry of lipids

Mass spectrometry is proving to be an extremely important tool for the analysis of intact lipids and this has produced a revolution in the approach to the problem for those with access to the required equipment. Indeed, this is frequently described in the literature as a new branch of science — "lipidomics" — in which the kinetics of lipid metabolism and the interactions of lipids with cellular proteins is studied via a detailed quantification of a cell's lipidome (lipid classes and individual molecular species), with the hope of obtaining new insights into health and disease. The topic has been the subject of reviews (Murphy et al., 2001; Han and Gross, 2003; 2005) and a monograph (Byrdwell, 2005). Fast atom bombardment (FAB), atmospheric pressure-chemical ionization (APCI), and matrix-assisted laser desorption/ionization linked to time-of-flight (MALDI-TOF) mass spectrometry can all have great value for specific lipid classes but electrospray ionization (ESI) is the most sensitive and appears to have the greatest general utility. The technique is developing so rapidly that these notes may soon be out of date.

Electron-impact ionization is still of value for GC-MS, when the lipids are sufficiently volatile for this technique to be suitable. For example, phospholipids can be analysed after conversion to simple diacylglycerol derivatives (see Section 6.4.6.3) and GC-MS of intact triacylglycerols is feasible but with some technical difficulty. In a typical electron-impact mass spectrum of a triacylglycerol, there is a rather small molecular ion, followed by a unique peak for an ester at $[M-18]^+$ (or loss of water). More importantly, there are intense ions that are characteristic of the various fatty acyl residues and these fall into two classes, i.e., those containing two acyl residues and those with only one. In the more important first class, there is an ion equivalent to the loss of an acyloxy group, i.e., $[M-RCOO]^+$, together with a related ion, but minus a further hydrogen atom. For example, the loss of a palmitoyl acyloxy group (equivalent to 255 amu) gives ions equivalent to $[M-255]^+$ and $[M-256]^+$, while the loss of an oleoyl moiety gives ions at $[M-281]^+$ and $[M-282]^+$. The relative intensity within each pair is dependent on whether the ion fragment contains an unsaturated residue (when the smaller ion is more intense). The other important class of diagnostic ions that contains the individual fatty acid moieties is of the form RCO^+, though if the fatty acid group is unsaturated an additional hydrogen atom is lost. Thus, an oleoyl moiety produces an ion at m/z = 264, while that from palmitate is at m/z = 239. Related ions with an additional 74 amu corresponding to the glycerol backbone, are found at m/z = 339 and 313, respectively; in this instance, the presence of a double bond has no effect.

The electron-impact mass spectra of acetate derivatives of diacylglycerols are equivalent to those of triacylglycerols, except that one of the acyl moieties is an acetyl residue. Published mass spectral data are sparse, but it is apparent that the molecular ion tends to be rather small or nonexistent, although an ion representing loss of water ($[M-18]^+$) can usually be seen. Loss of the acetyl group gives ions at $[M-59]^+$ or $[M-60]^+$, depending on the degree of unsaturation of the residual ion, and this is probably the best marker for determining the molecular weight. In addition, ions are seen for the loss of one or both of the other acyloxy moieties. TMS ether derivatives of 1,2-diacylglycerols tend to give much better spectra. Although the molecular ion is rarely seen, ions equivalent to $[M-15]^+$ (loss of a methyl group) and $[M-90]^+$ (loss of the TMS ether moiety) can be used to determine the molecular weight and, hence, the total carbon number and degree of unsaturation of the acyl moieties. An important diagnostic ion results from the loss of an acyloxy residue, i.e., $[M–RCOO]^+$ or $[M-RCOOH]^+$ if the residual acyl group is unsaturated. Other useful ions are equivalent to $[RCO + 74]^+$, $[RCO + 90]^+$, $[RCO]^+$, and $[RCO – 1]^+$. Characteristic ions at m/z = 145 and 129 contain the TMS group and parts of the glycerol backbone.

HPLC-MS with APCI has been widely used for the analysis of triacylglycerols. The technique results in relatively limited fragmentation, but with distinct diagnostic ions related to the nature of the acyl moieties. In particular, there is usually a protonated molecular ion ($[M+H]^+$) and a series of ions derived from diacylglycerol fragments ($[M-RCOO]^+$ or $[DG]^+$). The degree of unsaturation is the main factor that governs the intensities of the various ions and, in particular, the proportions of the diacylglycerol ions relative to the protonated molecular ion; the higher the degree of unsaturation the more intense is the protonated molecular ion, which becomes the base peak with four or more double bonds in the molecule. As an example, Figure 6.12 illustrates the mass spectrum of the triacylglycerol, 20:4 – 18:3 – 16:0. The protonated molecular ion is at m/z = 879, and ions at m/z = 574, 600, and 622 represent the diacylglycerols formed by loss of arachidonic, linolenic, and palmitic acids, respectively. Note that the spectra do not give information on the positional distributions of fatty acids on the glycerol moiety. The technique is especially compatible with reversed-phase chromatography, but it has also been used in conjunction with silver ion chromatography. The large differences in the response factors for each molecular species (depending on the degree of unsaturation of the acyl moieties) and instrumental factors must place a limit on the accuracy of quantification of triacylglycerols by APCI-MS. However, by careful calibration and the use of internal standards, acceptable results can be obtained, especially in routine analyses of similar samples.

While other ionization techniques continue to make a significant contribution, the development of electrospray ionization mass spectrometry (ESI-MS) has greatly simplified the task of analysis of triacylglycerols, phospholipids and sphingolipids. As this ionization technique does not cause extensive fragmentation, it has proved to be highly sensitive, accurate and reproducible without a need for complicated chromatographic steps, i.e., samples can be introduced to the instrument via a probe rather than via an HPLC system. With ESI, a high electric field is applied to nebulize a solution as it emerges from a needle. The field imparts a charge to the droplets and this builds up as the solvent evaporates until a point is reached where ions must be ejected from the surface. The ions are focused by an electronic lens and pass via a skimmer into the mass spectrometer. The correct pH and solvent composition are important, and lithium, sodium, or ammonium salts may be introduced to form $[M+Li]^+$, $[M+Na]^+$, or $[M+NH_4]^+$ ions, respectively. Both positive and negative ion spectra can be obtained and each may give useful structural information with specific lipid molecules. The technique works best at low concentrations of lipid in the infusion solution, e.g., fmol to pmol of total lipid per µl, when lipid-lipid interactions and ion suppression are not relevant and the response is highly linear.

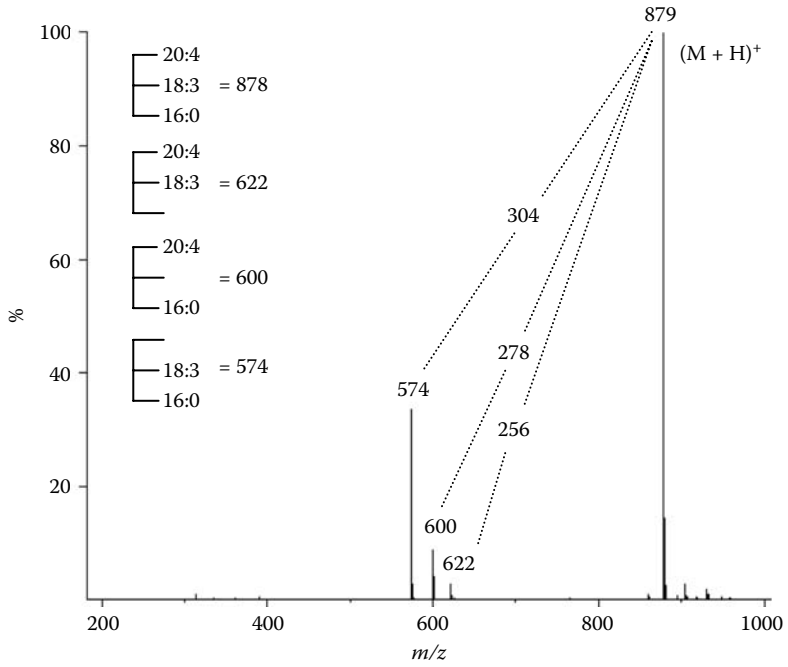


FIGURE 6.12 Mass spectrum (LC-MS with APCI detection) of the triacylglycerol, 20:4-18:3-16:0. (From Christie, W.W. (2003) *Lipid Analysis*, 3rd ed., Oily Press, Bridgwater, U.K. With permission.)

For example, Han and Gross (2003) favour the use of lithium ions in solution and positive ion mass spectrometry to analyse neutral or zwitterionic lipids, such as phosphatidylcholine, sphingomyelin, triacylglycerols and cerebrosides; the same technique with negative ion mass spectrometry is favoured for free acids, ceramides and phosphatidylethanolamine. Anionic lipids, such as cardiolipin, phosphatidylglycerol, phosphatidylinositols, phosphatidylserine, phosphatidic acid and sulfatides are analysed by negative-ion ESI-MS in the diluted chloroform extracts without added ions, and they are quantified by comparisons of the individual ion peak intensity with an internal standard. After identification of the main molecular species, tandem mass spectrometry is performed to estimate the molar ratios of any isobaric molecular forms. Variations in the methodology permit distinction of those fatty acids esterified to the sn-1 and sn-2 positions.

Similar principles apply to the analysis of the complex glycosphingolipids, although in a recent review (Merrill et al., 2005) HPLC, in both the normal- and reverse-phase modes, linked to tandem mass spectrometry is recommended over the direct inlet approach as this enables separation of isometric and isobaric species (such as glucosylceramides and galactosylceramides).

The main problem now lies in evaluating the vast amounts of data resulting from such measurements, especially when tandem mass spectrometry techniques are applied simultaneously. This has required the development and application of complex computational algorithms, most of which are not published in detail.

References

Ackman, R.G. (1972) The analysis of fatty acids and related materials by gas-liquid chromatography. *Prog. Chem. Fats Other Lipids*, **12**, 165–284.

Ackman, R.G. and Burgher, R.D. (1965) Cod liver oil fatty acids as secondary reference standards in GLC of polyunsaturated fatty acids of animal origin — analysis of a dermal oil of Atlantic leatherback turtle. *J. Am. Oil Chem. Soc.*, **42**, 38–42.

Andersson, B.A. (1978) Mass spectrometry of fatty acid pyrrolidides. *Prog. Chem. Fats Other Lipids*, **16**, 279–308.

Apon, J.M.B. and Nicolaides, N. (1975) The determination of the position isomers of the methyl branched FA esters by capillary gas chromatography/mass spectrometry. *J Chromatogr. Sci.,* **13**, 467–473.

Bell, M.V. (1997) Separations of molecular species of phospholipids by high-performance liquid chromatography. In *Advances in Lipid Methodology — Four*, Ed., W.W. Christie, Oily Press, Dundee, U.K., pp. 45–82.

Bergqvist, M. and Holmback, J. (2000) Nuclear magnetic resonance spectroscopy and reversed-phase high-performance liquid chromatography of peracetylated digalactosyldiacylglycerols. *J. Am. Oil Chem. Soc.*, **77**, 757–761.

Bitman, J. et al. (1984) Comparison of the phospholipid composition of breast milk from mothers of term and preterm infants. *Am. J. Clin. Nutr.*, **40**, 1103–1119.

Bligh, E.G. and Dyer, W.J. (1959) A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.*, **37**, 911–917.

Blomberg, L.G. et al. (1998) Characterization of lipids by supercritical fluid chromatography and supercritical fluid extraction. In *Lipid Analysis in Oils and Fats*, Ed., R.J. Hamilton, Blackie, London, pp. 34–58.

Bodennec, J. et al. (2000) A procedure for fractionation of sphingolipid classes by solid-phase extraction on aminopropyl cartridges. *J. Lipid Res.*, **41**, 1524–1531.

Brouwers, J.F.H.M. et al. (1999) Rapid separation and identification of phosphatidylethanolamine molecular species. *J. Lipid Res.*, **40**, 164–169.

Buchgraber, M. et al. (2003) Capillary GLC: a robust method to characterise the triglyceride profile of cocoa butter — results of an intercomparison study. *Eur. J. Lipid Sci. Technol.*, **105**, 754–760.

Buchgraber, M. et al. (2004) Triacylglycerol profiling by using chromatographic techniques. *Eur. J. Lipid Sci. Technol.*, **106**, 621–648.

Byrdwell, W.C. Ed. (2005) *Modern Methods for Lipid Analysis by Liquid Chromatography/Mass Spectrometry and Related Techniques*, AOCS Press, Champaign, IL.

Christie, W.W. (1985) Rapid separation and quantification of lipid classes by high performance liquid chromatography and mass (light-scattering) detection. *J. Lipid Res.*, **26**, 507–512.

Christie, W.W. (1987) A stable silver-loaded column for the separation of lipids by high-performance liquid chromatography. *J. High Resol. Chromatogr. Chromatogr. Commun.*, **10**, 148–150.

Christie, W.W. (1988) Separation of molecular species of triacylglycerols by HPLC with a silver ion column. *J. Chromatogr.*, **454**, 273–284.

Christie, W.W. Ed. (1992) Detectors for high-performance liquid chromatography of lipids with special reference to evaporative light-scattering detection. In *Advances in Lipid Methodology — One*, Oily Press, Bridgwater, U.K., pp. 239–271.

Christie, W.W. Ed. (1993a) Preparation of ester derivatives of fatty acids for chromatographic analysis. In *Advances in Lipid Methodology — Two*, Oily Press, Bridgwater, U.K., pp. 69–111.

Christie, W.W. Ed. (1993b) Preparation of lipid extracts from tissues. In *Advances in Lipid Methodology — Two,* Oily Press, Bridgwater, U.K., pp. 195–213.

Christie, W.W. Ed. (1996) Separation of phospholipid classes by high-performance liquid chromatography. In *Advances in Lipid Methodology —Three*, Oily Press, Bridgwater, U.K., pp. 77–107.

Christie, W.W. Ed. (1997) Structural analysis of fatty acids. In *Advances in Lipid Methodology — Four*, Oily Press, Bridgwater, U.K., p. 119–169.

Christie, W.W. (1998) Gas chromatography mass spectrometry methods for structural analysis of fatty acids. *Lipids*, **33**, 343–353.

Christie, W.W. (2003) *Lipid Analysis*, 3rd ed., Oily Press, Bridgwater, U.K.

Christie, W.W. et al. (1998) New procedures for rapid screening of leaf lipid components from Arabidopsis. *Phytochem. Anal.*, **9**, 53–57.

Christopherson, S.W. and Glass, R.L. (1969) Preparation of milk fat methyl esters by alcoholysis in an essentially nonalcoholic solution. *J. Dairy Sci.,* **52**, 1289–1290.

Craske, J.D. (1993) Separation of instrumental and chemical errors in the analysis of oils by GC — a collaborative evaluation. *J. Am. Oil Chem. Soc.*, **70**, 325–334.

Cremesti, A.E. and Fischl, A.S. (2000) Current methods for the identification and quantitation of ceramides: an overview. *Lipids*, **35**, 937–945.

Demandre, C. et al. (1985) Analysis of molecular species of plant polar lipids by high-performance and gas-liquid chromatography. *Phytochemistry*, **24**, 481–485.

Deschamps, F.S. et al. (2001) Assessment of the retention properties of poly(vinyl alcohol) stationary phase for lipid class profiling in liquid chromatography. *J. Chromatogr. A*, **928**, 127.

Fellenberg, A.J. et al. (1987) Simple mass spectrometric differentiation of the n–3, n–6 and n–9 series of methylene interrupted polyenoic acids. *Biomed. Environ. Mass Spectrom.*, **14**, 127–137.

Foglia, T.A. and Jones, K.C. (1997) Quantitation of neutral lipid mixtures using high-performance liquid chromatography with light scattering detection. *J. Liq. Chromatogr. Rel. Technol.*, **20**, 1829–1838.

Folch, J. et al. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, **226**, 497–509.

Gaudin, K. et al. (2002) Structure-retention diagrams of ceramides established for their identification. *J.Chromatogr.A,* **973**, 69–83.

Geurts van Kessel, W.S.M. et al. (1977) High performance liquid chromatographic separation and direct ultraviolet detection of phospholipids. *Biochim. Biophys. Acta*, **486**, 524–530.

Goiffon, J-P. et al. (1981a) High-pressure liquid-chromatography application for fatty triglycerides analysis. 2. Retention parameters of triglycerides. *Rev. Franc. Corps Gras,* **28**, 199–207.

Goiffon, J-P. et al. (1981b) High-pressure liquid-chromatography application for fatty triglycerides analysis. 1. A study of the best operating conditions for soyabean oil. *Rev. Franc. Corps Gras*, **28**, 167–170.

Greenspan, M.D. and Schroeder, E.A. (1982) Separation and detection of neutral lipids and free fatty acids in a liver extract by high-performance liquid chromatography. *Anal. Biochem.,* **127**, 441–448.

Gunstone, F.D. (1992) High resolution ^{1}H and ^{13}C NMR. In *Lipid Analysis. A Practical Approach*, Eds., R.J. Hamilton and S. Hamilton, IRL Press, Oxford, pp. 243–262.

Gunstone, F.D. (1993) High resolution 13C NMR spectroscopy of lipids. In *Advances in Lipid Methodology — Two,* Ed., W.W. Christie, Oily Press, Bridgwater, U.K., pp. 1–68.

Hakomori, S.-I. (1983) Chemistry of glycosphingolipids. In *Sphingolipid Biochemistry, Handbook of Lipid Research*, Vol. 3, Eds., J.N. Kanfer and S-I. Hakomori, Plenum Press, New York, pp. 1–165.

Han, X.L. and Gross, R.W. (2003) Global analyses of cellular lipidomes directly from crude extracts of biological samples by ESI mass spectrometry: a bridge to lipidomics. *J. Lipid Res.*, **44**, 1071–1079.

Han, X.L. and Gross, R.W. (2005) Shotgun lipidomics: electrospray ionization mass spectrometric analysis and quantitation of cellular lipidomes directly from crude extracts of biological samples. *Mass Spectrom. Rev.*, **24**, 367–412.

Harvey, D.J. (1992) Mass spectrometry of picolinyl and other nitrogen-containing derivatives of fatty acids. In *Advances in Lipid Methodology — One*, Ed., W.W. Christie, Oily Press, Dundee, U.K., pp. 19–80.

Heinz, E. (1996) Plant glycolipids: structure, isolation and analysis. In *Advances in Lipid Methodology — Three*, Ed., W.W. Christie, Oily Press, Dundee, U.K., pp. 211–332.

Heinze, F.J. et al. (1984) Release of diacylglycerol moieties from various glycosyl diacylglycerols. *Anal. Biochem.*, **139**, 126–133.

Herslöf, B. (1981) HPLC of triglycerides using UV detection. *J. High Resolut. Chromatogr. Chromatogr. Commun*, **4**, 471–473.

Herslöf, B. et al. (1990) Characterization of lecithins and phospholipids by HPLC with light scattering detection. In *Phospholipids*, Eds., I. Hanin and G. Pepeu, Plenum Press, New York, pp. 295–298.

Hirabayashi, Y. et al. (1986) An improved method for the separation of molecular species of cerebrosides. *Lipids*, **21**, 710–714.

Holman, R.T. and Rahm, J.J. (1971) Analysis and characterization of polyunsaturated fatty acids. *Prog. Chem. Fats Other Lipids*, **9**, 15–90.

Jungalwala, F.B. et al. (1976) High-performance liquid-chromatography of phosphatidylcholine and sphingomyelin with detection in the range of 200 nm. *Biochem. J.*, **155**, 55–60.

Kaufmann, P. (1995) Multivariate optimisation strategy for liquid chromatography. II. Exploring and finding optimal conditions in the search area of the multidimensional solvent space. *Chemometr. Intell. Lab. Systems*, **27**, 105–114.

Kesselmeier, J. and Heinz, E. (1985) Separation and quantitation of molecular species from plant lipids by high-performance liquid chromatography. *Anal. Biochem.*, **144**, 319–328.

Kramer, J.K.G. and Hulan, H.W. (1978) A comparison of procedures to determine free fatty acids in rat heart. *J. Lipid Res.*, **19**, 103–106.

Kuksis, A. et al. (1993) Quantitation of plasma lipids by gas-liquid chromatography on high-temperature polarizable capillary columns. *J. Lipid Res.*, **34**, 1029–1038.

Kuksis, A. et al. (1990) Gas-liquid chromatographic profiling of plasma lipids using high-temperature polarizable capillary columns. *J. Chromatogr.*, **500**, 427–441.

Laakso, P. and Manninen, P. (1998) Mass spectrometric techniques in the analysis of triacylglycerols. In *Spectral Properties of Lipids*, Eds., R.J. Hamilton and J. Cast, Sheffield Academic Press, Sheffield, U.K., pp. 141–190.

McCluer, R.H. et al. (1989) High-performance liquid chromatography of membrane lipids, glycosphingolipids and phospholipids. *Meth. Enzymol.*, **172**, 538–575.

Merrill, A.H. et al. (2005) Sphingolipidomics: high-throughput, structure-specific, and quantitative analysis of sphingolipids by liquid chromatography tandem mass spectrometry. *Methods*, **36**, 207–224.

Miwa, T.K. et al. (1960) Gas chromatographic characterization of fatty acids — identification constants for monocarboxylic and dicarboxylic methyl esters. *Anal. Chem.*, **32**, 1739–1742.

Mourey, T.H. and Oppenheimer, L.E. (1984) Principles of operation of an evaporative light-scattering detector for liquid chromatography. *Anal. Chem.*, **56**, 2427–2434.

Murphy, R.C. et al. (2001) Analysis of nonvolatile lipids by mass spectrometry. *Chem. Rev.*, **101**, 479–526.

Myher, J.J. and Kuksis, A. (1989) Relative gas-liquid chromatography retention factors of trimethylsilyl ethers of diradylglycerols on polar capillary columns. *J. Chromatogr.*, **471**, 187–204.

Nakagawa, Y. and Horrocks, L.A. (1983) Separation of alkenylacyl, alkylacyl and diacyl analogues and their molecular species by high performance liquid chromatography. *J. Lipid Res.*, **24**, 1268–1275.

Nikolova-Damyanova, B. (1992) Silver ion chromatography and lipids. In *Advances in Lipid Methodology — One*, Ed., W.W. Christie, Oily Press, Bridgwater, U.K., pp. 181–237.

Nikolova-Damyanova, B. (1997) Reversed-phase high-performance liquid chromatography: general principles and application to the analysis of fatty acids and triacylglycerols. In *Advances in Lipid Methodology — Four*, Ed., W.W. Christie, Oily Press, Bridgwater, U.K., pp. 193–251.

Olsson, N.U. et al. (1992) HPLC separation of molecular species of intact sphingomyelin, utilizing multivariate design and optimization. *Chromatographia*, **34**, 529–534.

Palmer, D.N. et al. (1984) Separation of some neutral lipids by normal-phase high-performance liquid chomatography on a cyanopropyl column: ubiquinone, dolichol and cholesterol levels in sheep liver. *Anal. Biochem.*, **140**, 315–319.

Patton, G.M. et al. (1982) Separation of phospholipids and individual molecular species of phospholipids by high performance liquid chromatography. *J. Lipid Res.* **23**, 190–196.

Perrin, J-L. and Naudet, M. (1985) Identification and quantification of triglycerides from natural fats by HPLC. *Rev. Franc. Corps Gras*, **32**, 301–303.

Raith, K. et al. (2000) Ceramide analysis utilizing gas chromatography-mass spectrometry. *J. Chromatogr. A*, **876**, 229–233.

Ratnayake, W.M.N. (1998) Analysis of trans fatty acids. In *Trans Fatty Acids in Human Nutrition*, Eds., J.L. Sébédio and W.W. Christie, Oily Press, Bridgwater, U.K., pp. 115–161.

Renkonen, O. and Luukkonen, A. (1976) Thin-layer chromatography analysis of complex lipids. In *Lipid Chromatographic Analysis*, 2nd ed., Vol.1, Ed., G.V. Marinetti, Marcel Dekker, New York, pp. 1–58.

Ritchie, A.S. and Jee, M.H. (1985) High-performance liquid chromatographic technique for the separation of lipid classes. *J. Chromatogr. A*, **329**, 273–280.

Rouser, G. et al. (1976) Column chromatographic and associated procedures for separation and determination of phosphatides and glycolipids. In *Lipid Chromatographic Analysis,* 2nd ed., Vol. 3, Ed., G.V. Marinetti, Marcel Dekker, New York, pp. 713–776.

Schnaar, R.L. and Needham, L.K. (1994) Thin-layer chromatography of glycosphingolipids. *Meth. Enzymol.*, **230**, 371–389.

Sébédio, J.L. (1995) Classical chemical techniques for fatty acid analysis. In *New Trends in Lipid and Lipoprotein Analyses*, Eds., J.-L. Sebedio and E.G. Perkins, AOCS Press, Champaign, IL, pp. 277–289.

Skipski, V.P. (1975) Thin-layer chromatography of neutral glycosphingolipids. *Meth. Enzymol.*, **35**, 396–425.

Sonnino, S. et al. (1985) Preparation of GM1 ganglioside molecular species having homogeneous fatty acid and long chain base moieties. *J. Lipid Res.*, **26**, 248–257.

Spitzer, V. (1997) Structure analysis of fatty acids by gas chromatography — low resolution electron impact mass

spectrometry of their 4,4-dimethyloxazoline derivatives — a review. *Prog. Lipid Res.*, **35**, 387–408.

Suzuki, A. et al. (1976) Separation of molecular species of glucosyl ceramide by high-performance liquid chromatography of their benzoyl derivatives. *J. Biochem*. (Tokyo), **80**, 1181–1183.

Takamura, H. et al. (1986) Quantitative analysis of polyenoic phospholipid molecular species by high-performance liquid chromatography. *Lipids*, **21**, 356–361.

van Echten-Deckert, G. (2000) Sphingolipid extraction and analysis by thin-layer chromatography. *Meth. Enzymol*., **312**, 64–79.

Vitiello, F. and Zanetta, J-P. (1978) Thin-layer chromatography of phospholipids. *J. Chromatogr. A,* **166**, 637–640.

Wells, M.A. and Dittmer, J.C. (1965) A preparative method for the isolation of brain cerebroside, sulfatide and sphingomyelin. *J. Chromatogr*., **18**, 503–511.

6.5 Nuclear Magnetic Resonance Spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy is one of the most common analytical methods in chemistry and the related sciences due to the wealth of information available from the spectra. Accordingly, both ^{1}H- and ^{13}C-NMR spectroscopy are routinely used in lipid chemistry. This section briefly discusses the basics of analyzing lipids by NMR but also touches on subjects such as quantification. The NMR phenomenon was discovered independently and simultaneously by Purcell and his associates at Harvard University and by Bloch and coworkers at Stanford University, for which they were jointly awarded the Nobel prize in physics in 1952. The theoretical background of NMR is described in numerous textbooks and websites and therefore will not be discussed here. A basic discussion is also available in the second edition of *The Lipid Handbook* (Gunstone et al., 1994). The website (www.lipidlibrary.co.uk) and the literature cited here (e.g., Gunstone, 1993) contain additional detailed information.

For the purpose of obtaining NMR spectra, the lipid sample is usually dissolved in a deuterated solvent or one that does not contain hydrogen. Deuterated chloroform ($CDCl_3$) is currently the most common solvent used for this purpose. In less recent literature, often spectra obtained in CCl_4 or other solvents were reported. The data from less recent literature using the τ scale (TMS: $\tau = 10$) have been recalculated to conform to the δ- scale.

6.5.1 ^{1}H-NMR spectroscopy

All values reported here use the δ scale, in which the signal of tetramethylsilane (TMS) has been assigned the value $\delta = 0$. In practice, many spectra are now obtained using the known shift values of the solvent signal(s) as reference.

Table 6.6 contains information on the most common functional groups in lipid chemistry and their ^{1}H-NMR chemical shifts and is taken largely from the second edition of *The Lipid Handbook* (Gunstone et al., 1994). To illustrate these values, the ^{1}H-NMR spectrum of methyl linoleate (Figure 6.13) is given as an example since it contains most of the common functional groups in a fatty acid chain. The assignments in Figure 6.13 are shown at the bottom of this page.

The abbreviations in parentheses refer to the splitting of the signals, s = singlet, d = doublet, t = triplet, q4 = quartet, q5 = quintet, m = multiplet (usually broad; br). The H-3 quintet is often not well resolved.

From the information in Table 6.6 and Figure 6.13, the spectra of other types of fatty acids can be deduced. For example, a saturated fatty acid chain (such as in methyl palmitate or methyl stearate) will show only five signals, namely those designated with the letters a, b, c, d, and h. In a monounsaturated fatty acid chain, such as in methyl oleate, the signals designated a, b, c, d, e, f, and h will be observed since the bis-allylic protons designated g are missing. In free fatty acids, of course, the singlet caused by the methyl ester protons disappears and is replaced by the OH of the acid. The nature of the ester does not affect the position of the signals of the protons in0 the fatty acid chain; however, signals of the ester moiety may overlap those of the fatty acid chain. For example, in ethyl esters the signal of the ethyl CH_3 group is contained within the large signal of the CH_2 groups of the fatty acid chain, in propyl esters the signal of H-3 in the fatty acid chain is overlapped by the second methylene group in the propyl moiety, and in butyl esters the signal of one methylene group is contained in the large methylene signal of the fatty acid methylene units.

The exact position of the signals caused by the protons in the fatty acid chain depends on the proximity of other functional groups. For example, in monounsaturated fatty acids, the signal of the olefinic protons "migrates" downfield and is split when the double bond is at C2 (Gunstone and Ismail,1967; Frost and Gunstone, 1975). In the case of proximity to C1, shifts are moved downfield for acids vs. methyl esters and *trans* vs. *cis* double bond configuration. For example, in CCl_4 the signals of 2(*Z*)-octadecenoic acid were observed at 6.285 (C3) and 5.735 (C2) ppm, while the corresponding ester showed peaks at 6.145 (C3) and 5.680 (C2) ppm, and 2(*E*)-octadecenoic acid displayed shifts at 7.01 (C3) and 5.75 (C2) ppm (Frost and Gunstone, 1975). With the signals toward the middle of the chain, shift values around 5.30 ppm were observed in CCl_4 (Gunstone and Ismail, 1967; Frost and Gunstone, 1975). Coupling constants are a facile method to distinguish *cis* and *trans* double bonds in ^{1}H-NMR, especially for monounsaturated compounds.

CH_3–O–CO–CH_2 – CH_2 – $(CH_2)_4$ – CH_2 – CH = CH – CH_2 – CH = CH – CH_2 – $(CH_2)_3$ – CH_3

a	b	c	d	e	f	f	g	f	f	e	d	h
(s)	(t)	(q5, br)	(m)	(q4)	(m)	(m)	(t)	(m)	(m)	(q4)	(m)	(t)

TABLE 6.6 Assignments of proton signals in the ^{1}H-NMR spectra of fatty compounds; all values relative to tetramethylsilane (TMS) = 0 ppm

Structure	Shift values [a]
— C*H*$_2$ — (cyclopropane)	(-0.3) – 0.6
— C*H*$_2$ — (cyclopropene)	0.6 (singlet)
— C*H*$_3$ (terminal methyl in alkyl chain)	0.85–0.90 (triplet)
— C*H*$_3$ (branched, saturated isoprenoid)	0.85–0.90 (singlet or doublet)
— C(C*H*$_3$)$_2$ isopropyl methyl	1.2–1.3
(ω1)C*H*$_2$, saturated alkyl chain	1.21.3
— C*H*$_2$ — , acyl C-3, saturated chains	1.58
— C*H*$_2$ — , acyl C-4 to C-(ω3). saturated chains; (ω2)CH$_2$, saturated chain	1.2–1.3
RS*H* (sulfhydryl)	1.1–1.5[b]
RN*H*$_2$ (amino)	1.1–1.5 (1.8)[b]
R$_2$N*H* (imino)	0.4–1.6 (2.2)[b]
R$_3$C-*H* (saturated)	1.4–1.7
— C=C — C*H*$_3$ (allylic methyl)	1.6–1.9 (doublet)
— C=C—C*H*$_2$ — (allylic methylene)	2.04 (doublet)
— C=C — C*H*$_2$ — C=C — (diallylic methylene)	2.8 (triplet)
— C*H*$_2$ — COOR, acyl C2	2.1–2.3 (triplet)
— C*H*$_2$ — CO — (α-metbylene in ketone)	2.2–2.5
COOR-C*H*$_3$ (methyl in acetoxy)	1.9–2.6 (singlet)
Ar — C*H*$_3$	2.1–2.5
— C–C — *H* (terminal acetylene, nonconjugated)	2.5–2.7
— O — C*H*$_3$ (methoxy ether, aliphatic)	3.3–3.8 (singlet)
— O — C*H*$_3$ (methyl ester, aliphatic)	3.6–3.8 (singlet)
— C*H* — OH, *sn*-2 in glycerol	3.75 (multiplet)
— C*H*$_2$ — OH, *sn*-1 or *sn*-3 in glycerol	3.6 (doublet)
— O — C*H*$_2$ — (aliphatic saturated alcohol or ether)	3.4–3.7
— C*H*$_2$ — O — CO — R (*sn*-1 or *sn*-3 esterified glycerol)	4.2–4.4
— C*H* — O — CO — R (*sn*-2 esterified glycerol)	5.1–5.2 (quintet)
— C*H*$_2$ — O — R (*sn*-1- or *sn*-3-*O*-alkylglycerol)	3.5–3.6
— C*H* — O — R (*sn*-2-*O*-alkylated glycerol)	3.6–3.7
— C*H* — O — P (*O*-acylglycerol; *sn*-1 or *sn*-3)	3.9
— C*H*$_2$ — O — P (*O*-alkylglycerol; *sn*-1 or *sn*-3)	3.9
— C*H*$_2$ — O — P (choline or sulfocholine)	4.3–4.4
R — O*H* (hydroxyl proton)	3.0–5.3
R — C*H* = C*H* — O (vinyl ether)	5.8 (*cis*), 6.0 (*trans*)
C=C*H*$_2$ (terminal vinyl, nonconjugated)	4.6–5.0
H — C = C — *H* (olefinic or cyclic; nonconjugated)	5.1–5.9 (multiplet)
— C*H* = C*H* — R, *cis*-Δ^2 in fatty acid chain	7.0 (-), 5.8 (-)
cis-Δ^3 in fatty acid chain	5.6
cis-Δ^4 in fatty acid chain	5.5
cis-Δ^5 in fatty acid chain	5.4
cis-Δ^6 in fatty acid chain	5.4
cis-Δ^9 in fatty acid chain	5.3
cis-Δ^{12} in fatty acid chain	5.3

(Continued)

TABLE 6.6 Continued

Structure	Shift values [a]
— $(CH_3)C$=C — *H* (olefinic isoprenoid)	5.0–5.1
— C = CH_2 (terminal vinyl, conjugated)	5.3–5.7 (6.2)
H — C = C — *H* (olefinic, conjugated; diene or triene)	5.8–6.5 (7.1)
— CO — N — *H* (amide NH and CO)	5.5–8.5
Ar - *H* (benzenoid)	7.3 - 8.5
RC*H*O (aldehyde proton aliphatic saturated)	(9.5) 9.7–9.8
aliphatic, α,β-unsaturated	9.5–9.7
R-COO*H* (carboxyl)	10.5–12.0

[a] Values in parentheses apply to compounds that may absorb outside this range.
[b] Concentration-dependent; higher δ when diluted.
Source: Gunstone, F.D., Harwood, J.L., and Padley, F.B. (1994) *The Lipid Handbook*, 2nd ed., Chapman & Hall, London. With permission.)

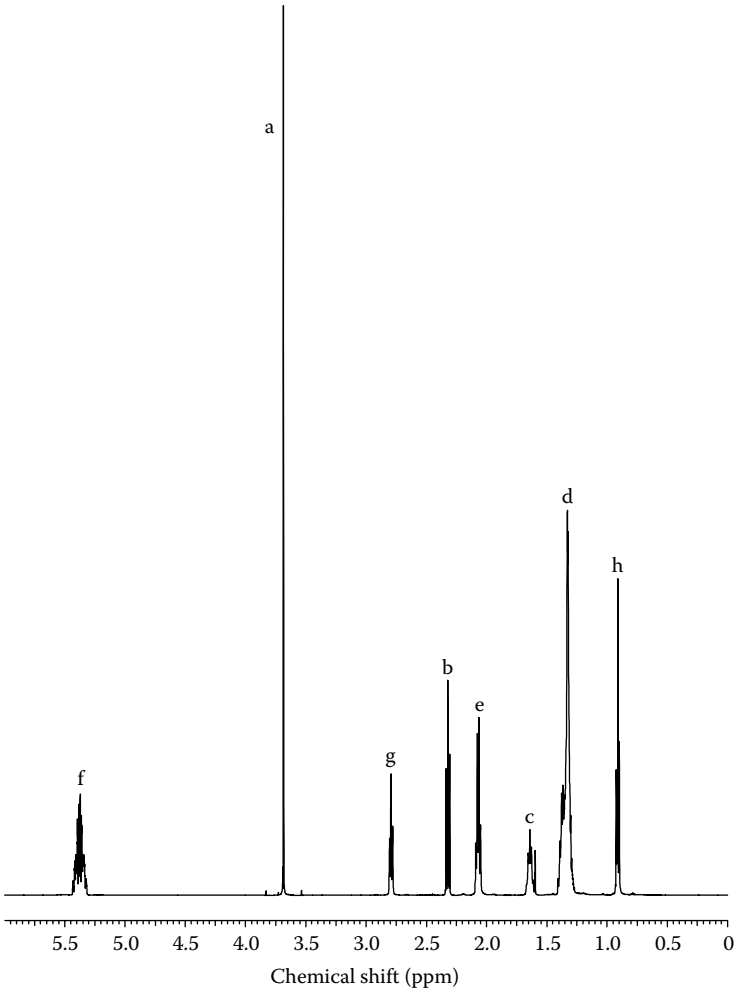


FIGURE 6.13 ^{1}H-NMR spectrum of methyl linoleate. (See text for explanation of letters.)

In contrast to methyl linoleate with methylene-interrupted unsaturation, in conjugated linoleic acid (CLA) the peaks of the olefinic protons are split. The number of signals depends on the double bond configuration. Two peaks are observed when the two double bonds have identical configuration and four signals when the configuration is not identical. Thus, in methyl 9*(Z)*,11*(Z)*-octadecadienoate, a multiplet assigned to the "outer" positions

9 and 12 and doublet of doublets at 6.22 caused by the "inner" protons 10 and 11 is observed), while for the corresponding all-*E* isomer the shift values are 5.56 ppm ("outer" protons) and 5.96 ppm ("inner" protons) (Lie Ken Jie et al., 1997). The 9*(Z)*,11*(E)* isomer displayed two multiplets at 5.32 and 5.56 ppm and two triplets at 5.82 ppm and 6.24 ppm attributable to H-9, H-12, H-10, and H-11, respectively (Lie Ken Jie et al., 1997).

In fatty compounds with triple bonds, the signals of the protons adjacent to the triple bonds are those of major interest. These propargylic protons resonate at 2.05 to 2.3 ppm in monoacetylenic fatty acid chains with the downfield shifts occurring in case of greater proximity of the triple bond to C1 (Gunstone and Ismail, 1967; Frost and Gunstone, 1975). The signals of the propargylic protons can be split depending on the proximity of one of the triple bonds to C1 or the proximity of triple bonds to each other. When the triple bond is located at C3, the shift of C2 was observed at 3.205 ppm (acid) or 3.135 ppm (methyl ester) and for the triple bond at C4 the shifts were 2.445 ppm for the acid and 2.33 ppm for the ester (Gunstone and Ismail, 1967; Frost and Gunstone, 1975). With increasing distance from C1, the shift of C2 approximates its "usual" value of about 2.25 ppm. For bis-propargylic protons in compounds with two triple binds, shift values around 2.98 to 3.07 ppm (in CCl_4) are observed (Frost and Gunstone, 1975). Results for chains with mixed unsaturation (mainly one triple and one double bond, etc.) were also reported (Frost and Gunstone, 1975).

The introduction of other functional groups into the fatty acid chain induces changes to the NMR spectrum. Probably the most common additional moieties contain oxygen. For example, in saturated hydroxy fatty acids, an additional signal for the proton attached to the hydroxy-bearing carbon arises. This signal moves downfield, similar to the discussion above on olefinic protons when the OH group "moves" closer to either end of the molecule. In CCl_4, the signal of the proton attached to the hydroxy-bearing carbon was observed at 3.93 ppm for 2-hydroxystearate, 3.80 ppm for 3-hydroxystearate, 3.48 ppm for 4-hydroxystearate, while for the stearates with the OH at C7-C14 this signal was observed at 3.38 to 3.40 ppm (Tulloch, 1966). For 15-, 16-, 17-, and 18-hydroxystearate, the signals were observed at 3.42, 3.34, 3.58, and 3.46 ppm, respectively (Tulloch, 1966). The introduction of the hydroxy group into unsaturated fatty acids leads to more additional peaks. For example, in methyl ricinoleate an additional signal (triplet) at around 2.1 ppm (in $CDCl_3$), while the signal of the olefinic protons is split due to the proximity of the OH group into signals at about 5.45 (H-10) ppm and 5.60 ppm (H-9).

In epoxy fatty acids, the shift values of the protons attached to the carbons carrying the epoxy group depend on the *cis*- or *trans* configuration of the epoxy group. The shifts of the *cis* epoxy isomers are downfield from those of the *trans* isomers. For methyl *cis*-2,3-epoxyoctadecanoate, the signals are observed at 3.30 and 2.99 ppm, while for the *trans* isomer a value of 2.99 ppm is observed (solvent CCl_4; Gunstone and Jacobsberg, 1972). When the epoxy group is located towards the middle of the chain, the shift values in CCl_4 are 2.68 to 2.70 ppm for the *cis*-epoxy group and 2.45 to 2.49 for *trans*-epoxy. For the methyl ester of vernolic acid (*cis*-12,13-epoxy-9(*Z*)-octadecenoic acid), the protons at C-12 and C-13 give a signal at 2.82 ppm, while olefinic protons signal is split at 5.33 ppm (H-10) and 5.43 ppm (H-9) and the signal of the diastereotopic H-11 protons is split at 2.10 and 2.27 ppm for one proton each (Fürmeier and Metzger, 2003). When the epoxy group is located directly next to the double bond, the signal of the epoxy group is split; for example, in methyl *trans*-11,12-epoxy-9*(Z)*-octadecenoate, the shifts are observed as multiplets 3.31-3.35 ppm (H-11) and 2.79-2.83 ppm (H-12) and the olefinic protons at 5.64 to 5.73 ppm (H-9) and 5.00 to 5.08 ppm (H-10) (Lie Ken Jie et al., 2003). For methyl *cis*-9,10-epoxy-11(E)-octadecenoate, the values were 5.92 ppm (H-12), 5.25 to 5.34 ppm (m, H-11) for the olefinic protons and 3.36 ppm (q, H-10) as well as 3.01 to 3.09 (m, H-9) for the epoxy protons (Lie Ken Jie et al., 2003).

In fatty compounds with oxo (keto) moieties, the protons α to this functionality are often observed around 2.50 ppm. Thus, in methyl 3-oxo-hexadecanoate, the signal of the protons at C2 was a singlet at 3.45 ppm (Lie Ken Jie and Lam, 1996). The protons to the oxo group at C4 were observed at 2.53 ppm. With an oxo group α to the terminal methyl group, the signal of the latter became a singlet downfield at 2.13 ppm in methyl 12-oxo-3(*E*)-truidecenoate (Lie Ken Jie and Lam, 1996). The protons to the oxo groups in fatty compounds with 1,2-dioxo groups gave triplets at 2.70 ppm (Knothe 2002). If an oxo group is close to a double bond the signal of the olefinic protons splits and is moved downfield considerably, for example, in 9(*E*)-11-oxooctadecenoic acid the olefinic signals were observed at 6.8 ppm (H-9) and 6.1 ppm (H-10) with the protons α to the oxo group again resonating at 2.5 ppm (Porter and Wujek, 1987). Similar results are available from other literature.

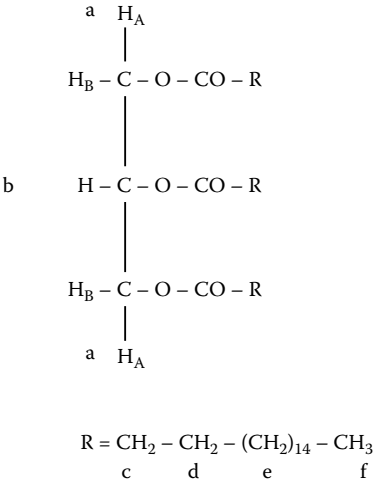
For isostearic acid (16-methylheptadecanoic acid), the two equivalent terminal methyl groups show a doublet. If the branching is in the middle of the chain, for example, a lone methyl group, then the branched methyl group displays a doublet slightly upfield of the triplet generated by the terminal methyl group. If the methyl group is close to C1, such as at C3, the signal of the distereotopic protons at C2 is split. If the methyl grouup is close to a double bond, such as in 9-methyl-10(*Z*)-octadecenoic acid, the signal of the olefinic protons is split again, as it is with other groups, in this case giving signals at 5.08 ppm (H-10) and 5.24 to 5.37 (broad multiplet of H-11) (Carballeira et al., 1999).

In fatty acids with a cyclopropene unit in the chain, the protons of the ring methylene caused a singlet at 0.73 to

0.75 ppm (Gosalbo et al., 1993). Protons β to the ring overlapped with the C3 protons to give a signal at 1.57 ppm while a signal at 2.37 ppm was assigned to CH_2 adjacent to the ring (Gosalbo et al., 1993). In fatty acids with a terminal cyclopentene ring, the olefinic protons in the ring gave a signal at 5.58 ppm and the methine proton of the carbon carrying the ring was observed at 2.60 ppm (Blaise et al., 1997). The signal of the methylene protons to the carbon in the cyclopentene ring gave two signals at 2.02 and 1.27 ppm, while the two protons on the carbon to the ring also gave a split signal at 1.25 and 1.35 ppm (Blaise et al., 1997).

Signal integration yields information on the number of protons causing the signals. Thus, the number of double bonds, etc., can be determined in this fashion. In methyl linolenate, which contains an ω-3 double bond, the signal of the terminal methyl group at C18 is shifted slightly downfield and in mixtures can be integrated separately of the corresponding signal of other components. This forms the basis of some quantifications, such as determination of the fatty acid profile.

As an example of a triacylglycerol, the ^{1}H-NMR spectrum of tristearin is shown in Figure 6.14. The assignments in the figure are as follows:



The ^{1}H-NMR spectra of numerous triacylglycerols have been discussed in the literature (Lie Ken Jie and Lam, 1995).

The ^{1}H-NMR spectrum of the corresponding 1,3-diacylglycerol (1,3-distearin) also shows the resonance of the H_A and H_B protons at around 4.2 ppm, but in this case it almost overlaps the proton at the C2 carbon of the glycerol backbone. In the 1-monoacylglycerol, numerous peaks can be identified. The most upfield signals (doublet of doublets) are those of the two C1 glycerol protons at 4.18 and 4.25 ppm. The other protons give individual signals at around 3.95 ppm (C2 glycerol proton) with the two protons at C3 split at about 3.65 and 3.73 ppm.

6.5.2 ^{13}C-NMR spectroscopy

Similar to the discussion of ^{1}H-NMR above, an unsaturated compound shall serve as starting point for the discussion. ^{13}C-NMR data for linoleic acid have been given in the literature (Gunstone, 1993) and are shown at the bottom of this page.

The ^{13}C-NMR spectrum of the methyl ester of linoleic acid is shown in Figure 6.15. Major differences to the values for linoleic acid are the presence of the peak of the methyl ester moiety at 51.41 ppm and the upfield shift of the carbonyl carbon to 174.27 ppm.

Generally, many effects observed in ^{1}H-NMR are also found in ^{13}C-NMR. For example, the methyl and methylene signals are upfield in the spectrum, while signals of olefinic carbons are farther downfield. The number and nature of double bonds affects the chemical shifts as does the proximity of multiple double bonds to each other and the presence of functional groups. In case of acylglycerols, it plays a role if the double bond is in the α or β chain with detailed data given by Lie Ken Jie and Lam (1995). Again, qualitatively the spectra of other fatty compounds can be reasonably deduced from the information given above for linoleic acid. For example, the spectrum of oleic acid (or methyl oleate) will not show the signal of the bis-allylic carbon and there will be only two olefinic carbon signals. The number of signals in the "methylene envelope" at around 29 to 30 ppm, however, will obviously increase. With the increasing number of closely spaced signals, correctly carrying out assignments of the signals in the methylene envelope to individual carbons becomes increasingly difficult and other more sophisticated experiments, such as those described briefly below, may not always help. Table 6.7 is a compilation of the chemical shifts of functional groups in ^{13}C-NMR. While most authors report ^{13}C-NMR data to two decimal places, these data can vary slightly from laboratory to laboratory and even when repeating the same experiment in a certain laboratory. Therefore, ranges are given in Table 6.7, similar to Table 6.6.

6.5.2.1 Double bonds

Since there are numerous combinations of double bond positions and configurations possible in unsaturated fatty acids with more than one double bond and the relatively narrow range in which they are observed (with the exception of some functional groups inducing shift changes), these individual combinations are not listed separately in Table 6.7. The shifts of *trans* double bonds are slightly downfield from those of *cis* double bonds, for example, if a lone double bond is located towards the

$HOOC - CH_2 - CH_2 - (CH_2)_4 - CH_2 - CH = CH - CH_2 - CH = CH - CH_2 - CH_2 - CH_2 - CH_2 - CH_3$

180.54 34.15 24.70 29.08-29.63 27.22 130.02 128.12 25.67 127.95 130.21 27.25 29.19 31.58 22.62 14.09

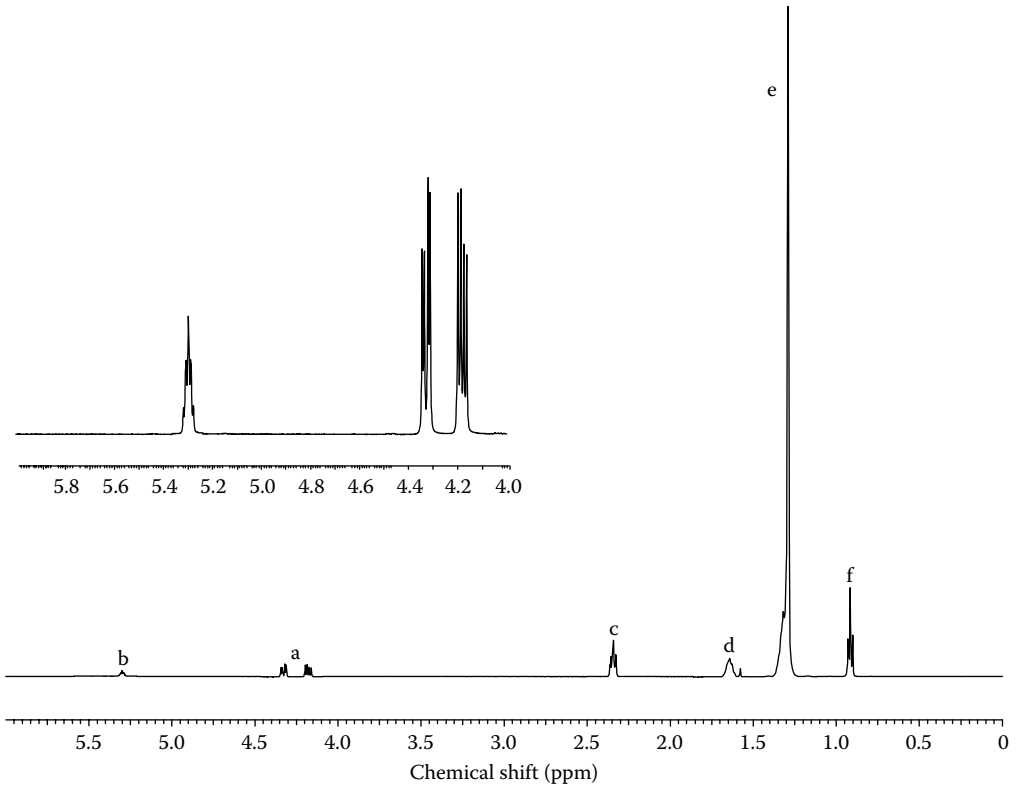


FIGURE 6.14 ^{1}H-NMR spectrum of tristearin. (See text for explanation of letters.)

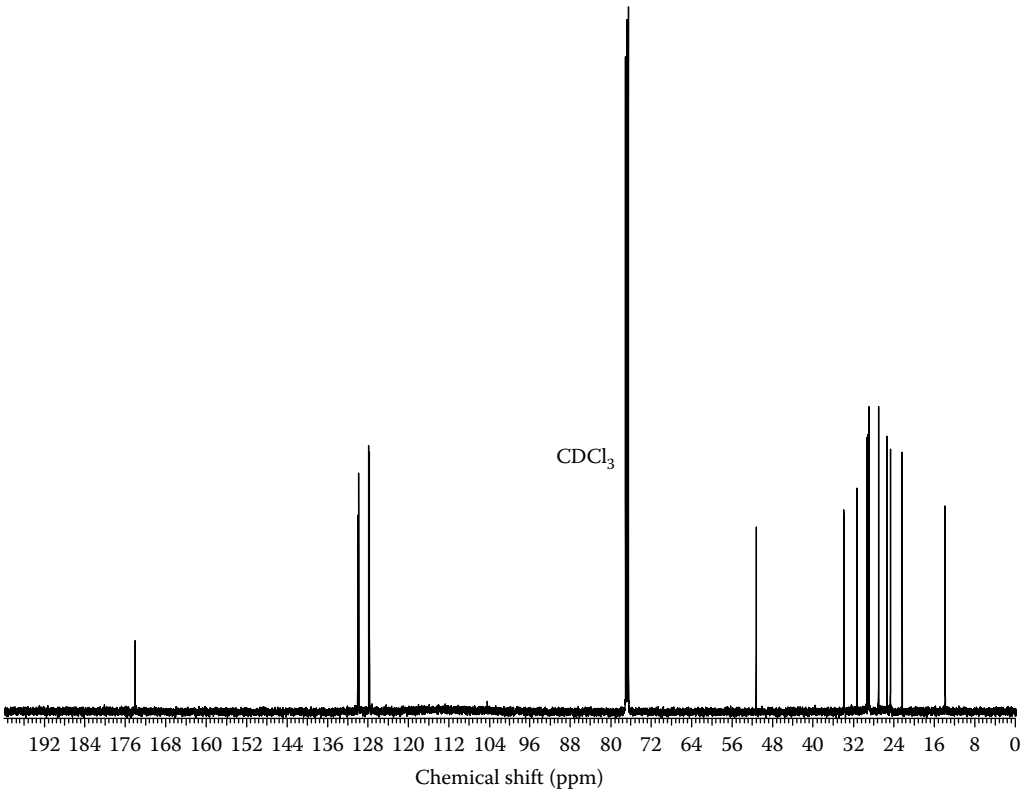


FIGURE 6.15 ^{13}C-NMR spectrum of methyl linoleate.

TABLE 6.7 Assignments of carbon signals in the ^{13}C-NMR spectra of fatty compounds ($CDCl_3$)

Acids and Esters	**Assignment**
HOO***C*** – $(CH_2)_x$	179–181
HOO***C*** – $CHCH_3$ – $(CH_2)_x$	183–184
CH_3O – ***C***O – ; other alkyl esters	172–175
CH_3O – CO	51–52
Glycerol esters	
Triacylglycerols	
Glyc-O-***C***O-$(CH_2)_x$ – CH_3	173–173.2 (-chain); 172.7-172.9 (-chain)
Glyc-O-***C***O-$(CH_2)_x$ – CH = CH - R	172.5–172.9 (-chain); 172.1–172.5 (-chain)
CH_2 – O – CO – R	62–62.5
|	
CH – O – CO – R	68.5–69
|	
CH_2 – O – CO – R	62–62.5
Diacylglycerols	
CH_2 – O – CO – R	ca. 61.5–62
|	
CH – O – CO – R	72–72.5
|	
CH_2OH	ca. 62
CH_2 – O – CO – R	ca. 65
|	
CH_2	68–68.5
|	
CH_2 – O – CO – R	ca. 65
Monoacylglycerols	
CH_2 – O – CO – R	63–63.5
|	
CHOH	70-70.5
|	
CH_2OH	65.65.5
CH_2 OH	61.5–62
|	
CH – O – CO – R	74.5–75
|	
CH_2OH	61.5–62
Methyl esters	
– $(CH_2)_x$ – ***C***H_3 ; x > 1	13.5–14.5
Methylene in saturated chains	
$(C H_2)_x$	29–30
HOOC – ***C***H_2 – $(CH_2)_x$ – or CH_3O – CO – ***C***H_2 – $(CH_2)_x$ –	34-35
HOOC – CH_2 – ***C***H_2 – $(CH_2)_x$ – or CH_3 O – CO – CH_2 – ***C***H_2 – $(CH_2)_x$ –	23–26
– $(CH_2)_x$ – ***C***H_2 – CH_3	22–23
– $(CH_2)_x$ – ***C***H_2 – CH_2 – ***C***H_3	31.5–32.5
Double bonds	
– ***C***H = ***C***H –	125–135 (exceptions given below)
$(CH_2)_x$ – CH = ***C***H_2	114–115
$(CH_2)_x$ – ***C***H = CH_2	139–140
$(CH_2)_x$ – ***C***H_2 – CH = ***C***H_2	33–35
$(CH_2)_x$ – ***C***H_2 – C = C – ***C***H_2 – $(CH_2)_y$	27–28 (*cis*); 32–33 (*trans*)
HOOC – ***C***H_2 – C = C	33–34 (*cis*)
HOOC – CH_2 – ***C***H_2 – C = C	22–23 (*cis*)
– CH=CH – ***C***H_2 – CH_3	20–21(*cis*); 25–26 (*trans*)

(Continued)

TABLE 6.7 Continued

	Assignment
– CH=CH – ***C***H_2 – ***C***H_2 – ***C***H_3	29-30 (*cis*); 34–35 (*trans*)
– C = C – ***C***H_2 – CH = CH –	25–26 (all *cis*); 35–36 (all *trans*); 30–31 (*cis, trans*)
– CH = ***C*** = CH –	200–205
– CH = C = ***C***H –	90–92
Triple bonds	
$(CH_2)_x$ – ***C*** ≡ ***C*** – $(CH_2)_y$	79–81
$(CH_2)_x$ – ***C***H_2 – C ≡ C – ***C***H_2 – $(CH_2)_y$	18–19
– C ≡ ***C*** – CH_2 – ***C*** ≡ C –	74–75
– C ≡ C – ***C***H_2 – C ≡ C –	9–10
– C ≡ C – ***C***H_2 – ***C***H_2 – C ≡ C –	19–20
– ***C*** ≡ C – C ≡ ***C*** –	77–78
– C ≡ ***C*** –***C*** ≡ C –	65–66
–***C***H_2-C ≡ C –	18–20
Hydroxy groups	
$(CH_2)_x$ – ***C***HOH – $(CH_2)_y$	71–73
$(CH_2)_x$ – ***C***H_2 – CHOH – ***C***H_2 – $(CH_2)_y$	37–38
$(CH_2)_x$ – CH = CH – ***C***HOH – $(CH_2)_y$	67–68 (*cis*); 73–74 (*trans*)
$(CH_2)_x$ – ***C***H = ***C***H – CHOH – $(CH_2)_y$	131–134; (*trans*; CH adjacent to CHOH downfield)
$(CH_2)_x$ – ***C***H = CH – CH_2 – CHOH – $(CH_2)_y$	133–134 (*cis*)
$(CH_2)_x$ – CH = ***C***H – CH_2 – CHOH – $(CH_2)_y$	125–126 (*cis*)
$(CH_2)_x$ – CH = CH – ***C***H_2 – CHOH – $(CH_2)_y$	35–36 (*cis*)
$(CH_2)_x$ – ***C***H = CH – CH_2 – CH_2 – CHOH – $(CH_2)_y$	130–131 (*cis*)
$(CH_2)_x$ – CH = ***C***H – CH_2 – CH_2 – CHOH – $(CH_2)_y$	129–130 (*cis*)
$(CH_2)_x$ – CH = CH – ***C***H_2 – CH_2 – CHOH – $(CH_2)_y$	23–24 (*cis*)
$(CH_2)_x$ – CH = CH – CH_2 – ***C***H_2 – CHOH – $(CH_2)_y$	37–38 (*cis*)
$(CH_2)_x$ – ***C***HOH – ***C***HOH – $(CH_2)_y$	74–75 (erythro slightly > threo)
$(CH_2)_x$ – ***C***H_2 – CHOH – CHOH – ***C***H_2 – $(CH_2)_y$	31–32 (erythro), 33–34 (threo)
$(CH_2)_x$ – CHOH – ***C***H_2 – ***C***H_2 – CHOH – $(CH_2)_y$	32–33 (erythro), 33–34 (threo)
$(CH_2)_x$ – ***C***H_2 – CHOH – CH_2 – CH_2 – CHOH – ***C***H_2 – $(CH_2)_y$	37–38 (threo slightly > erythro)
$(CH_2)_x$ – CHOH – ***C***H = ***C***H – CHOH – $(CH_2)_y$	133–134; (*trans*; threo slightly > erythro)
Hydroperoxy compounds	
$(CH_2)_x$ – CH_2 – ***C***H(OOH) – CH = CH – CH= CH – $(CH_2)_y$	86–88
$(CH_2)_x$ – ***C***H_2 – CH(OOH) – CH = CH – CH = CH – $(CH_2)_y$	25–26
Oxo compounds	
$(CH_2)_x$ – ***C***O – $(CH_2)_y$	209–213
$(CH_2)_x$ – ***C***H_2 – CO – $(CH_2)_y$	42–43
$(CH_2)_x$ CH = CH – ***C***H_2 – CO – $(CH_2)_y$	41–42
$(CH_2)_x$ ***C***H = CH – CH_2 – CO – $(CH_2)_y$	133–134
$(CH_2)_x$ CH = ***C***H – CH_2 – CO – $(CH_2)_y$	121–122
$(CH_2)_x$ – CO – ***C***H_2 – ***C***H_2 – CO – $(CH_2)_y$	36–37
$(CH_2)_x$ – *C*O – CH = CH – ***C***O – $(CH_2)_y$	203-204 *cis*; 200–201 *trans*
$(CH_2)_x$ – CO – *C*H = *C*H – CO – $(CH_2)_y$	135–136 *cis*; 136–137 *trans*
Epoxy and furanoid compounds	
$(CH_2)_x$–CH(O)CH–$(CH_2)_y$	56–57 (*cis*); 58–59 (*trans*)
$(CH_2)_x$–CH_2–CH(O)CH–CH_2–$(CH_2)_y$	27–28 (*cis*); 31–32 (*trans*)

(Continued)

TABLE 6.7 Continued

	Assignment
$(CH_2)_x-CH=CH-\boldsymbol{C}H_2-\underset{\diagdown O \diagup}{CH-CH}-\boldsymbol{C}H_2-(CH_2)_y$	25–26 (*cis; cis*-epoxy)
$(CH_2)_x-\boldsymbol{C}H=CH-CH_2-\underset{\diagdown O \diagup}{CH-CH}-\boldsymbol{C}H_2-(CH_2)_y$	132–133 (*cis; cis*-epoxy)
$(CH_2)_x-CH=\boldsymbol{C}H-CH_2-\underset{\diagdown O \diagup}{CH-CH}-\boldsymbol{C}H_2-(CH_2)_y$	123–124 (*cis; cis*-epoxy)
$-(CH_2)_2-\underset{\boldsymbol{C}-\boldsymbol{C}}{\overset{O}{\boldsymbol{C}-\boldsymbol{C}}}-(CH_2)-$	104–105 154–155
Acetoxy	
$(CH_2)_x - \boldsymbol{C}O(COCH_3) - (CH_2)_y$	73–75
$(CH_2)_x - CO(\boldsymbol{C}OCH_3) - (CH_2)_y$	170–171
$(CH_2)_x - CO(CO\boldsymbol{C}H_3) - (CH_2)_y$	20–22
$CH_3 - O - CO - \boldsymbol{C}O(COCH_3) - (CH_2)_y$	72–73
$CH_3 - O - CO - \boldsymbol{C}O(COCH_3) - (CH_2)_y$	70–71
$(CH_2)_x - \boldsymbol{C}H_2 - COCH_3$	64–65
Cyclic compounds (cyclopropene fatty acids)	
$(CH_2)_x - \underset{\diagup \quad \diagdown}{\overset{CH_2}{\boldsymbol{C}H=\boldsymbol{C}H}} - (CH_2)_y$	7–8 109–110
$(CH_2)_x-\boldsymbol{C}H_2-\underset{\diagup \quad \diagdown}{\overset{CH_2}{CH=CH}}-\boldsymbol{C}H_2-(CH_2)_y$	25.5–26.5
$HOOC-(CH_2)_x-CH_2-\underset{\diagup \quad \diagdown}{\overset{CH_2}{\boldsymbol{C}H=CH}}-CH_2-(CH_2)_y$; x = 2–3	107–109
$HOOC-(CH_2)_x-CH_2-\underset{\diagup \quad \diagdown}{\overset{CH_2}{CH=\boldsymbol{C}H}}-CH_2-(CH_2)_y$; x = 2–3	110–112
Branched compounds	
$(CH_2)_x - CH(\boldsymbol{C}H_3) - (CH_2)_y$	19–20
$(CH_2)_x - \boldsymbol{C}H(CH_3) - (CH_2)_y$	32–33
$(CH_2)_x - \boldsymbol{C}H_2 - CH(CH_3) - \boldsymbol{C}H_2 - (CH_2)_y$	3637
$HOOC - \boldsymbol{C}H(CH)_3 - (CH_2)_x -$ or $CH_3 - O - CO - CH(CH)_3 - (CH_2)_x$	39–40
$HOOC - CH(\boldsymbol{C}H)_3 - (CH_2)_x -$ or $CH_3 - O - CO - CH(CH)_3 - (CH_2)_x$	16.5–17.5
$CH_3 - O - CO - CH_2 - \boldsymbol{C}H(CH)_3 - (CH_2)_x$	30–31
$CH_3 - O - CO - CH_2 - CH(\boldsymbol{C}H)_3 - (CH_2)_x$	19–20
$CH_3 - O - CO - \boldsymbol{C}H_2 - CH(CH)_3 - (CH_2)_x$	41–42
$CH_3 - O - CO - CH_2 - CH_2 - \boldsymbol{C}H(CH)_3 - (CH_2)_x$	31–32
$CH_3 - O - CO - CH_2 - CH_2 - CH(\boldsymbol{C}H)_3 - (CH_2)_x$	19–20

middle of a long chain, the values for *cis* are about 129.9 ppm and *trans* 130.4 ppm. Similar to ^{1}H-NMR spectroscopy, when the double bond approaches one end of the molecule, the signals of the olefinic protons become more strongly separated. Such differences are usually evaluated in terms of additive increments, but rational functions can be used (Knothe and Nelsen 1998). In practice, the number of double bonds can be determined by the number of signals and intensity (in case of signal overlap). The positions and configuration of double bonds may, in many cases, be better determined by the shifts of the allylic methylene carbons, although for lone double bonds the shifts of the olefinic carbons and their differences can be definitive, especially if the lone double bond approaches one end of the molecule. Extensive reports on the ^{13}C-NMR spectra of unsaturated fatty acids and esters are Gunstone et al. (1976, 1977). Other extensive reports exist on fatty acids

with functional groups, for example, those with oxo (Tulloch, 1977), hydroxy and acetoxy groups (Tulloch, 1978) and epoxy groups (Gunstone and Schuler, 1975).

Generally, the shifts of methylene carbons cover a greater range of ppm values and are very sensitive to the nature of the adjacent functional group(s). For this reason, they are dealt with more explicitly in Table 6.7, being arranged according to the functional group influencing the shift. Methylene carbons allylic to *trans* double bonds often show downfield shifts of 4 to 5 ppm compared to those methylenes allylic to *cis* double bonds.

The fact that ^{13}C-NMR spectra cover a wider ppm range (typically 0 to 200 ppm and even beyond) facilitates some aspects of evaluation compared to ^{1}H-NMR. A major aspect is that some functional groups impart shift values in parts of the spectrum where they do not overlap with the peaks of other moieties. Salient examples in this respect are epoxy groups, which show peaks in the range of 53 to 60 ppm and oxo groups, which cause significant downfield shifts beyond 200 ppm, around 210 to 212 for an oxo group in the middle of a fatty acid chain (see also Table 6.7). To some extent, this effect can also be found in methylenes adjacent to a group. For example, when an oxo group is present, the adjacent methylenes are shifted downfield to about 42 ppm. The influence of other moieties with more upfield signals, however, can then shift the signal of these methylenes upfield. Free fatty acid and methyl esters can be distinguished in this fashion, too, with the signals of C1 in free fatty acids around 179 to 181 ppm and in alkyl esters around 172 to 175 ppm and the methyl carbon in methyl esters causing a peak at 51 to 52 ppm. Again, such shifts may be perturbed by the presence of other functional groups. Double and triple bonds can be recognized in this fashion, too. For triple bonds under the influence of other unsaturation or other groups, it may need to be considered that the signal of chloroform in ^{13}C-NMR is close to their range. Diastereomers, such as those resulting from the presence of two hydroxy groups in the chain, can also be distinguished by ^{13}C-NMR with some data given in Table 6.7.

A full discussion of all possible changes to a spectrum for various combinations of functional groups is certainly beyond the scope of this section. Instead the reader is referred to Table 6.7 for some common combinations, including those not discussed in the text, and the literature cited in this chapter, which gives more details including numerous other functional group combinations and provides additional references.

6.5.3 Other NMR experiments

Besides acquiring the "normal" ^{1}H- and ^{13}C-NMR spectra, other NMR experiments can prove to be very helpful in assigning peaks and removing remaining ambiguities. Not all possible experiments in NMR are discussed, rather the reader is referred to the specialized literature for those not discussed here. However, the application and possible results of some methods will be briefly outlined here, but without discussion of their physical background.

DEPT (distortionless enhancement of polarization transfer) is an experiment that is useful for determining the number of protons attached to a carbon atom. In the usual DEPT experiment (DEPT-135; the number referring to the pulse angle of the experiment), the ^{13}C signals appear either as positive or negative peaks or disappear. Positive peaks correlate with carbons carrying an odd number of protons, in other words methine or methyl carbons. Negative peaks correlate with carbons carrying an even number of protons, in other words methylene carbons. Carbons without an attached hydrogen do not give a visible peak. This experiment, therefore, is useful for distinguishing carbons in areas of the spectrum where there can be significant overlap of the type of signal-causing carbons. C1 carbons in fatty acids and esters are easily distinguishable because their signal disappears in comparison to the "normal" proton-decoupled ^{13}C-NMR experiment. Figure 6.16 depicts the DEPT-135 spectrum of methyl linoleate. A comparison of this spectrum with the "normal" ^{13}C-NMR spectrum in Figure 6.15 shows the differences between the two experiments as discussed above. In the probably less routinely performed DEPT-90, only the peaks of methine are determined, thus allowing to distinguish between these species and methyl, which both give positive peaks in DEPT-135. In DEPT-45, all protonated carbons give positive peaks. Therefore, DEPT-45 appears less useful because DEPT-135, the "routine" DEPT, has the same feature but distinguishes carbons based on odd or even numbers of protons attached. However, DEPT-45 is more sensitive than the "normal" ^{13}C experiment, making it useful when less sample is available, but caution must be used in interpretation because of the missing peaks of nonprotonated carbons.

Two-dimensional experiments provide a means of identifying nuclei that are mutually coupled. In the COSY (COrrelation SpectroscopY) experiment, homonuclear-coupled protons are identified. The ^{1}H spectrum serves as x- and y-axis for a contour plot. The diagonal of the contour plot results from the cross peaks of an individual signal with itself. Besides the peaks in the diagonal, various cross peaks "scattered" across the contour plot, but symmetrical to the diagonal as mirror plane, signal coupling of specific protons. This is often very useful in identifying which kind of carbon a specific functional group is bound. For example, in a COSY experiment of a fatty acid, the signal of the terminal methyl group will show a contour outside the diagonal that can be correlated with the broad peak caused by the methylene protons, showing that the terminal group is connected to a CH_2 group.

Because of the low natural abundance of ^{13}C (ca. 1.1%), correlation spectroscopy between carbons is of no significance. Instead, heteronuclear correlation (HETCOR)

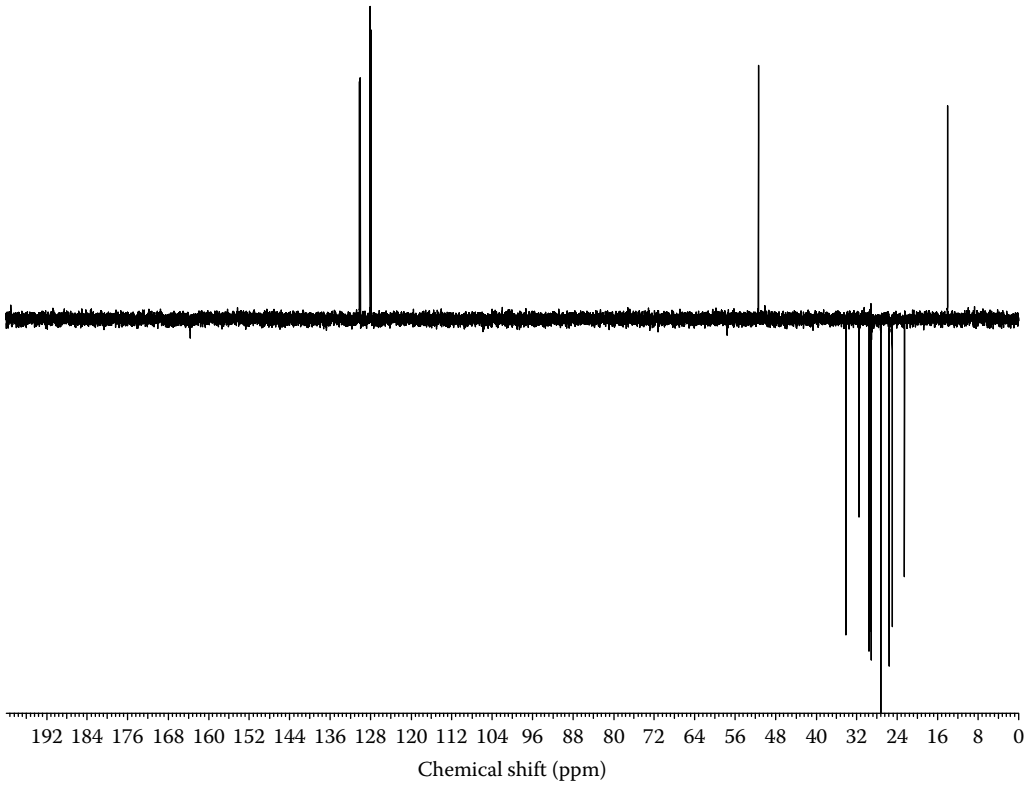


FIGURE 6.16 DEPT-135 (^{13}C) spectrum of methyl linoleate.

between ^{1}H and ^{13}C is now a common experiment yielding useful information. The common experiment is termed HMQC (heteronuclear multiple-quantum correlation). The experiment yields a contour map similar to the one described for COSY above, except that one axis is now the ^{13}C spectrum instead of the ^{1}H spectrum, and, of course, there is no diagonal of cross peaks. The contour plot indicates which ^{1}H-NMR peaks correlate with which ^{13}C peaks. An application is the following: in methyl linoleate shown above, several peaks are found in the region of about 24.5 to 27.27 ppm in ^{13}C. Fortunately all of these peaks can be correlated with distinct peaks in the ^{1}H-NMR spectrum, enabling their assignment. One potential difficulty is, however, that peaks that are close together can often only be assigned to the type of carbon with this procedure, not necessarily the specific carbon. An example is the allylic carbons in linoleic acid with signals at 27.22 and 27.25 ppm. The exact assignment to a specific carbon is often the result of careful comparison with other known model compounds and/or of theoretical considerations. Another example is the terminal methyl group of long-chain fatty acids usually exhibits its ^{13}C peak at 14.00 to 14.10 ppm. In a saturated fatty acid chain with a methyl group in the middle of the chain, this methyl group will generate another signal separate from that of the terminal methyl group. The branched methyl group will generate a doublet instead of the triplet of the terminal methyl group and this doublet is slightly upfield of the terminal CH_3 triplet. By heteronuclear correlation, a ^{13}C peak at 19.7 ppm correlates with the doublet of the mid-chain methyl group. Two-dimensional (2D) methods are also useful for identifying diastereotopic protons and assigning the ^{13}C signals of such species.

Thus, the combination of "regular" spectra, DEPT and 2D experiments is a powerful tool for determining the structure of lipids and organic compounds in general.

6.5.4 ^{31}P-NMR spectroscopy

Phosphorus NMR (^{31}P-NMR) spectroscopy is of interest for the analysis of phospholipids, but can also be employed when quantifying lipid components by means of phosphorus-containing derivatives. An advantage of ^{31}P-NMR is that phosphorus is monoisotopic, rendering it sensitive compared to other nuclei with less natural abundance. Signals in ^{31}P-NMR spectroscopy are distributed over almost 700 ppm and sometimes beyond that, thus facilitating separation of signals based on different environments and subtle structural differences. However, most signals of phospholipids are found in the relatively narrow range of + 5 to – 1 ppm, with each phospholipid giving a characteristic signal. Triphenyl-phosphate (TPP) is the commonly used internal standard in quantitative ^{31}P-NMR, an advantage being that its shift value of –17.8 ppm is beyond that of natural phospholipids, although artificial phospholipids, such as distearoylphosphatidylglycerol (DSPG) are also employed (Diehl 2002). ^{31}P-NMR is a reference method of the

International Lecithin and Phospholipid Society and was preferred over HPLC methods (Diehl, 2002).

Recent overviews of the applications of ^{31}P-NMR to problems in phospholipid research were provided by Diehl (2001, 2002). For some earlier papers on the analysis of phospholipids by ^{31}P-NMR, see Meneses and Glonck (1988) and London and Feigenson (1979). The applications of ^{31}P-NMR as discussed in the articles by Diehl (2001, 2002) are summarized below.

For quantification of phospholipids in a mixture, absolute quantities are obtained by adding an internal standard. This is of significance for the composition of lecithins and body fluids such as lung surfactant and human blood plasma. An example is the ratio of phosphatidylcholine to lysophosphatidylcholine in human plasma indicating the level of inflammation in rheumatoid arthritis (Fuchs et al., 2005).

Determination of the fatty acid profile of phospholipids: ^{31}P-NMR distinguishes double unsaturated and saturated/unsaturated phospholipids. This determination takes advantage of signal splitting in ultra-high resolution ^{31}P-NMR when phospholipids from bilayers and liposomes in aqueous solution, which yield broad signals. The splitting depends on the pH value of the solution with greatest splitting occurring at the pK of the investigated phospholipid. Thus, the composition of phosphatidic acid is 40% saturated/unsaturated and 60% saturated/unsaturated.

For phospholipid structure determination, in combination with enzymatic reactions, ^{31}P-NMR can be used to determine the chiral center of a racemic phospholipid. Also, signal splitting indicates diastereomeric phospholipids while lack of splitting indicates pure enantiomers.

Besides analyzing phospholipids, ^{31}P-NMR can be used for other purposes in lipid chemistry. For example, phosphorus-containing derivatives used for quantification purposes may exhibit signals in other spectral regions than phospholipids. Thus, the relevant peaks of mono- and diacylglycerols derivatized with 2-chloro-4,4,5,5-tetramethyldioxaphospholane are observed around 145 to 150 ppm (Spyros and Dais, 2000; Schiller et al., 2002). ^{31}P-NMR can be used accordingly to quantify the mono- and diacylglycerols in olive oils by phosphorylizing the free hydroxy groups with 2-chloro-4,4,5,5-tetramethyldioxaphospholane and integrating the appropriate peaks (Spyros and Dais, 2000). The method may be applicable to other oil constituents with labile protons. Thus, thermally stressed vegetable oils can be analyzed with ^{31}P-NMR (Schiller et al., 2002).

6.5.5 Quantification and applications of NMR

Fatty Acid Profile and Related Issues. This method utilizes integration values in ^{1}H-NMR applied to equations for determining the amounts of the unsaturated fatty acids. The results agree well with GC conducted as a control, although reportedly ^{13}C NMR results correlate even better with GC (Miyake et al., 1998). When determining the fatty acid profile of oil, the integration values of the protons need to be divided by 3. Using the equations (Knothe and Kenar, 2004):

$$A_{C18:3} = I_{exper,methyl,C18:3}/(I_{exper,methyl,C18:3} + I_{exper,methyl,rest})$$

$$A_{C18:2} = 0.5\ (I_{exper,bisallylic} - 4A_{C18:3})$$

$$A_{C18:1} = (I_{exper,allylic}/4) - A_{C18:2} - A_{C18:3}$$

in which A indicates the amounts of the subscripted fatty acids and I indicates the experimentally determined integration values of the terminal methyl, bisallylic and allylic protons, the fatty acid composition of a vegetable oil can be determined. Other related work is Miyake et al. (1998a).

Docosahexaenoic acid and ω-3 fatty acids in fish oils can be determined by ^{1}H NMR in good agreement with GC analyses (Igarashi et al., 2000). Other authors also reported on the determination of ω-3 fatty acids in fish oils (Aursand et al., 1993; Sacchi et al., 1993). Related, although less specific, parameters, such as the iodine value, which reflects the total amount of unsaturated fatty acids in an oil or fat, can also be determined by ^{1}H NMR (Miyake et al., 1998b). The key to this determination is that the signal of the terminal methyl group in ω-3 fatty acids is shifted slightly downfield from that of fatty acids without this double bond. The triacylglycerols in whole vegetable seeds were quantitatively determined using a magic angle spinning technique (Wollenberg, 1991).

Similar kinds of analyses can be carried out with ^{13}C-NMR. While regiospecific analysis (determination of the α or β positions of the fatty acids attached to the glycerol backbone of triacylglycerols) was carried out by ^{1}H-NMR in presence of shift reagents (Frost et al., 1975), extensive ^{13}C-NMR reports exist in this matter, although the two α fatty acid chains cannot be distinguished. The shifts of the carbonyl carbons are employed for this purpose. Semiquantitative analysis of mixtures is possible in this fashion. Some literature is Wollenberg (1990), Gunstone (1991b), van Calsteren et al. (1996), and Vlahov (1998). The levels of Δ5 or Δ6 unsaturated fatty acids (petroselinic and linoleic) (Gunstone 1991a) or γ-linolenic acid (Gunstone, 1990a) were determined with reasonable accuracy in a variety of less common oils by ^{13}C-NMR as were polyene esters (Gunstone, 1990b) individual acids in fish oils (Gunstone, 1991c). Intact soybean seeds can be analyzed for their fatty acid composition by ^{13}C-NMR (Yoshida et al., 1989). A semiquantitative determination of the composition of hydrogenated fats was achieved using the shift differences of allylic signals as discussed above (Gunstone, 1993b). Related quantitative aspects of ^{31}P-NMR are discussed above in the section on ^{31}P-NMR.

Identification/verification of vegetable oils: While oils or fats may be deliberately mixed for specific reasons, the adulteration of high-value oils with oils of

lesser value is a problem of economic and commercial significance when the adulterated oil is marketed as the pure, high-value oil. This constitutes a major problem for olive oil. Accordingly, numerous publications from major olive oil-producing Mediterranean countries are concerned with identifying lower-value oils, such as hazelnut oil, used for adulterating olive oil. The adulteration problem is complicated by the fact that the lower-value oils usually have fatty acid profiles similar to olive oil. Among the methods used for analyzing potentially adulterated olive oil is NMR, both ^{1}H and ^{13}C. For example, NMR was utilized in a study applying multivariate statistical methods to certain peaks of olive oil diluted with hazelnut or sunflower oil (Fauhl et al., 2000). Besides analyzing olive oil for diluents, ^{1}H NMR, together with analytical data, can be used in assessing the variety and geographical origin of the oil (Sacco et al., 2000). Even when not quantitating components, the peak differences in NMR can be used to distinguish vegetable oils by visual inspection of the spectra (Guillén and Ruiz, 2003). An example of the application of ^{13}C NMR is the detection of soybean oil in olive oil with the DEPT procedure (Vlahov, 1997). The concentration dependence of the triacylglycerols in olive oil can also be used for its analysis (Mannina et al., 2000). Overviews of methods for assessing the quality and adulteration status of olive oil have been published (Sacchi et al., 1997; Mannina and Segre, 2002).

Monitoring of oxidation: the oxidation of vegetable oils or their derivatives is an important quality problem and can lead to further deterioration of the oil. Especially, unsaturated fatty acids with *bis*-allylic methylene groups are susceptible to oxidation. In ^{1}H-NMR-based oxidation studies, primary oxidation products, such as hydroperoxides, and secondary oxidation products, such as aldehydes, were detected (Guillén and Ruiz, 2001; Guillén and Ruiz, 2005). Oxidation of ethyl docosahexaenoate was also evaluated by ^{1}H-NMR and compared with traditional methods (Falch et al., 2004). ^{1}H NMR is especially useful for such studies since the samples do not require treatment, which could cause changes to the samples themselves. 2D techniques also utilizing ^{13}C NMR have also been reported for studying the oxidation products in autoxidized linoleoyl/linolenoylglycerols (Silwood and Grootveld, 1999).

Reaction monitoring (mixtures of vegetable oils with other fatty compounds): NMR can be used for reaction monitoring, which actually constitutes analyzing mixtures of vegetable oils with other fatty compounds. An example is the transesterification reaction of a vegetable oil to its corresponding methyl esters (Knothe, 2001a), a reaction that is steadily gaining significance due to the increasing production of biodiesel to which both ^{1}H-NMR (Knothe, 2001a) and ^{13}C-NMR (Dimmig et al., 1999) have been applied. In the case of ^{1}H-NMR, the strong singlet peak of the resulting methyl esters is useful in quantification.

Solid fat content (SFC): While the other methods discussed here usually rely on high-resolution, research-grade NMR instruments, a benchtop, low-resolution pulsed NMR instrument can be used for determining the SFC of an oil or fat. The method determines the amount of solid triacylglycerols in the oil or fat at different temperatures, with only the pulsed NMR signal of the liquid fat being measured (van Duynhoven et al., 1999). The low-temperature signal is proportional to the total liquid at 60°C. The method is used for quality control purposes in hydrogenation, blending, and interesterification. The SFC is used to assess properties of food products, such as hardness and mouth feel. Crystallization mechanisms of fat blends can be studied by kinetic SFC measurements. The NMR-based SFC method is considered to be more accurate than the older dilatometric method giving the solid fat index (SFI) as shown by an interlaboratory collaborative study. The results of SFC cannot be directly compared to the dilatometric SFI. The dilatometric procedure is also more labor-intensive and cumbersome than SFC determination by NMR.

Nonfatty (extraneous) materials: nonfatty or extraneous materials in fatty compounds can also be determined by ^{1}H NMR. An example is the quantitation of other lipidic materials, such as sterols, in vegetable oils (Sacco et al., 2000). The signal of a methyl group at C-18 of sterols is reportedly especially useful for this purpose. Another example is for blends of biodiesel with petroleum-derived diesel fuel. ^{1}H NMR may be applied to verifying the blend level of biodiesel with the petroleum-derived fuel (Knothe, 2001b), in which case again the strong peak of the methyl ester moiety is useful.

References

(Additional information is also available at the website: www.lipidlibrary.co.uk.)

Aursand, M. et al. (1993) Quantitative high-resolution ^{13}C and ^{1}H nuclear magnetic resonance of δ-3 fatty acids from white muscle of Atlantic salmon (*Salmo salar*). *J. Am. Oil Chem. Soc*. **70**, 971–981.

Blaise, P. et al. (1997) Identification of cyclopentenyl fatty acids by ^{1}H and ^{13}C nuclear magnetic resonance. *J. Am. Oil Chem. Soc*., **74**, 727–730.

Carballeira, N.M. et al. (1999) Synthesis of racemic 9-methyl-10-hexadecenoic acid. *Chem. Phys. Lipids*, **97**, 87–91.

Diehl, B.W.K. (2001) High resolution NMR spectroscopy, *Eur. J. Lipid Sci. Technol*., **103**, 830–834.

Diehl, B.W.K. (2002) ^{31}P-NMR in phospholipid analysis. *Lipid Technol*., **14**, 62–65.

Dimmig, T. et al. (1999) ^{13}C-NMR spectroscopic determination of the conversion and reaction kinetics of transesterification of triglycerides to methyl esters. *Chem. Tech. (Leipzig)*, **51**, 326–329.

Falch, E. et al. (2004) Correlation between ^{1}H and traditional methods for determining lipid oxidation of ethyl docosahexaenoate. *J. Am. Oil Chem. Soc.,* **81**, 1105–1110.

Fauhl, C. et al. (2000) ^{1}H NMR as a tool for the analysis of mixtures of virgin olive oil with oils of different botanical origin. *Magn. Reson. Chem.,* **38**, 436–443.

Frost, D.J. and Gunstone, F.D. (1975) The PMR analysis of non-conjugated alkenoic and alkynoic acids and esters. *Chem. Phys. Lipids*, **15**, 53–85.

Frost, D.J. et al. (1975) PMR analysis of unsaturated triglycerides using shift reagents. *Chem. Phys. Lipids,* 14, 189–192.

Fuchs, B. et al. (2005) The phosphatidylcholine/lysophosphatidylcholine ratio in human plasma is an indicator of the severity of rheumatoid arthritis: investigations by ^{31}P NMR and MALDI-TOF MS. *Clin. Biochem.,* **38**, 925–933.

Fürmeier, S. and Metzger, J.O. (2003) Fat-derived aziridines and their n-substituted derivatives: biologically active compounds based on renewable raw materials. *Eur. J. Org. Chem*., 649–659.

Gosalbo, L. et al. (1993) Synthesis of deuterated cyclopropene esters structurally related to palmitic and myristic acids. *Lipids*, **28**, 1125–1130.

Guillén, M.D. and Ruiz, A. (2001) High resolution ^{1}H nuclear magnetic resonance in the study of edible oils and fats. *Trends Food Sci. Technol.,* **12**, 328–338.

Guillén, M.D. and Ruiz, A. (2003) Edible oils: discrimination by ^{1}H nuclear magnetic resonance. *J. Sci. Food Agric*., **83**, 338–346.

Guillén, M.D. and Ruiz, A. (2005) Monitoring the oxidation of unsaturated oils and formation of oxygenated aldehydes by proton NMR. *Eur. J. Lipid Sci. Technol*. **107**, 36–47.

Gunstone, F.D. (1991a) The ^{13}C-NMR spectra of six oils containing petroselinic acid and of aquilegia oil and meadowfoam oil which contain δ-5 acids. *Chem. Phys. Lipids*, **58**, 159–167.

Gunstone, F.D. (1991b) ^{13}C-NMR studies of mono-, di- and triacylglycerols leading to qualitative and semiquantitative information about mixtures of these glycerol esters. *Chem. Phys. Lipids,* **58**, 219–224.

Gunstone, F.D. (1991c) High resolution NMR studies of fish oils. *Chem. Phys. Lipids,* **59**, 83–89.

Gunstone, F.D., (1993a) High resolution ^{13}C-NMR spectroscopy of lipids. In *Advances in Lipid Methodology — Two,* Ed., W.W. Christie, Oily Press, Dundee, U.K., pp. 1–68.

Gunstone, F.D. (1993b) The composition of hydrogenated fats by high-resolution ^{13}C nuclear magnetic resonance spectroscopy. *J. Am. Oil Chem. Soc*., **70**, 965.

Gunstone, F.D. and Ismail, I.A. (1967) Fatty acids, Part 15. Nuclear magnetic resonance spectra of the cis octadecenoic acids and of some acetylenic acids. *Chem. Phys. Lipids,* **1**, 337–340.

Gunstone, F.D. and Jacobsberg, F.R. (1972) The preparation and properties of the complete series of methyl epoxyoctadecanoates. *Chem. Phys. Lipids,* **9**, 26–34.

Gunstone, F.D. and Schuler, H.R. (1975) Fatty acids, Part 46. PMR spectra of several epoxyoctadecenoic, epoxyoctadecynoic, and diepoxyoctadecanoic esters. *Chem. Phys. Lipids,* **15**, 189–197.

Gunstone, F.D. et al. (1976) Fatty acids. Part 48. ^{13}C nuclear magnetic resonance of acetylenic fatty acids. *Chem. Phys. Lipids,* **17**, 1–13.

Gunstone, F.D. et al. (1977) Fatty acids. Part 50. ^{13}C nuclear magnetic resonance studies of olefinic fatty acids and esters. *Chem. Phys. Lipids,* **18**, 115–129.

Gunstone, F.D. et al. Eds. (1994) *The Lipid Handbook*, 2nd ed., Chapman & Hall, London.

Igarashi, T. et al. (2000) Nondestructive quantitative determination of docosahexaenoic acid and n-3 fatty acids in fish oils by high-resolution ^{1}H nuclear magnetic resonance spectroscopy. *J. Am. Oil Chem. Soc*., **77**, 737–748.

Knothe, G. (2001a) Monitoring a progressing transesterification reaction by fiber-optic near-infrared spectroscopy with correlation to ^{1}H nuclear magnetic resonance spectroscopy. *J. Am. Oil Chem. Soc.,* **77**, 489–493.

Knothe, G. (2001b) Determining the blend level of mixtures of biodiesel with conventional diesel fuel by fiber-optic near-infrared spectroscopy and ^{1}H nuclear magnetic resonance spectroscopy. *J. Am. Oil Chem. Soc*., **78**, 1025–1028.

Knothe, G. (2002) Synthesis and characterization of long chain 1,2 dioxo compounds. *Chem. Phys. Lipids*, **115**, 85–91.

Knothe, G. and Kenar, J.A. (2004) Determination of the fatty acid profile by ^{1}H-NMR spectroscopy. *Eur. J. Lipid Sci. Technol.*, **106**, 88–96.

Knothe, G. and Nelsen, T.C. (1998) Evaluation of the ^{13}C NMR signals of saturated carbons in some long-chain compounds. *J. Chem. Soc., Perkin Trans*., **2,** 2019–2026.

Lie Ken Jie, M.S.F. and Lam, C.C. (1995a) ^{1}H-Nuclear magnetic resonance spectroscopic studies of saturated, acetylenic and ethylene triacylglycerols. *Chem. Phys. Lipids,* **77**, 155–171.

Lie Ken Jie, M.S.F. and Lam, C.C. (1995b) ^{13}C-nuclear magnetic resonance spectroscopic studies of triacylglycerols of type AAA and mixed triacylglycerols containing saturated, acetylenic and ethylenic acyl groups. *Chem. Phys. Lipids,* **78**, 1–13.

Lie Ken Jie, M.S.F. et al. (1997) Synthesis and nuclear magnetic resonance properties of all geometric isomers of conjugated linoleic acids. *Lipids,* **32**, 1041–1044.

Lie Ken Jie, M.S.F. et al. (2003) Epoxidation of a conjugated linoleic acid isomer. *Eur. J. Lipid Sci. Technol*., **105,** 391–396.

London, E. and Feigenson, G.W. (1979) Phosphorus NMR analysis of phospholipids in detergents. *J. Lipid Res.*, **20**, 408–412.

Mannina, L. et al. (2000) Concentration dependence of ^{13}C NMR spectra of triglycerides: implications for the NMR analysis of olive oils. *Magn. Reson. Chem*., **38**, 886–890.

Mannina, L. and Segre, A. (2002) High resolution nuclear magnetic resonance: from chemical structure to food authenticity. *Grasas y Aceites*, **53**, 22–33.

Meneses, P. and Glonek, T. (1988) High resolution ^{31}P NMR of extracted phospholipids. *J. Lipid Res.,* **29**, 679–689.

Miyake, Y. et al. (1998a) Determination of unsaturated fatty acid composition by high-resolution nuclear magnetic resonance spectroscopy. *J. Am. Oil Chem. Soc.,* **75**, 1091–1094.

Miyake, Y. et al. (1998b) Rapid determination of iodine value by ^{1}H nuclear magnetic resonance spectroscopy. *J. Am. Oil Chem. Soc.,* **75**, 15–19.

Porter, N.A. and Wujek, J.S. (1987) Allylic hydroperoxide rearrangement: ß-scission or concerted pathway? *J. Org. Chem.,* **52**, 5085–5089.

Sacchi, R. et al. (1993) Proton nuclear magnetic resonance rapid and structure-specific determination of T-3 polyunsaturated fatty acids in fish lipids. *J. Am. Oil Chem. Soc.,* **70**, 225–228.

Sacchi, R. et al. (1997) ^{1}H and ^{13}C NMR of virgin olive oil: an overview. *Magn. Reson. Chem*., **35**, S133–S145.

Sacco, A. et al. (2000) Characterization of Italian olive oils based on analytical and nuclear magnetic resonance determinations. *J. Am. Oil Chem. Soc.,* **77**, 619–625.

Schiller, J. et al. (2002) Effects of thermal stressing on saturated vegetable oils and isolated triacylglycerols — product analysis by MALDI-TOF mass spectrometry, NMR and IR spectroscopy. *Eur. J. Lipid Sci. Technol.,* **104**, 496–505.

Silwood, C.J.L. and Grootveld, M. (1999) Application of high-resolution, two-dimensional ^{1}H and ^{13}C nuclear magnetic resonance techniques to the characterization of lipid oxidation products in autoxidized linoleoyl / linolenoylglycerols. *Lipids,* **34**, 741–756.

Spyros A. and Dais, P. (2000) Application of ^{31}P NMR spectroscopy in food analysis. 1. Quantitative determination of the mono- and diglyceride composition of olive oils. *J. Agric. Food Chem*., **48**, 802–805.

Tulloch, A.P. (1977) Deuterium isotope effects and assignment of ^{13}C chemical shifts in spectra of methyl octadecanoate and the sixteen isomeric oxooctadecanoates. *Can. J. Chem*., **55**, 1135–1142.

Tulloch, A.P. (1978) Carbon-13 NMR spectra of all the isomeric methyl hydroxy- and acetoxyoctadecanoates. Determination of chemical shifts by deuterium isotope effects. *Org. Magn. Res.,* **11**, 109–115.

van Calsteren, M.R. et al. (1996) ^{13}C NMR of triglycerides. *Bull. Magn. Reson*., **18**, 175–177.

van Duynhoven, J. et al. (1999) Solid fat content determination by NMR. *INFORM,* **10**, 479–484.

Vlahov, G. (1997) Quantitative ^{13}C NMR method using the DEPT pulse sequence for the detection of olive oil adulteration with soybean oil. *Magn. Reson. Chem*., **35**, S8–S12.

Vlahov, G. (1998) Regiospecific analysis of natural mixtures of triglycerides using quantitative ^{13}C nuclear magnetic resonance of acyl chain carbonyl carbons. *Magn. Reson. Chem*., **36**, 359–362.

Wollenberg, K.F. (1990) Quantitative high resolution ^{13}C nuclear magnetic resonance of the olefinic and carbonyl carbons of edible vegetable oils. *J. Am. Oil Chem. Soc*., **67**, 487–494.

Wollenberg, K. (1991) Quantitative triacylglycerol analysis of whole vegetable seeds by ^{1}H and ^{13}C magic angle sample spinning NMR spectroscopy. *J. Am. Oil Chem. Soc*., **68**, 391–400.

Yoshida, M. et al. (1989) Non-destructive analysis of the oil composition of soybean seeds by natural abundance carbon-13 nuclear magnetic resonance spectroscopy. *Agric. Biol. Chem*., **53**, 1395–1400.

7

PHYSICAL PROPERTIES: STRUCTURAL AND PHYSICAL CHARACTERISTICS

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7.1 Introduction

Food products in which fat crystallisation is important include chocolate and confectionery coatings, dairy products, such as butter and cream, and shortenings, margarine, and spreads. An understanding of fat crystallisation processes in these products plays a critical role in determining overall product quality (Bailey, 1950; Hartel, 1992; Walstra, 2003).

Fat crystallisation largely determines the following important properties of a food:

- The consistency of high-fat products like butter, margarine, and chocolate during storage and handling. This determines the shape retention of the product during storage and its plasticity during handling (e.g., when spreading on a slice of bread or mixing it into a dough).
- Eating properties: This may concern fracture properties and also meltdown, sticky mouthfeel and coolness.
- Physical stability: This concerns formation and sedimentation of crystals in oil, oiling out (oil separates under the influence of gravity), coalescence of aqueous droplets in butter and margarine, and partial coalescence in some oil-in-water emulsions, such as cream when fat crystals that slightly protrude from the fat globules trigger coalescence of two adjacent globules.
- Visual appearance: Examples are the appearance of bloom on chocolate, gloss of chocolate and margarine, and turbidity in oils.

Fat crystallisation is also important in fractionation processes, when products with different melting and physical properties are made (Hartel, 1992; Walstra, 2003).

Section 7.2 of this chapter will deal with crystallisation, polymorphism, and melting. It will discuss nucleation, crystal growth, crystal size, and morphology, polymorphism, solid fat content (SFC), microstructural development, macroscopical and mechanical properties, and melting behaviour. In the section on polymorphism, triglycerides, diglycerides, and monoglycerides are treated separately. The next section of this chapter (7.3) deals with phase behaviour. Phase diagrams of mixtures of triglycerides, real fats, and mixtures of triglycerides with mono- and diglycerides are discussed. Section 7.4 of this chapter deals with lipid/water interaction. After explaining the concept of a liquid crystalline phase, the interaction of water with monoglycerides, phospholipids, triglycerides, diglycerides, and soaps will be discussed.

References

Bailey, A.E. (1950), *Melting and Solidification of Fats*, Interscience, New York.

Hartel, R.W. (1992), Solid-liquid equilibrium in foods, in *Physical Chemistry of Foods*, Schwartzberg, H.G. and Hartel, R.W., Eds., Marcel Dekker, Inc., New York, 47–81.

Walstra, P. (2003), *Physical Chemistry of Foods*, Marcel Dekker, New York.

7.2 Crystallisation and melting

Crystals can be formed from the vapour phase, the melt, or a solution. No special theories have been developed to treat each of these different phase changes, but general theories have been adapted to apply to specific cases (Garside, 1987).

Crystallisation comprises several steps. First of all, a sufficiently large thermodynamic driving force must be provided. Once this has been attained, nucleation (Section 7.2.1) can occur, whereby crystals are generated as a result of bringing growth units together so that a crystal lattice can be formed. From then on, proper crystal growth (Section 7.2.2) can proceed. Although it is convenient to treat nucleation and crystal growth as consecutive events, it should be noted that nucleation does not stop when growth starts. So, in a crystallising fat, nucleation and growth occur side by side (Hartel, 1992; Timms, 2003).

The combination of nucleation and crystal growth leads to crystals with a certain size distribution and morphology (Section 7.2.3). Polymorphism (Section 7.2.4) complicates the crystallisation of lipids. Ultimately a certain solid:liquid ratio (Section 7.2.5) is obtained. Various models have been developed to describe the rate of the crystallisation process. They will be compared and models describing multistep crystallisation and nonisothermal crystallisation will also be discussed (Section 7.2.6). When the crystals are large enough, they form aggregates. This aggregation leads to the formation of a continuous network of crystals, which may alter in several different ways, often involving sintering (i.e., growing together) of adjacent crystals (Walstra et al., 2001) (Section 7.2.7). All processes mentioned above determine the macroscopic and mechanical properties of a fat (Section 7.2.8). The melting behaviour, which is reverse of crystallisation, is discussed in Section 7.2.9.

7.2.1 Nucleation

7.2.1.1 Types of nucleation

Three types of nucleation can be distinguished: primary nucleation, which can be either homogeneous or heterogeneous, and secondary nucleation.

If nucleation is not catalysed by fat crystals or foreign solid surfaces, it is called primary, homogeneous nucleation. In this case, supercooling up to 30 K should be applied before crystallisation can occur (Kloek, 1998).

If foreign surfaces (e.g., dust, container walls, molecules of different compounds) are present and catalyse the nucleation process, this gives rise to primary, heterogeneous nucleation occurring at lower levels of supersaturation (1 to 3 K) than homogeneous nucleation (Garside, 1987). Most natural fats contain enough catalytic impurities for heterogeneous nucleation to take place.

However, when the fat is divided into emulsion droplets, these impurities are also divided among the various droplets and not every droplet will contain an impurity that can give rise to heterogeneous nucleation (e.g., if a bulk fat contains one catalytic impurity per mm^3 and is divided into emulsion droplets of 100 μm^3, only about 1 in 10^7 droplets will contain an impurity). An example of the crystallisation of an oil-in-water emulsion is the physical ripening of dairy cream prior to the churning process.

Crystallisation in one droplet cannot induce crystallisation in another droplet, so that a material in a finely dispersed state can be undercooled to a far lower temperature than a bulk material, and its nucleation rate will generally be much slower. The nucleation is in this case mostly primary and homogeneous (Walstra, 2003). Secondary nucleation occurs in the presence of crystals of the material being crystallised, and, therefore, can only occur after primary homogeneous or heterogeneous nucleation and subsequent crystal growth (Kloek, 1998).

7.2.1.2 Classical nucleation theory for homogeneous nucleation

The driving force for crystallisation is the difference in chemical potential $\Delta\mu$ [J mol^{-1}] (or partial molar Gibbs free energy) between the liquid phase (melt or solution) and the solid phase. The larger this difference in chemical potential, the larger the driving force for crystallisation. To obtain nucleation in a solution, it is necessary to supersaturate this solution by arriving at a concentration C [m^{-3} or equivalent] that is higher than the solubility, in other words the concentration at saturation C_s [m^{-3} or equivalent]. The difference in chemical potential then equals:

$$\Delta\mu = R_g \times T_K \times \ln(C/C_s) \tag{7.1}$$

where R_g denotes the ideal gas constant [8.314 J mol^{-1} K^{-1}] and T_K the absolute temperature [K]. The term $ln(C/C_s)$ is called the supersaturation, $ln(\sigma)$ [-], while C/C_s is the supersaturation ratio σ [-]. For ideal solutions, the concentration at saturation C_s is given by the Hildebrand equation:

$$\ln C_s = \frac{\Delta H_m}{R_g}\left(\frac{1}{T_{Km}} - \frac{1}{T_k}\right) \tag{7.2}$$

where T_{Km} denotes the absolute melting temperature [K] and ΔH_m stands for the molar enthalpy variation in the system during the transition [J mol^{-1}]. It should be noted that the values of T_{Km} and ΔH_m are polymorph dependent and, therefore, the values for the specific polymorph concerned should be used.

At low values, the supersaturation is often approximated by expanding the logarithmic term in Equation 7.1 by a Taylor series and using only the first term. This results in:

$$\ln(\sigma) \approx \frac{C - C_s}{C_s} = \frac{\Delta C}{C_s} = \sigma_r = \sigma - 1 \tag{7.3}$$

The term $\Delta C/C_S$ is called the relative supersaturation σ_r [-].

For crystallisation from the melt to occur, supercooling should be induced and the resulting difference in chemical potential can then be written as:

$$\Delta\mu = \Delta H_m \frac{T_{Km} - T_K}{T_{Km}} \tag{7.4}$$

The difference $T_{Km} - T_K = \Delta T$ is called supercooling [K]. In this case the relative supersaturation can be written as:

$$\sigma_r = \frac{\Delta T}{T_{Km}} \tag{7.5}$$

Nucleation occurs when growth units meet, giving rise to a distribution of clusters, called embryos. The Gibbs free energy change ΔG [J] for the formation of such an embryo is determined by a surface term ΔG_s [J] due to surface tension that is positive, and a volume term ΔG_v [J]. This Gibbs free energy change ΔG is also called the free activation energy. For a spherical embryo with radius r [m], ΔG equals:

$$\Delta G = 4\pi r^2\gamma - \frac{4\pi r^3 \Delta\mu}{3V_m} \tag{7.6}$$

where γ is the surface free energy per unit of surface area [J m^{-2}], and V_m is the molar volume [m^3 mol^{-1}]. Figure 7.1 illustrates how ΔG depends on the embryo radius and shows that a critical radius r^* [m] exists for which ΔG is at a maximum so that the value of the critical radius r^* [m] can be calculated by determining the first derivative of the function with respect to r and setting this to zero:

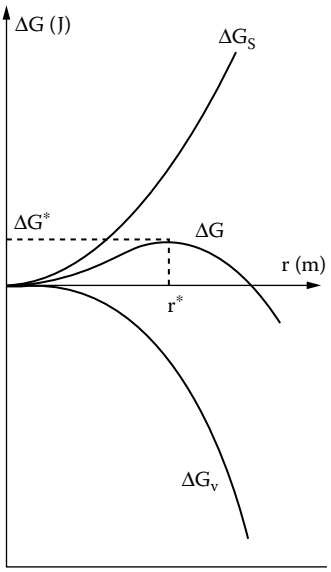

FIGURE 7.1 Variation of the Gibbs free energy change ΔG [J] for three-dimensional nucleation as a function of nucleus size r [m]. (From Boistelle, R. (1988), in *Crystallization and Polymorphism of Fats and Fatty Acids*, Garti, N. and Sato, K., Eds., Marcel Dekker, New York, 189–276. With permission.)

$$r^* = \frac{2\gamma V_m}{\Delta\mu} \tag{7.7}$$

Since growth of the embryo only leads to a decrease of ΔG once the embryo radius is higher than the critical radius r^* [m], the embryo is stable above the critical radius and unstable below it.

Inserting Equation 7.7 into Equation 7.6 yields the critical free energy of activation for nucleation ΔG^* [J]:

$$\Delta G^* = \frac{16\,\pi\, V_m^2 \gamma^3}{3\,(\Delta\mu)^2} \tag{7.8}$$

The factor $\frac{16\pi}{3}$ results from the spherical shape attributed to the nucleus and can, in general, be replaced by a dimensionless shape factor.

The nucleation rate J [s^{-1} m^{-3}] at which new nuclei are formed is a problem of kinetics and is determined by the rate at which embryos surmount the maximum in the free energy curve. Supposing the embryos follow a Boltzmann distribution as a function of their free energy, the nucleation rate can be written as:

$$J = A_J \times e^{-\frac{\Delta G^*}{k_B T_K}} \tag{7.9}$$

where A_J represents the global kinetic coefficient [s^{-1} m^{-3}] and k_B denotes the Boltzmann constant [1.380 10^{-23} J K^{-1}]. A_J can also be written as:

$$A_J = \frac{N_m \times k_B \times T_K}{h_P} \tag{7.10}$$

with N_m the number of molecules per m^3 [m^{-3}] and h_P Planck's constant [6.626 10^{-34} J s].

If nucleation occurs in the melt or a high viscosity solution, the difficulty encountered by a molecule in crossing the interface between the liquid and solid phases must be taken into account. Therefore, an additional critical free energy of activation for volume diffusion ΔG^*_{vd} [J] is added to Equation 7.9, which then becomes:

$$J = A_J \times e^{-\frac{\Delta G^*}{k_B T_K}} \times e^{-\frac{\Delta G^*_{vd}}{k_B T_K}} \tag{7.11}$$

Equation 7.11 is generally known as the Turnbull–Fisher equation. When the temperature decreases, the volume diffusion term may become rate determining,

causing the nucleation rate to drop off at these lower temperatures.

7.2.1.3 Heterogeneous nucleation

In heterogeneous nucleation, the process of molecular orientation is enhanced by the presence of a foreign surface, e.g., a dust particle or a microscopic structure in the vessel wall, which orients molecules preferentially so that a crystal lattice is more easily formed.

To derive the critical free energy of activation for heterogeneous nucleation, ΔG^*_{het} [J], it is convenient to consider a cap-shaped nucleus making a contact angle with the foreign surface (Figure 7.2). The value of ω depends on the way the foreign surface is wetted by the nucleus.

The surface-free energies involved in this process are γ_0 [J m^{-2}] between foreign surface and mother liquor, γ_1 [J m^{-2}] between nucleus and mother liquor, and γ_2 [J m^{-2}] between nucleus and foreign surface. When the nucleus is in contact with the foreign surface, two excess energies have been expended in the creation of the new surfaces, whereas one has been gained in losing a fraction of the area of the foreign surface. The Gibbs free energy change for heterogeneous nucleation ΔG_{het} [J], therefore, equals their sum:

$$\Delta G_{het} = -\Delta G_v + A_1\gamma_1 + A_2\gamma_2 - A_2\gamma_0 \qquad (7.12)$$

By calculating the volume of the cap and the surfaces and taking into account that the different surface free energies depend on each other according to:

$$\gamma_0 = \gamma_2 + \gamma_1 \cos\omega \qquad (7.13)$$

the free energy for heterogeneous nucleation, ΔG_{het} can be written as:

$$\Delta G_{het} = -\frac{\pi r^3 \Delta\mu (2 - 3\cos\omega + \cos^3\omega)}{3V_m} + 2\pi r^2 (1-\cos\omega)\gamma_1 - \pi r^2 (1-\cos^2\omega)\gamma_1 \cos\omega \qquad (7.14)$$

in which r [m] is the radius of the underlying sphere.

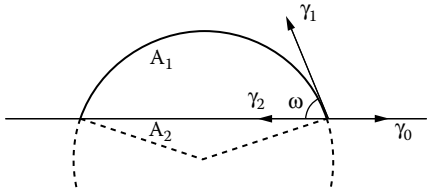

FIGURE 7.2 Cap-shaped nucleus formed by heterogeneous nucleation on a foreign substrate. The arrows represent the different surface-free energies between foreign surface, mother phase, and nucleus. (From Boistelle, R. (1988), in *Crystallization and Polymorphism of Fats and Fatty Acids*, Garti, N. and Sato, K., Eds., Marcel Dekker, New York, 189–276. With permission.)

Then the critical radius r^* for which ΔG_{het} is at its maximum can be calculated; it is the same as for homogeneous nucleation (*cf.* Equation 7.7):

$$r^* = \frac{2\ \gamma_1 V_m}{\Delta\mu} \qquad (7.15)$$

However, since the spherical cap contains fewer molecules than the full sphere, the energy barrier is lower for heterogeneous than for homogeneous nucleation.

Inserting Equation 7.15 in Equation 7.14 yields the critical free energy of activation for heterogeneous nucleation:

$$\Delta G^*_{het} = \Delta G^* \times \left(\frac{1}{2} - \frac{3}{4}\cos\omega + \frac{1}{4}\cos^3\omega \right) \qquad (7.16)$$

Accordingly, ΔG^*_{het} equals the product of ΔG^* (the critical free energy of activation for homogeneous nucleation) and a term depending on the magnitude of the contact angle ω. For ω = 90° (the contact is limited to a geometrical point), ΔG^*_{het} equals ΔG^* / 2 and for ω tending towards zero (perfect wetting of the substrate), ΔG^*_{het} also tends towards zero, which means that in this borderline case, no free energy of activation at all is required for nucleation.

7.2.1.4 Secondary nucleation

According to most studies, the main source of secondary nuclei is the crystal surface. Secondary nuclei form whenever tiny crystallites, embryos, are removed from the surface and exceed the critical size. Two main causes are invoked to explain the removal of embryos or nuclei from the surface. Except for partial dissolution, which gives rise to spontaneous removal, removal is due either to fluid shearing forces or to collisions between a crystal and its neighbours or with the walls, the agitator or other parts of the crystalliser (Boistelle, 1988).

In some cases, it has also been assumed that the source of the secondary nuclei lies in the supersaturated solution itself (Boistelle, 1988). Walstra (2003) even states that true secondary nucleation means that nuclei are formed in the vicinity of a crystal of the same phase and not on its surface.

Secondary nucleation has been studied mainly under conditions that are typical of industrial crystallisers and involve considerable agitation. However, Walstra (1998) has shown that secondary nucleation can also happen under quiescent conditions. He considered droplets of milk fat of typically a few μm in diameter at a temperature of around 20°C. No precooling to a lower temperature was applied. Under these conditions, earlier studies (Walstra and Beresteyn, 1975) showed that the number of catalytic impurities would be of the order of one per 100 μm^3. As this is about the size of the droplets, there should be zero, one or occasionally two crystals in a droplet. However,

a typical droplet was measured to contain about 25% solid fat. Electron micrographs from earlier work (Mulder and Walstra, 1974), on the other hand, have shown that, under comparable conditions, crystals are typically 0.005 μm^3, which would mean some 5000 crystals per droplet. There is, thus, an enormous discrepancy between the number of crystals present and the number that can originate from heterogeneous nucleation. As homogeneous nucleation is out of the question under these circumstances, the conclusion must be that the numerous small crystals formed originated from secondary nucleation.

Walstra (1998) has also given a tentative explanation for this phenomenon. Clusters of more or less oriented molecules, which Larsson (1972) postulated to exist in liquid triglycerides, may diffuse away from the growing crystal and subsequently form a new nucleus. Two conditions should be fulfilled for this secondary nucleation to take place: the crystal surface must be rough and the crystal growth rate must be so slow that the clusters of more or less oriented molecules can diffuse away from the crystal face before they become incorporated in the crystal.

There, however, is no generally accepted theory for secondary nucleation (Walstra, 2003). In the industrial dry fractionation process, secondary nucleation is considered to be responsible for the formation of additional small crystals that badly affect the filtration characteristics of the stearin cake and, thus, make the process less selective (Timms, 1997). It has been linked to the rate of agitation, but again, no mechanism has been put forward.

7.2.2 Crystal growth

Once nuclei are formed and exceed the critical size, they become crystallites, whose growth depends not only on external factors (supersaturation, solvent, temperature, impurities), but also on internal factors (structure, bonds, defects) (Boistelle, 1988). Consequently, the rate of crystal growth can vary by several orders of magnitude. Growth occurs by attachment of molecules to a crystal surface. On the other hand, molecules will also become detached. There is a continuous movement of molecules across the surface of the crystal in both directions. The net result of these two processes determines the growth rate (Walstra, 2003).

The mechanism by which a crystal surface grows is determined by the nature of the interface between the crystal and the liquid (Garside, 1987). As illustrated in Figure 7.3, there are three types of faces: kinked (K), stepped (S), and flat (F) (Boistelle, 1988).

7.2.2.1 Growth of a kinked (K) face

A kinked (K) face looks like an infinite population of kink sites. Continuous growth will occur since there are no thermodynamic barriers to the growth process: Each growth unit reaching the surface in a supersaturated state will immediately be incorporated into the crystal.

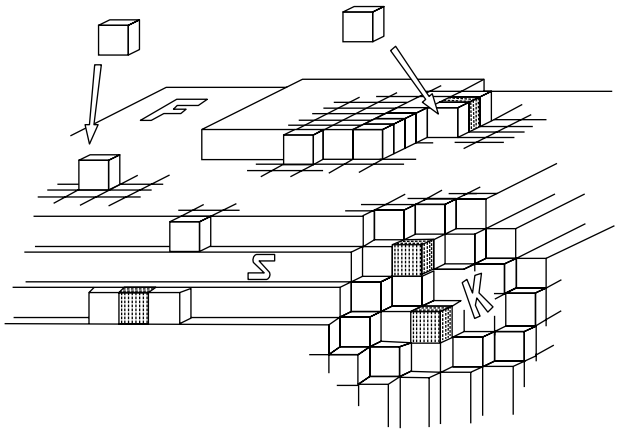

FIGURE 7.3 Schematic picture of different growth faces. Each small cube represents a growth unit. (From Aquilano, D. and Sgualdino, G. (2001), in *Crystallization Processes in Fats and Lipid Systems*, Garti, N. and Sato, K., Eds., Marcel Dekker, New York, 1–51. With permission.)

As a consequence, the growth rate of a K face is proportional to the relative supersaturation σ_r (Garside, 1987; Aquilano and Sgualdino, 2001).

However, when growth occurs from the melt, the free energy of activation for self-diffusion of the molecules in the melt plays an essential role in the growth kinetics. Consequently, the growth rate is also inversely proportional to the viscosity. If the viscosity increases drastically with decreasing temperature, it may happen that at a given temperature, the growth rate passes through a maximum and begins to decrease with increased supercooling (Boistelle, 1988).

7.2.2.2 Growth of a stepped (S) face

From a kinetic viewpoint a stepped (S) face is similar to a K face. However, the number of kink sites per unit area is lower compared to a K face. Just as for a K face, the growth rate for an S face is proportional to the relative supersaturation σ_r, but due to the lower number of kink sites, the growth rate of an S face will be lower than that of a K face (Aquilano and Sgualdino, 2001).

7.2.2.3 Growth of a flat (F) face

Flat (F) faces grow layer after layer, either by a two-dimensional nucleation mechanism or by a spiral growth mechanism (Boistelle, 1988).

If the crystal surface is without any defect, growth takes place by two-dimensional nucleation. The growth units that adsorb on the surface must diffuse, make contact, and coalesce to form a stable two-dimensional nucleus (Boistelle, 1988). Once a surface nucleus is formed, the new crystal layer can be filled by attachment of growth units near the kink or by repeated surface nucleation and subsequent surface diffusion to existing surface nuclei (Kloek, 1998). Consequently, the growth rate of a perfect flat face is proportional to the two-dimensional nucleation rate and, hence, it is an exponential function of supersaturation. The growth rate is near zero below a certain

critical value of the relative supersaturation and increases dramatically above this value. This type of growth mechanism is rather rare (Boistelle, 1988; Aquilano and Sgualdino, 2001).

The second mechanism involving layer growth is the spiral growth mechanism; this occurs much more frequently than the previous one. When a screw dislocation emerges on a face, it provides a step. When the growth units adsorb onto the face, they first diffuse towards and along the step. As soon as they encounter a kink, they are fixed more firmly to the surface. The step advances by rotating around the emergence point of the dislocation. After a complete rotation, one or several layers of growth units have been added to the crystal. There are several theoretical expressions for the growth rate and the general growth rate equation is very complex. In general, it can be said that at low supersaturation, the growth rate of the face is a quadratic function of supersaturation, whereas at high supersaturation it becomes proportional to the supersaturation (Boistelle, 1988).

7.2.3 Crystal size and morphology

Crystal size is controlled by the relative rates of nucleation and crystal growth. Since nucleation depends more strongly on supersaturation than crystal growth, the rate of nucleation mainly determines the size of the crystals. Since the nucleation rate increases roughly exponentially with increasing supersaturation, many nuclei are formed when the supersaturation is high, and this also means that small crystals will result. On the other hand, at low supersaturation only a few, large crystals are formed. Secondary nucleation can complicate this picture as it leads to a larger number of crystals and consequently a smaller average crystal size (Walstra, 2003; Timms, 2003).

It should be noted that as nucleation and crystal growth proceed, the extent of supersaturation necessarily decreases causing the critical size for a stable crystal or nucleus to increase. Smaller crystals, which were stable at higher levels of supersaturation, now become unstable and redissolve. In theory, this process, which is called Ostwald ripening, would continue indefinitely until eventually only one large crystal would be left in the presence of a slightly supersaturated liquid. In practice, once crystals grow to about 10 μm, the thermodynamic driving force for Ostwald ripening has become negligible (Walstra, 2003; Timms, 2003).

Crystal size may be an important quality parameter in foods. In many soft solid foods, it is often undesirable that crystals are felt in the mouth, a sensation described as sandiness when the crystals also exhibit a high melting point. To prevent this, crystals generally have to be smaller than about 10 μm. The macroscopic and mechanical properties of plastic fats also depend to a large extent on fat crystal size, among other factors, but this will be discussed further in Section 7.2.7 and Section 7.2.8, respectively. If the crystallisation is intended to isolate the crystalline fraction, as in the dry fractionation process, it is usually desirable to obtain large crystals, to ensure their efficient separation from the mother liquor (Walstra, 2003).

Crystal morphology is controlled by the relative rates of growth of the different crystal faces, which in turn depend on internal and external factors. Crystals may grow with flat, well-defined faces or with rounded-off, roughened faces. An intermediate situation, when spherulitic crystals form, is common in fat crystallisation. The crystals then appear as many-faceted crystals growing out of an ill-defined nucleus. Like crystal size, crystal morphology can also play an important role in the functionality of a crystallised fat and during the dry fractionation of fats (Timms, 2003).

Whether crystal faces become rough depends on the temperature. There is a critical temperature, the roughening temperature, above which crystal growth leads to rough crystal faces. Below this critical temperature, even at the same degree of supersaturation, flat, well-defined faces form. This effect is known as thermal roughening. Additionally, above a critical degree of supersaturation, a flat face can become rough. This effect is known as kinetic roughening. An excellent review of this complex topic and its theoretical treatment has been published by Bennema et al. (2001).

7.2.4 Polymorphism

7.2.4.1 General principles

Polymorphism is defined as the existence of several crystalline phases with the same chemical composition that have a different structure, but yield identical liquid phases on melting. Polymorphism leads to the existence of multiple melting points. Two crystalline forms are enantiotropic when each has a definite range of stability. Either modification may be the stable one and transition can go in either direction, depending on the conditions. Two crystalline forms are monotropic if one is stable and the other metastable under all conditions. Transition will only take place in the direction of the more stable form. Natural fats are invariably monotropic (Nawar, 1996).

Larsson (1966) proposed a classification of the basic polymorphs of triglycerides on the basis of both infrared spectroscopy and x-ray diffraction, which is now generally accepted. The polymorphs are classified into three crystallographic types: α (alpha), β′ (beta prime), and β (beta), according to their hydrocarbon subcell packing. A subcell is the smallest spatial unit of repetition along the chain axis (Nawar, 1996). The α-polymorph is associated with the hexagonal subcell packing in which the fatty acid chains are perpendicular to the methyl end group plane and are assumed to be oscillating with a high degree of molecular freedom. The β′-polymorph is associated with the orthorhombic subcell packing in which the fatty acid

chains are tilted with respect to the methyl end group plane and where adjacent zigzag fatty acid chains are in different planes. The β-polymorph is associated with the triclinic subcell packing where unlike the orthorhombic subcell packing, all zigzag fatty acid chains are in the same plane (Timms, 1984). Figure 7.4 shows schematic diagrams of these three basic polymorphs.

Because each of the subcell packings is characterized by a unique set of x-ray diffraction (XRD) lines in the wide angle region between 3.5 and 5.5 Å (the short spacings), the three basic polymorphs can be unambiguously identified on the basis of wide angle x-ray diffraction (WAXD). The hexagonal subcell packing of the α-polymorph is easy to identify as it exhibits one strong diffraction line around 4.15 Å. The orthorhombic subcell packing of the β′-polymorph is characterized by two strong diffraction lines around 3.7 and 4.2 Å. The triclinic subcell packing of the β-polymorph gives a whole series of diffraction lines with one prominent line at 4.6 Å and two other, less intense lines around 3.6 and 3.8 Å. (Kellens, 1991).

The polymorphs differ in stability, melting point, melting enthalpy, and density. The α-polymorph is the least stable and has the lowest melting point, melting enthalpy, and density. The β-polymorph is the most stable and has the highest melting point, melting enthalpy, and density. The β′-polymorph has intermediate properties (Walstra, 1987).

Although differential scanning calorimetry (DSC) can accurately determine melting points and would be a useful technique to determine polymorphism (as each polymorph has its own melting point), this technique can only give an indication of the polymorphic form due to the presence of mixtures of polymorphs and the fact that the melting point of each polymorph depends on the chemical composition (Walstra and Beresteyn, 1975; Walstra, 2003).

According to Equation 7.9, the polymorph with the lowest critical free activation energy for nucleation ΔG^* shows the highest nucleation rate. The surface-free energy γ in the equation for ΔG^* (Equation 7.8) will vary considerably between the most unstable polymorph, which has a structure close to that of the liquid phase, and the most stable polymorph with a structure very much different from that of the liquid phase. This leads to a lower critical free energy of activation and, thus, a higher nucleation rate for the α-polymorph, despite the fact that the difference in chemical potential Δμ is greater for the β-form (Rousset, 1997). According to Loisel (1996) the rate of crystal growth increases with the stability of the polymorph, while Kellens (1991) stated that the growth rate of the unstable polymorph is higher than that of the stable polymorph. However, this order reverses at higher driving forces.

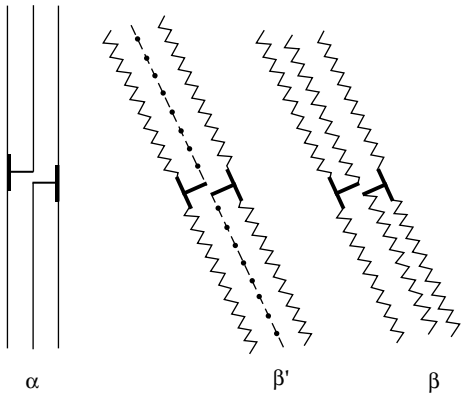

FIGURE 7.4 Schematic diagrams comparing the polymorphs α, β′ and β as exemplified by tristearin. (From Timms, R.E. (1984), *Prog. Lipid Res.*, 23, 1–38.)

7.2.4.2 Polymorphism of triglycerides

Most triglycerides exhibit an α-form, although it is often very unstable. Some manifest both a β′- and a β-form, others only a stable β′- and no β-form or a stable β-form and no intermediate form. The existence or nonexistence of a polymorph depends heavily on the composition and the position of the fatty acids on the glycerol unit (Kellens, 1991). For mixed saturated/unsaturated triglycerides, the β′-polymorph is usually the most stable one if the triglyceride is asymmetrical, i.e., two saturated or two unsaturated acids occupy the 1,2- or 2,3-positions, the third position being occupied by an unsaturated or saturated acid, respectively. Examples of asymmetrical triglycerides are PPO and POO, with P standing for palmitic acid and O for oleic acid. The packing requirements for the β′-polymorph are less stringent than for the β-polymorph so that mixed fatty acid triglycerides of various types tend to be β′-stable (Timms, 1984).

The polymorphism of triglycerides is complicated even further by the presence of other polymorphs and multiple submodifications of the basic polymorphs. Low-temperature XRD experiments have demonstrated the existence of the sub-α-polymorph, sometimes also called the γ-polymorph. The XRD pattern of this polymorph is similar to that of the β'-form, but its melting point is lower than that of the α-form (Hagemann, 1988; Kellens, 1991). For saturated monoacid triacylglycerols, it is generally accepted that at least two β'-polymorphs exist, which are denoted as β'_2 and β'_1 in order of increasing stability, and several authors have also revealed the presence of multiple β-forms (Kellens, 1991). In mixed-acid triglycerides, these submodifications are even more prevalent (Sato et al., 1999).

SOS, with S standing for stearic acid, for example, has five polymorphs: α, γ, β′, β_2, and β_1. The two β-forms, which only show very subtle differences in their subcell structure, are also observed in POP, AOA, and BOB (A denotes arachidic acid and B behenic acid, saturated fatty acids with 20 and 22 carbon atoms, respectively). They correspond to the two β-forms, form V and VI, in cocoa butter. In the γ form, the hexagonal subcell structure of the α-form is also present in the oleoyl leaflets, but a specific type of subcell occurs in the stearoyl leaflet (Sato et al., 1999).

7.2.4.3 Polymorphic behaviour in commercial fats

It is evident that the polymorphic behaviour of a fat is largely influenced by its fatty acid composition and the positional distribution of these fatty acids on the glycerol backbone, in other words by its triglyceride composition. In general, fats comprising relatively few and closely related triglyceride species tend to transform rapidly to stable β-forms. Conversely, heterogeneous fats tend to transform more slowly to stable forms since compound β′-crystals can host fewer different molecules than α-crystals and compound β-crystals hardly exist. Fats that tend to crystallise in β-forms include soya bean oil, peanut oil, low erucic acid rapeseed oil (canola), corn oil, olive oil, coconut oil, cocoa butter, and lard. On the other hand, cottonseed oil, palm oil, high erucic acid rapeseed oil (HEAR), milk fat, and tallow tend to produce β′-crystals that tend to persist for long periods (Nawar, 1996; Walstra, 2003).

7.2.4.4 Polymorphic behaviour of diglycerides

Hagemann (1988) has reviewed the polymorphism of diglycerides. All 1,3-diglycerides ranging from CC (dicaprate), EE (dielaidate), OO (dioleate) to SM (M stands for myristic acid) possess stable β-polymorphs and no α-form. Even chain length 1,3-diglycerides from dilaurate to distearate can form two β–polymorphs, which Baur (1949) called a and b. Shannon et al. (1992) confirmed the existence of these two β-forms in PP and SS, but renamed them β_2 and β_1, respectively to be in accordance with the polymorph nomenclature rules of Larsson (1966). The two forms can be distinguished on the basis of subtle differences in short spacing values.

A quite different polymorphism is displayed by 1,2-diglycerides. Even monoacid 1,2-diglycerides have an α- and a stable β′-polymorph; this was erroneously called a β-form in earlier studies. The optically active and the optically inactive racemic 1,2-diglycerides exhibit very similar polymorphism but show contrasting thermal behaviour (Hagemann, 1988; Shannon et al., 1992).

Di and Small (1993; 1995) studied the polymorphism of mixed chain diglycerides in which a saturated 18 carbon chain is attached to the 1-position and an unsaturated 18 carbon chain to the 2-position. This 1-stearoyl-2-oleoyl-*sn*-glycerol was shown to have eight polymorphs: γ_2, γ_1, α, β_4, β_3, β_2, β_1 and β′ in order of increasing melting point. In this case the β'-polymorph is thus the most stable. The γ_2- and γ_1-polymorphs are formed reversibly from the α-polymorph on cooling. The β′-polymorph can only be formed by crystallisation from hexane at 4°C by slow evaporation. The β_1-polymorph is only formed when a sample that is crystallised from a solvent is melted at 30°C for 1 h to remove the seeds of β′ and then cooled to below 17.4°C.

The diglyceride 1-stearoyl-2-linoleoyl-*sn*-glycerol has four polymorphs: sub-α_2, sub-α_1, α, and β′ in order of increasing melting point. The polymorphs sub-α_2 and sub-α_1 have a pseudo-hexagonal chain packing, while the other polymorphs display their usual subcell chain packing. It is evident from the large number of polymorphic forms of rather similar energy in these mixed chain diglycerides, that the two chains have difficulty in deciding what is the most favourable composition. However, considering the difference in the number of polymorphs, it seems that the polyunsaturated linoleate chains pack marginally more effectively than monounsaturated chains with the saturated stearate chains.

7.2.4.5 Polymorphic behaviour of monoglycerides

For 1-monoglycerides it is generally agreed that four polymorphs exist: sub-α, α, β′, and β. Sub-α forms are a characteristic feature of 1-monoglycerides since they occur in all samples on which polymorphic studies have been performed. They form at low temperatures from the α-form via a solid-state transition. Some studies do not report a β'-form. A possible explanation for this is the difficulty of preparing this form (Hagemann, 1988; Krog and Sparso, 2004). But it is also true that unlike pure 1-monoglycerides, industrial distilled monoglycerides do not form any β′-polymorph (Krog, 2001).

It seems that 2-monoglycerides are free from polymorphism, although Malkin (1954) has stated that the 2-monoglycerides probably separate from the melt in the α-form, but transform very rapidly into the β-form. However, it has to be stressed that the polymorphism of these compounds has not been studied frequently (Hagemann, 1988).

7.2.4.6 Phase transitions

Most transitions are monotropic or irreversible and characterised by first-order kinetics. Only the sub-α to α transition is assumed to be reversible and of the second-order type. Second-order transitions occur over a wide temperature range and do not involve heat exchange. Large changes in crystal structures do not occur in second-order transitions, which merely involve an increase in molecular movement (Hagemann, 1988; Kellens, 1991). However, Loisel et al. (1998) stated that, at least in cocoa butter, the sub-α to α-transition is also irreversible. They deduced this from the observation that cooling of the α-polymorph, obtained by transition of the sub-α polymorph on heating, did not restore the latter even after 1 day at low temperature.

A metastable crystal can change into a stable one through the rearrangement of its structural unities until a complete transformation occurs (solid-state phase transition) or by melting and recrystallisation (melt-mediated phase transition). If a suitable solvent is involved, the metastable phase dissolves and a new stable phase is allowed to form from its supersaturated solution (solvent-mediated phase transition). The kind of transition may depend on the thermal history of the sample as is the case for 1,2-dipalmitoyl-*sn*-glycerol. For this diglyceride, Takahashi (1999) showed that when the diglyceride is incubated at around 3°C for more than 10 h, the α-phase

is converted directly into the β′-phase, but that without incubation at low temperatures, the α-phase is transformed into the β′-phase by way of a melted phase. If a number of different phase transitions from a less stable state to more and more stable states are possible, the closest more stable phase is usually formed and not the most stable one (Aquilano and Sgualdino, 2001). Phase transition kinetics can vary widely, ranging from almost instantaneous to extremely slow for some solid-state transitions (Aquilano and Sgualdino, 2001).

7.2.4.7 Arrangement in crystal space (cf. long spacings)

The x-ray scattering patterns at small angles (SAXS) of the various polymorphs show a series of lines that are related to the thickness of the layers formed by the side-by-side arrangement of the chains (long spacings). The layer thickness depends on the length of the molecule and, hence, on the number of carbon atoms in the fatty acid chains, and on the angle of tilt between the chain axis and plane of the methyl end groups. The triacylgly-cerols are arranged head to tail and form a chair-shaped structure with the fatty acid at the 2-position forming the back of the chair. Two packing modes are possible, resulting in pairs of two (2L packing) or three (3L packing) fatty acid chains (Figure 7.5) (Kellens, 1991). Pairs of three fatty acid chains are formed when the fatty acid chains are mixed, i.e., when there are large differences between the number of carbon atoms of the different chains and when saturated and unsaturated fatty acids form part of the same triacylglycerol (Sato and Kuroda, 1987).

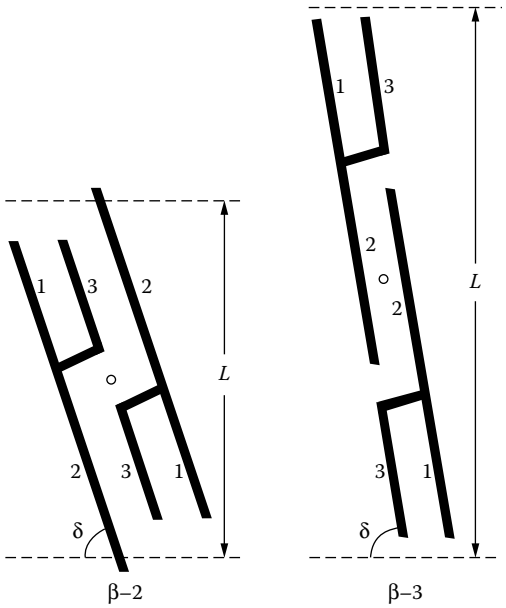

FIGURE 7.5 Arrangement of the triacylglycerol molecules in the crystalline phase: double and triple chair arrangements of the form. L is the layer thickness and δ is the angle of tilt. (From Walstra, P. (1987), in *Food Structure and Behaviour*, Blanshard, V.M.V. and Lillford, P., Eds., Academic Press, London, 67–85.)

7.2.5 Solid Fat Content

The solid fat content of a sample (SFC) is a measure of the percentage of solid, crystalline fat in a sample at a selected temperature. Often, the SFC is measured at selected points within a temperature range (Timms, 2003).

A measure of the SFC can be determined by a variety of methods: dilatometry, pulsed nuclear magnetic resonance (p-NMR), or differential scanning calorimetry (DSC). The method used and differences in the way it is executed can seriously affect the final result. This should always be taken into account when comparing different results. Over the years several authors have compared the various methods, e.g., Walker and Bosin (1971), Lambelet (1983), Van Duynhoven et al. (1999), and Marangoni et al. (2000).

Until the early 1970s, dilatometry was the standard method. It is based on the difference in specific volume of the liquid and the crystalline phase. It assumes all fats to have the same melting dilatation, which is incorrect since the melting dilatation of a fat varies according to the molecular weight of the triglycerides, the type of fatty acids and the polymorphic form of the fat. The result of a dilatometric measurement, called the Solid Fat Index (SFI) and expressed as [mm^3/g] or [$mm^3/25$ g] is fundamentally different from the solid fat content expressed as [%]. Moreover, a dilatometric determination takes a long time.

In the early 1970s, Unilever research workers (Enden et al., 1978; 1982) pioneered the practical use of a pulsed NMR technique, also referred to as "time-domain" NMR (Todt et al., 2001) as a routine method for determination of the SFC that is expressed as a percentage between 0 and 100%. The principle of the pulsed NMR technique is as follows: After a short radio-frequency pulse, which rotates the magnetic field by 90°, the magnetisation signal in the detector decays over several hundred milliseconds. The decay of the signal from protons in the solid state is rapid, occurring over tens of microseconds, whereas the decay of the signal from protons in the liquid state is much slower, occurring over tens to hundreds of milliseconds. Accordingly, a distinction can be made between crystalline and liquid triglycerides.

The SFC can be measured using the Direct Method, which involves the determination of the solid + liquid and of the liquid signals. Unfortunately, a "dead time," during which no measurements can be made, occurs after the pulse, which makes it is impossible to measure the solid + liquid signal. To overcome this problem, an f-factor was introduced, which is often set at a value of 1.4 (f-factor for β′-margarine fats of Unilever). However, this f-factor depends on several factors, but most importantly on the polymorphic form of the fat. Because for many fat blends the actual f-factor is not known, it may be preferable to use the Indirect Method. In this method, the solid signal is ignored and the SFC is calculated by comparing the measured liquid signal to the liquid signal of a reference that is completely liquid at the temperature of measurement.

When using calorimetry, the heat of melting per unit mass is measured. This value, however, varies significantly among triglycerides. When a constant average value is taken, this means that the resulting proportion of solid fat is significantly biased. The value of the latent heat also depends on the polymorph, which can further bias the result.

An ultrasonic technique has also been proposed as an alternative way to measure the solid fat content (Povey, 1995). It is based on the observation that the velocity of ultrasound is greater in solid fat than in liquid fat. With this technique, the polymorphic form of the sample can bias the results, since polymorphic transitions lead to more densely packed crystals and, therefore, lower compressibility, resulting in a higher ultrasound velocity.

Before its solid fat content can be determined, the fat must be exposed to a prescribed temperature profile: first it has to be melted completely to destroy all traces of crystals, then cooled to achieve virtually complete crystallisation, and finally it has to be held at the measuring temperature to come to equilibrium at that temperature. Sometimes, depending on the fat used, an extra step is introduced where the fat is held at a particular temperature, which is not the measuring temperature. This step is referred to as a tempering step. For confectionery fats, a tempering step of 40 hours at 26°C is mentioned in the standard methods to ensure that cocoa butter and similar fats like cocoa butter equivalents (CBEs) are converted to their β-polymorph before the SFC is measured.

7.2.6 Modelling crystallisation kinetics

The crystallisation kinetics of fats (when, how fast, and to what extent fat components crystallise under certain conditions) is the basis for controlling operations in which (re)crystallisation is of concern. From the late 1970s onwards, and especially in the last few years, quite a number of articles have been published in which the isothermal single-step crystallisation of fats is mathematically modelled to enable the quantification of differences in crystallisation behaviour of different products and under different crystallisation circum-stances. A model is constructed on the basis of the experimental data sets, and providing parameters with a physical meaning.

The most frequently used model to describe the isothermal one-step crystallisation kinetics of fats is the Avrami model. Some authors use a modified Avrami equation, also called the Avrami–Erofeev equation. Kloek (1998) and Vanhoutte (2002) used a reparameterised Gompertz equation to describe their crystallisation curves. Foubert et al. (2002) developed a new model, which is available as both an algebraic and a differential equation.

In the following sections, further details of these four models used to describe the isothermal one-step crystallisation of fats will be given. This is followed by a paragraph comparing the different models with respect to curve fitting and their theoretical background. Finally, attention is given to some recent work concerning the modelling of multistep processes and the modelling of nonisothermal crystallisation.

7.2.6.1 The Avrami model

The Avrami model is the most widely used approach for the description of isothermal phase transformation kinetics. In the 1940s, various authors independently developed this kinetic formulation, which is sometimes also called the Johnson–Mehl–Avrami–Kolmogorov equation. The theory was initially developed for low molecular weight materials such as metals. Later it was extended to the crystallisation of polymers.

Avrami (1939; 1940) stated that there is overwhelming evidence pointing to the conclusion that phases are nucleated by tiny germ nuclei that already exist in the liquid phase and whose effective number equals $\overline{N}_0$ per unit nucleation region. The number of germ nuclei per unit region at time t decreases from $\overline{N}_0$ in two ways: (1) some of them become active growth nuclei (N at time t) as a consequence of free energy fluctuations and with a probability of occurrence p per germ nucleus, and (2) some of them get swallowed by growing grains of the new phase. The number of growth nuclei may increase linearly with time (sporadic nucleation), or the large majority of the growth nuclei can be formed near the beginning of the transformation process (instantaneous nucleation). The variable V represents the volume of the crystalline phase per unit volume of space. Avrami also introduced a characteristic time scale, defined by $pdt = d\tau$. This characteristic time scale is in fact rescaled time taking into account the value of p.

Furthermore, Avrami made the assumption that growth ceases when one grain impinges upon another. The volume at rescaled time of any grain that began growth from a nucleus at rescaled time z is denoted as $v(\tau, z)$. The number of such grains is expressed by $N(z)$. Thus, the total extended volume (the term "extended" refers to the volume the grains would have had, if growth had remained unimpeded) equals:

$$V_{ext} = \int_0^{\tau} v(\tau, z) N(z) dz \tag{7.17}$$

Let r, the "radius," be a one-dimensional measure of the size of a grain and let G be the direction average of the rate of growth of r. For a grain, which started to grow at time y, the radius r at time t is given by:

$$r(t, y) = \int_y^t G(x) dx \tag{7.18}$$

or, if the rescaled time τ is introduced:

$$r(\tau, z) = \int_z^{\tau} \frac{G}{p} du \tag{7.19}$$

Note that G differs from $G(x)$ since G is expressed in the rescaled time.

The grain volume then becomes:

$$v(\tau,z)=\sigma r^3=\sigma\left[\int_z^\tau \frac{G}{p}du\right]^3 \tag{7.20}$$

where σ is a shape factor, equal to $4\pi/3$ for a sphere.

Since the factors which govern the tendency of the growth nuclei to grow out of the germ nuclei are similar to those which govern further growth, Avrami assumed that p and G are approximately proportional throughout a considerable temperature and concentration range called the isokinetic range. Thus, if G/p is constant for a given substance in the isokinetic range, Equation 7.17 can be integrated according to:

$$V_{ext}=\sigma\frac{G}{p^3}^3\int_0^\tau(\tau-z)^3N(z)dz \tag{7.21}$$

In any region, selected arbitrarily, the part of the volume still without crystallised matter is designated as the "nonoverlapped" volume. Then, on average, the ratio of the nonoverlapped volume v' to the extended volume v_{ext} of a randomly selected region is equal to the density of untransformed matter $1\text{-}V$ at that time, so that:

$$\frac{v'}{v_{ext}}=1-V \tag{7.22}$$

The same reasoning may be applied to the nonoverlapping and extended portions of the increments of single grains in an element of time. The following equation is obtained for the average grain:

$$\frac{dv}{dv_{ext}}=1-V \tag{7.23}$$

since the nonoverlapped decrease of a grain is the same as the increment in transformed volume of that grain.

For the unit volume this leads to:

$$\frac{dV}{dV_{ext}}=1-V \tag{7.24}$$

Integrating and rearranging, this gives:

$$V=1-e^{-V_{ext}} \tag{7.25}$$

Thus, the entire problem of determining the kinetics of the crystallisation has been reduced to finding V_{ext} in any particular case. To obtain the value for V_{ext}, Equation 7.21 is integrated taking into account that $N(z)=\bar{N}_0e^{-z}$ and

$$\begin{aligned}E_q(-x)&=\frac{1}{q!}\int_0^x(x-z)^qe^{-z}dz\\&=(-1)^{q+1}\left[e^{-x}-1+x...(-1)^{q+1}\frac{x^q}{q!}\right]\end{aligned} \tag{7.26}$$

$$\begin{aligned}V_{ext}=&\frac{6\sigma G^3\bar{N}_0}{p^3}\\&\left[e^{-\tau}-1+\tau-\frac{\tau^2}{2!}+\frac{\tau^3}{3!}\right]=\beta E_3(-\tau)\end{aligned} \tag{7.27}$$

where the following abbreviation has been introduced:

$$\beta=\frac{6\sigma G^3\bar{N}_0}{p^3} \tag{7.28}$$

This equation is valid up to $\tau=\bar{\tau}$, the time corresponding to the exhaustion of the supply of germ nuclei. Beyond this, the upper limit of the integral should be replaced by $\bar{\tau}$ and the result of integration may be expressed by:

$$V_{ext}=\beta\left\{E_3(-\tau)-e^{-\bar{\tau}}E_3\left[-(\tau-\bar{\tau})\right]\right\} \tag{7.29}$$

When $\bar{N}_0$ is very large, i.e., the supply of germ nuclei is not exhausted until the end of crystallisation, two borderline cases can be considered. When τ is very small, i.e., when p is very small and t is not too large, i.e., in the case of sporadic nucleation, the first four terms of the series expansion of the exponential term $e^{-\tau}$ in Equation 7.27 cancel out against the other terms between the square brackets. Hence, only the term of the fourth power in τ has to be taken into account, being the first term that does not cancel. By inserting the equation for V_{ext} thus obtained in Equation 7.25 the following equation results:

$$V=1-e^{(-\beta\tau^4/4!)}=1-e^{(-\sigma G^3\bar{N}_0pt^4)/4} \tag{7.30}$$

Note that a not too large value for p and a very small value for t lead to similar values of τ and, therefore, support the same reasoning. However, Avrami did not take this case into account.

On the other hand, when τ is very large, i.e., for p very large and t not too small, i.e., instantaneous nucleation, the exponential term $e^{-\tau}$ and the terms up to the order of two in Equation 7.27 can be disregarded in comparison

with the last term between square brackets; this results in the following equation for V:

$$V = 1 - e^{-\beta \tau^{3/3!}} = 1 - e^{-\sigma G^3 \bar{N}_0 t^3} \tag{7.31}$$

Again, τ can also be very large in the opposite case (t very large and p not too small), a case that Avrami did not take into account either.

For intermediate values of p the way V depends on t will lie in between Equation 7.30 and Equation 7.31.

In general, Equation 7.30 and Equation 7.31 can be written as:

$$V = 1 - e^{-kt^m} \tag{7.32}$$

which expression represents the equation that is generally known as the Avrami equation.

For plate-like and linear growth, an analysis similar to the previous one leads to other values for k and m (Table 7.1). As can be seen from Table 7.1, the rate constant k depends on the nucleation (amount of germ nuclei $\overline{N}_0$ for instantaneous nucleation and rate of nucleation $p\,\overline{N}_0$ for sporadic nucleation) and on the growth rate. The exact relationship depends on the specific case. The Avrami exponent m depends on the type of nucleation (sporadic or instantaneous) and the growth morphology of the crystallising particles. The meaning of the value of m, however, is not straightforward, since values for m of 2 and 3 can have two different meanings.

Theoretically, values for m should be an integer. However, analysis of experimental data frequently leads to Avrami exponents that are a noninteger. Several causes have been suggested (Long et al., 1995; Supaphol and Spruiell, 2001):

1. The ratio of the density of the crystalline phase over the density of the liquid phase varies during the process.
2. The true nucleation rate varies during the process.
3. The growth rate changes during the process.
4. The growth morphology changes during the process.
5. Crystalline aggregates grow concurrently from both instantaneous and sporadic nuclei.

Evans (1945), seemingly without knowledge of the prior work of Avrami, arrived at the same model by solving the problem of expanding waves created by raindrops falling on a pond.

7.2.6.2 Modified Avrami model

Apart from the original Avrami model as derived above, some authors also use a so-called modified Avrami model, also called the Avrami–Erofeev model. The modified equation is expressed by:

$$V = 1 - e^{(-k' t)^{m'}} \tag{7.33}$$

This equation differs from the original Avrami equation in that the rate constant k' is also raised to the power m' which is not the case in the original Avrami equation. This modified model originates from the work of Ng (1975) who described the development of the Erofeev model as well as from the work of Khanna and Taylor (1988) who modified the Avrami model to eliminate the dependence of k on m.

Ng (1975) described the development of the Erofeev model in a work on thermal decomposition in the solid state. This development was based on the Avrami theory. It can be deduced from Equation 7.25 and Equation 7.26 that for three-dimensional growth:

$$-\ln(1-V) = const.\left[e^{-\tau} - 1 + \tau - \frac{\tau^2}{2!} + \frac{\tau^3}{3!}\right] \tag{7.34}$$

When returning to the original time scale ($\tau = p*t$), one obtains:

$$-\ln(1-V) = const.\left[e^{-pt} - 1 + pt - \frac{p^2t^2}{2!} + \frac{p^3t^3}{3!}\right] \tag{7.35}$$

TABLE 7.1 Summary of the values obtainable for k and m in the Avrami model

Growth Morphology	Sporadic Nucleation		Instantaneous Nucleation	
	K[s^{-n}]	m [-]	k [s^{-n}]	m [-]
Linear	$\frac{\sigma''^* G^* \bar{N}_0^* p}{2}$	2	$\sigma''^* G^* \bar{N}_0$	1
Plate-like	$\frac{\sigma'^* G^2 * \bar{N}_0^* p}{3}$	3	$\sigma'^* G^2 * \bar{N}_0$	2
Spherical	$\frac{\sigma^* G^3 * \bar{N}_0^* p}{4}$	4	$\sigma^* G^3 * \bar{N}_0$	3

This equation can be transformed into a simplified form in two borderline cases (see also the deduction of Equation 7.30 and Equation 7.31). When $p \times t$ is much smaller than 1, Equation 7.35 changes into:

$$-\ln\ (1-V) = const\left(\frac{p^4 t^4}{4!}\right) \qquad (7.36a)$$

or

$$[-\ln\ (1-V)]^{1/4} = \frac{const}{24} pt = const' \times t \qquad (7.36b)$$

When $p \times t$ is much larger than 1, Equation 7.35 can be simplified into:

$$-\ln\ (1-V) = const\ \left(\frac{p^3 t^3}{3!}\right) \qquad (7.37a)$$

or

$$[-\ln\ (1-V)]^{1/3} = \frac{const}{6} pt = const' \times t \qquad (7.37b)$$

These equations can be represented by the generalised Erofeev equation:

$$[-\ln(1-V)]^{1/m'} = k't \qquad (7.38)$$

which is identical to Equation 7.33.

Khanna and Taylor (1988) claimed that the value of k resulting from the original Avrami model may not be correct, since k is a function of m. According to the authors, this problem can be eliminated by using a modified equation, such as Equation 7.33. What these authors did was transform the Avrami constant k from a complex constant of an m^{th} order process to a first order rate constant, despite the fact that crystallisation is not a first order process. It can be calculated that the k' value of the modified Avrami model is the m^{th} root of the k value of the original model (Marangoni, 1998):

$$k' = k^{\ 1/m} \qquad (7.39)$$

Therefore, the modified Avrami model is simply a reparameterised Avrami model.

Khanna and Taylor (1988) show that by modifying the Avrami model, more meaningful values for the reaction rate constant can be obtained. They compared, for example, the overall crystallisation rates of virgin Nylon 6 and extruded Nylon 6. By means of rate programmed DSC experiments, isothermal DSC experiments and optical microscopy, they showed that the crystallisation rate of virgin Nylon 6 is dramatically lower than the rate of extruded Nylon 6. Thus, if k is an overall rate constant, it should always be larger for the extruded Nylon 6 resin than for the virgin material. When calculating k by means of linear regression, it appeared that the value was higher for the extruded Nylon 6 at temperatures below 200°C, but lower at temperatures above 200°C. When calculating k' from the modified Avrami model, the value was always higher for the extruded Nylon 6, as expected. The authors also cite other work where the original Avrami model has yielded rate constants that differ from the expected values.

The authors further claim that despite the modification, the model retains its original connection to nucleation and crystal growth processes. The modification presented simply corrects the value of k by eliminating the influence of m. Khanna and Taylor (1988) conclude that attempts to obtain k values through the original Avrami model may lead to erroneous results, especially when comparing processes that have different values of m.

Marangoni (1998) does not agree with this modified Avrami model and argues that Khanna and Taylor (1988) arbitrarily suggested a modification of the Avrami model without providing any theoretical justification. The only justification the authors provided was their opinion that k and m were correlated and that the modification would solve this problem. However, no proof of this was given in their paper.

7.2.6.3 The Gompertz model

Kloek (1998) and Vanhoutte (2002) fitted their crystallisation curves to a reparameterised Gompertz equation as derived by Zwietering et al. (1990). The latter authors compared several sigmoid functions for their ability to describe a bacterial growth curve. Most of these functions contain mathematical fitting parameters rather than parameters with a biological meaning, making it difficult to provide initial guesses for these parameters. Moreover, it is difficult to calculate 95% confidence intervals for biologically meaningful parameters if these are not derived directly from the equation but calculated from mathematical fitting parameters.

Therefore, the growth models were rewritten to replace the mathematical fitting parameters with biologically meaningful parameters such as a_G (the maximum value reached), μ (the maximum specific growth rate that is defined as the tangent in the inflection point), and λ (the lag time, which is defined as the x-axis intercept of that tangent) (Figure 7.6). This reparameterisation was performed by deriving an expression for the biologically meaningful parameters as a function of the mathematical fitting parameters of the basic function.

The unmodified Gompertz equation is written as:

$$Y = A \times \exp\left[-\exp(B - D \times t)\right] \qquad (7.40)$$

with Y being the logarithm of the relative population size.

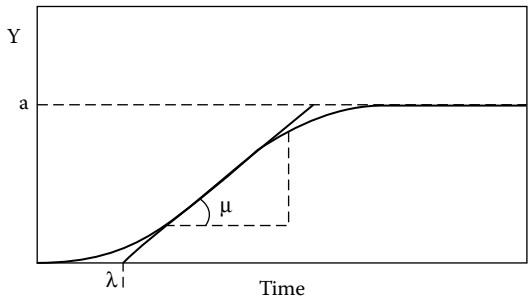

FIGURE 7.6 Bacterial growth curve.

To obtain the inflection point (at $t = ti$) of the curve, the second derivative of the function with respect to t is set at zero. This leads to:

$$ti = B / D \tag{7.41}$$

An expression for the maximum specific growth rate can be derived by calculating the first derivative at this inflection point:

$$\mu = \frac{A - D}{e} \text{ with } e \text{ equalling } 2.718281 \tag{7.42}$$

The parameter D in the unmodified Gompertz equation, thus, can be replaced by $\mu \times e/A$.

To determine the lag time, the slope of the tangent through the inflection point is calculated and, subsequently, the intercept with the t-axis is calculated:

$$\lambda = \frac{(B-1)}{D} \tag{7.43}$$

The parameter B can be replaced by $\frac{\mu \times e}{A}\lambda + 1$.

The a_G value equals the value of A since Y approaches A when t approaches infinity. Parameter A in the unmodified Gompertz equation can be substituted by a_G, yielding the reparameterised Gompertz equation:

$$Y = a_G \times \exp\left\{-\exp\left[\frac{\mu \times e}{a_G} * (\lambda - t) + 1\right]\right\} \tag{7.44}$$

Kloek (1998) and Vanhoutte (2002) used this reparameterised Gompertz equation after replacing Y by f, the extent of crystallisation at time t. At that point in time, A is the maximum fraction of solid fat. Kloek (1998) used this model because of several analogies between the crystallisation of fats and bacterial growth: production of bacteria is comparable with nucleation and growth of crystals, and consumption of nutrients is comparable with decrease of supersaturation.

7.2.6.4 Model of Foubert (2002)

Unlike the former models, the model developed by Foubert et al. (2002) was originally expressed in the form of a differential equation. This type of equation has the advantage that (1) it is often easier to interpret the equation mechanistically, (2) it is easier to make minor changes to the equation on the basis of acquired knowledge, and (3) by incorporation of secondary models describing the temperature dependency of the parameters, the model can be used to describe nonisothermal crystallisation kinetics. An algebraic solution for isothermal conditions, however, offers the advantage that the estimation of the parameters is facilitated by readily available software packages capable of nonlinear regression of algebraic equations. An algebraic solution assuming isothermal conditions, therefore, was also developed.

The differential equation of this model is expressed in terms of a variable h, which is the residual crystallisable fat:

$$h = \frac{a_F - f}{a_F} \tag{7.45}$$

where f is the extent of crystallisation at time t, and a_F is the maximum extent of crystallisation. In contrast to f, which increases sigmoidally over time, this variable h is related to the residual supersaturation (i.e., the driving force for crystallisation) and, thus, decreases sigmoidally over time.

To obtain the model, the crystallisation process is represented as if it is a combination of a first-order forward reaction and a reverse reaction of order n with rate constants K_i for each of the reactions. The dynamics of h can then be written mathematically as:

$$\frac{dh}{dt} = K_n \times h^n - K_1 \times h \tag{7.46}$$

K_1 and K_n are the rate constants of the first order forward reaction and the n^{th} order reverse reaction, respectively.

To calculate the values of h as a function of time according to Equation 7.46, the initial value for h, $h(0)$, needs to be elaborated according to:

$$h(0) = \frac{a_F - f(0)}{a_F} \tag{7.47}$$

with $f(0)$ representing the amount of crystals initially present, which can be related to the induction time of the crystallisation process.

Extensive parameter estimation studies revealed that the relative difference between K_1 and K_n amounts only to some 10^{-4} %. Furthermore, the quality of the 5-parameter

model was found to be not significantly better than that of a 4-parameter model for which $K_1 = K_n$. It was decided to simplify the model according to:

$$\frac{dh}{dt} = K \times (h^n - h) \quad h(0) = \frac{a_F - f(0)}{a_F} \qquad (7.48)$$

in which a_F is the maximum extent of crystallisation (expressed in percent [=solid fat potential] if measured by means of p-NMR, or expressed in J/g (latent heat) if measured by means of DSC), K is the rate constant (expressed in time unit^{-1}), n is the order of the reverse reaction (dimensionless) and $f(0)$ is the amount of crystals initially present (expressed in the same units as a).

To simplify the calculation of the parameters, the differential equation (4-parameter model) was converted to its algebraic solution. Since the physical interpretation of the parameter "induction time" is more straightforward than that of the parameter $h(0)$ (or the equivalent f(0)) and since the induction time can be more easily estimated from a crystallisation curve, it was decided to represent the equation as a function of t_x instead of $h(0)$. The parameter t_x is defined as the time needed to obtain x% of crystallisation.

$$h = \left[1 + ((1-x)^{1-n} - 1) \times e^{-(1-n) \times K \times (t - t_x)}\right]^{\frac{1}{1-n}} \qquad (7.49)$$

7.2.6.5 Comparison of models

Figure 7.7 compares the quality of fit of the Avrami, Gompertz, and Foubert models for a cocoa butter crystallisation followed by means of DSC. The modified Avrami model is not represented since it is not different from the original Avrami model with respect to curve-fitting qualities. Figure 7.7 shows that the Gompertz model provides a better fit than the Avrami model, a trend that could also be seen when the models were fitted to data pertaining to other cocoa butters at different temperatures and to data on milk fat and its fractions (Foubert et al., 2002). The Foubert model shows a better fit than both other models. The ability of the different models to describe isothermal fat crystallisation adequately has been tested statistically by Foubert et al. (2002). This study revealed that the Gompertz and Foubert models always perform better than the Avrami model and that the Foubert model performs better than the Gompertz model in the majority of cases.

The theory behind the Avrami model was developed on the basis of some assumptions that may not always be valid in the case of fat crystallisation. In addition to the fact that this may lead to noninteger values for the Avrami exponent m, it may also raise questions about the applicability of the Avrami model. The modified Avrami model, advocated by some authors, has been criticized by others as having no theoretical foundation. In our opinion, the modified Avrami model simply is a reparameterisation of the original model, possibly leading to better parameter estimates. The theoretical basis for using the Gompertz model for fat crystallisation is rather weak. Bacterial growth can intuitively be compared with fat crystallisation, but this provides no real theoretical justification. The very good fits obtained with the Foubert model make it a useful tool to obtain a better quantitative description of crystallisation processes. Later work (Foubert et al., 2005) has already given some more insight into the meaning of the different parameters.

7.2.6.6 Models to describe mult-step crystallisation

Since fats are complex mixtures of triglycerides, their crystallisation can lead to the formation of many crystal types, either due to polymorphism or concomitant growth of several crystal types. This may lead to crystallisation curves in which two steps can be identified.

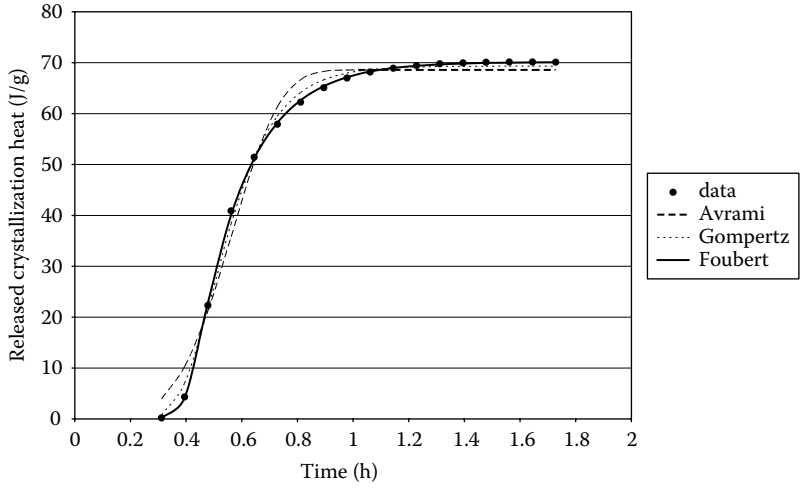

FIGURE 7.7 Comparison of fit.

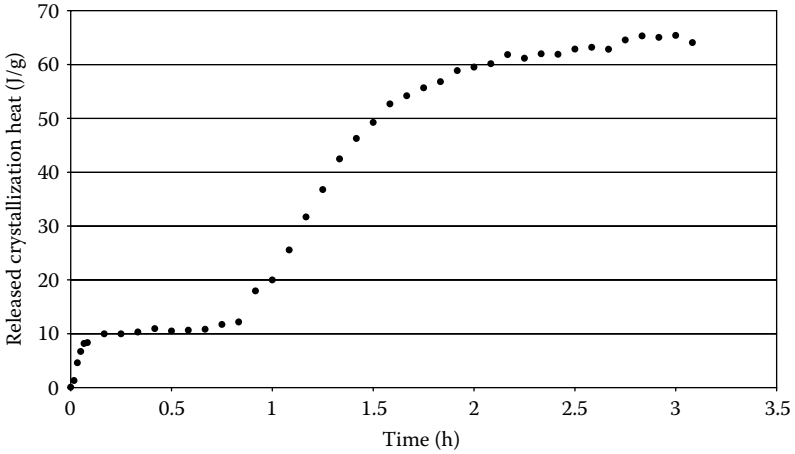

FIGURE 7.8 Two-step crystallisation curve.

Figure 7.8 shows an example of such a two-step process, which, of course, also makes the modelling more complex. To be able to fit a model to this kind of data Vanhoutte (2002) combined two Gompertz equations simply by numerically adding two algebraic Gompertz equations. Mazzanti et al. (2005) developed a quantitative model describing the growth of crystals and shear-related phase transitions in palm oil using a modified differential form of the Avrami model. Foubert et al. (2006) developed a model describing the isothermal two-step phase behaviour of cocoa butter as an extension to the original Foubert model. Both models have been developed on the basis of the known crystallisation mechanism and are combinations of several differential equations.

7.2.6.7 Models to describe nonisothermal crystallisation

A model able to describe nonisothermal crystallisation will be very interesting for the food industry, since most of their processes are of a nonisothermal kind. However, up to now most studies of nonisothermal fat crystallisation have been comparative in nature and have not applied a kinetic model. The difficulty in modelling nonisothermal crystallisation is that the rate of crystallisation depends upon the degree of crystallisation and the temperature, both of which vary. Smith et al. (2005) examined the determination of kinetic parameters from nonisothermal DSC crystallisation of a model fat, POP. They applied peak and isoconversional methods to determine activation energies and compared these techniques with a nonparametric method, which separates the temperature dependence and the degree of crystallisation dependence of the crystallisation rate. The Avrami model provides the best fit with the data, while the temperature dependence of the rate constant is best explained by a Vogel–Fulcher relationship, with the melting point of the crystallising species as the reference temperature.

7.2.7 Microstructural development

The various stages of the microstructural development (aggregation, network formation, and sintering) are illustrated in Figure 7.9.

Fat crystals attract each other by van der Waals' forces. The only repulsive interaction is hard-core repulsion, which only operates over very short distances. Random aggregation of particles that encounter each other due to Brownian movement and then stick together, leads to formation of fractal aggregates. A specific property of such fractal aggregates is that their structure is self-similar, implying that they have, on average, the same structure when observed at different magnifications. The time

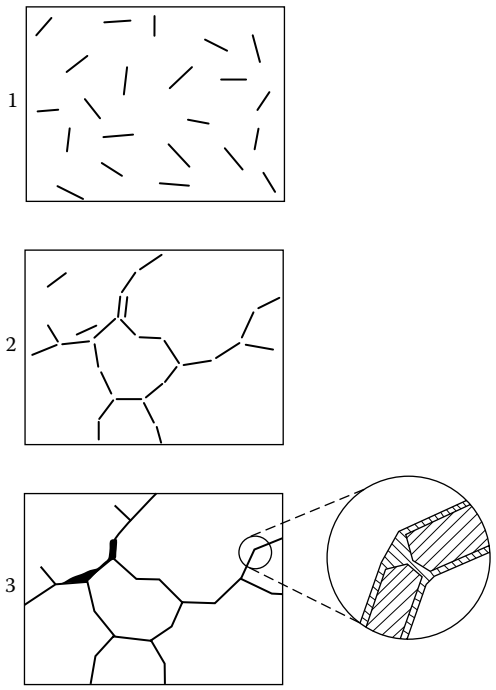

FIGURE 7.9 Stages in microstructural development.

needed to form aggregates of a few particles is in the range of between 10 and 100 seconds.

As soon as the volume fraction of particles in a fractal aggregate is about the same as the volume fraction of primary particles in the system, the aggregates start to impinge on each other. A continuous network or a gel is formed, giving the fat elastic properties. Values observed for the time it takes for a gel to be formed are between 2 and 5 minutes, i.e., at a very low fraction of solid fat (1%) (Kloek, 1998; Walstra et al., 2001).

After formation of the primary network, the major part of the fat, thus, still has to crystallise. This additional crystallisation will lead to compaction of the aggregates that form the primary network. Whether the fractal nature of the network still exists at these higher solid:liquid ratios is a controversial point (Kloek, 1998; Narine and Marangoni, 1999).

Another consequence of further crystallisation after aggregation is sintering, i.e., the formation of solid bridges between aggregated crystals and aggregates. Sintering of two crystals will occur if some triglyceride molecules are incorporated in the lattices of both crystals. This is more likely to occur at crystal surfaces that have defects due to lattice mismatches and may be related to the occurrence of compound crystals. Therefore, it is more likely that sintering occurs in fats that contain many different triglycerides (Kloek, 1998). Johansson (1994) showed that polymorphism is also important for the occurrence of sintering; sintering only occurs when the outer part of the crystals and the bridging molecules have the same polymorphic structure.

The properties of a fat crystal network are affected by several formation process parameters, such as temperature, concentration, and agitation. A lower temperature means smaller crystals in the early stages, which would mean a somewhat faster aggregation and a clearly shorter gelation time. However, these conclusions are not universally valid because factors, such as polymorphism and secondary nucleation, disturb this pattern. Mild agitation may speed up aggregation of crystals, but at high shear rates, the resulting forces cause disaggregation. Prediction of these effects, however, is far from easy because the prevailing strain rates tend to vary enormously according to place and time (Walstra et al., 2001).

7.2.8 Mechanical and macroscopic properties

The mechanical and macroscopic properties of a fat are influenced by all the structure levels described above (molecular structure, primary crystals, crystal aggregates, three-dimensional network). Each step in this structural hierarchy is influenced by the processing conditions (Narine and Marangoni, 1999). The interaction between these structure levels has been the subject of recent research, e.g., Kloek (1998), Narine and Marangoni (1999), and Vanhoutte (Vanhoutte, 2002).

This section gives an overview of the macroscopic and mechanical properties of crystallised fats together with some information on how they are measured.

7.2.8.1 Small deformations

When discussing small-scale deformation behaviour (mostly measured by oscillatory experiments in a rheometer), the modulus, be it elastic or viscoelastic, is the most important parameter. It is a measure of the stiffness (not the strength) of the system. One can only speak of a true modulus if the strain is proportional to the stress, i.e. when measurements are performed in the so-called linear region. This region comprises only strains in the order of 10^{-4}, which is a very low value. Outside this region, irreversible changes in the crystal network are induced (Kloek, 1998; Walstra et al., 2001).

The rheological properties of fats are primarily determined by the fraction of crystallised fat. Another important factor is the interaction between the crystals. Initially, crystals are aggregated due to van der Waals' attraction. Deformation then leads to an increasing distance between the crystals. However, if the crystals are sintered, the crystals have to bend when the network is deformed. In this case, the elastic modulus depends on the bending modulus of the crystals. Bending of crystals can also be important for nonsintered dispersions if the crystals are long and thin. The geometric arrangement of the crystals and the aggregates is also an important factor determining the elastic modulus. All these influences have been incorporated in various models that explain the elastic modulus of particle networks.

7.2.8.2 Large deformations

Although the theoretical background of small deformations has been studied quite extensively, the results are rarely of practical significance. Research on large-scale deformation behaviour, on the other hand, is of great practical importance as these experiments yield information about important quality characteristics, which are relevant to processing, handling, and eating. An example of such a characteristic is spreadability. A major criticism concerning these large-scale deformation tests, however, is that it is difficult to correlate the measured parameters with fundamental properties of the material (Shellhammer et al., 1997; Kloek, 1998). Care should also be taken when interpreting the results, since large-scale strain experiments can be applied in various deformation modes, which tend to give different results. Moreover, several factors may affect the results, such as deformation of the sample when transferring it in the measuring body, or inhomogeneous deformation (Walstra et al., 2001).

Figure 7.10 gives an idealized stress-strain curve for a margarine-type fat with a fairly low SFC. Four regions can be distinguished. In zone 1 the behaviour is linearly elastic, which means that the strain is proportional to the

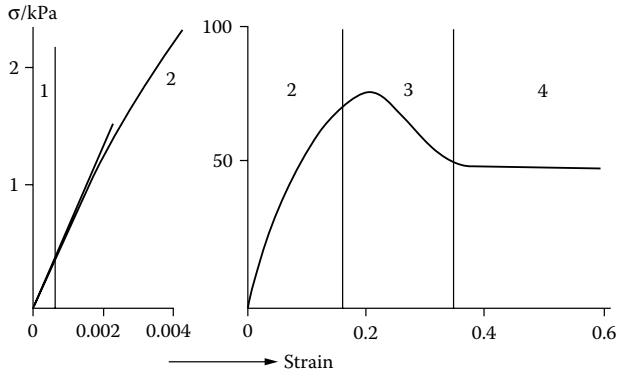

FIGURE 7.10 Stress–strain curve.

stress. The deformation is fully reversible, which implies that no bonds between crystals are broken. For larger strains, the behaviour becomes nonlinear (zone 2) and it is increasingly viscoelastic. Although most of the deformation is still reversible, irreversibility increases with increasing strain. This means that an increasing number of bonds are broken. Presumably, van der Waals' bonds are broken and re-form, whereas fairly weak sintered bonds are also broken, but do not re-form, at least not within a short time span. It should be realized that the inhomogeneity of the system leads to a wide variation in bond strength.

Zone 3 is the region of stress overshoot, a phenomenon that typically leads to yielding of the material. Yielding is the transition from viscoelastic to viscous behaviour. It only occurs in materials composed of a solid network interspersed with a continuous liquid phase. In local planes, all of the bonds in the network are broken, but the "cracks" are immediately filled with liquid and the system remains continuous. In most systems exhibiting yielding, removal of the stress quickly leads to the partial recovery of the elastic properties. This means that significant interaction forces must remain between the solid structural elements. The stress needed to cause yielding of the fat is especially important since cutting, spreading, and shaping all involve yielding.

Finally, plastic flow is attained in zone 4. The material flows, but it is very viscous. The high viscosity is primarily due to the presence of fairly large remnants of what was originally a continuous crystal network. Moreover, the irregular shape and spiky surface of these structural elements leads to their entanglement during flow. Possibly, van der Waals' attraction between crystals or crystal aggregates may also contribute to these properties.

The deformation rate can also be determined as a function of the stress applied. Often, this flow curve becomes virtually linear, and the Bingham yield stress σ_B can be obtained by extrapolation (Figure 7.11). It turns out that σ_B correlates well with the firmness (often called hardness) of the fats as determined by penetrometry, extrusion or wire-cutting tests. The firmness depends on the SFC, but the shape and steepness of the curve varies depending on the extent of sintering, the homogeneity of the network, and crystal size and shape. In practice, firmness tends to increase considerably when the temperature is decreased. This is because of an increase in solid fat content and an increase of sintering. Many fats increase in firmness during storage and the rate of increase tends to be faster at a higher storage temperature. The cause appears to be further sintering. Recrystallisation involving transition to the β-polymorph tends to lead to a decrease in firmness, often to a considerable extent. This is because β-crystals tend to be rather isometric and not very prone to sintering. The latter may be because hardly any compound crystal formation can occur in the β-polymorph.

Working can decrease the firmness of a plastic fat and this is called work softening. The extent of work softening can vary widely: a relative decrease in firmness of between 10 and 75% can be observed, depending on the type of fat and the intensity of the working. Considerable setting, i.e., increase in firmness, may occur after working. Initially, setting will be caused by aggregation due to van der Waals' attraction of the crystals and crystal network formation, but in a longer timescale, setting is presumed to be due to recrystallisation. The above considerations

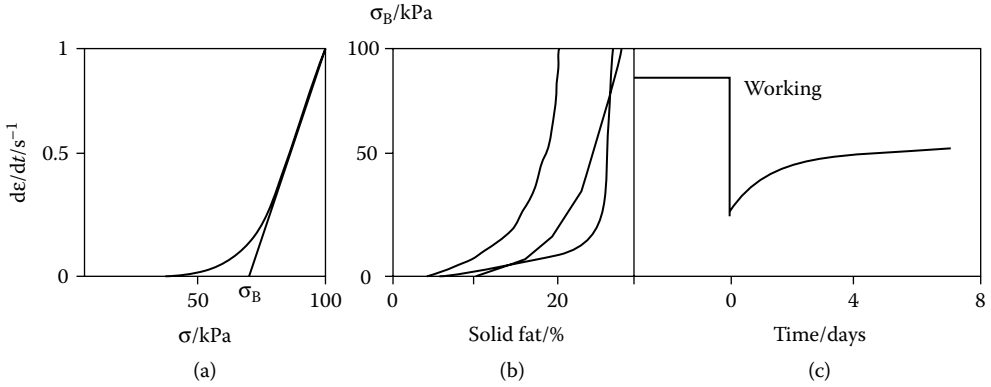

FIGURE 7.11 Bingham stress and firmness.

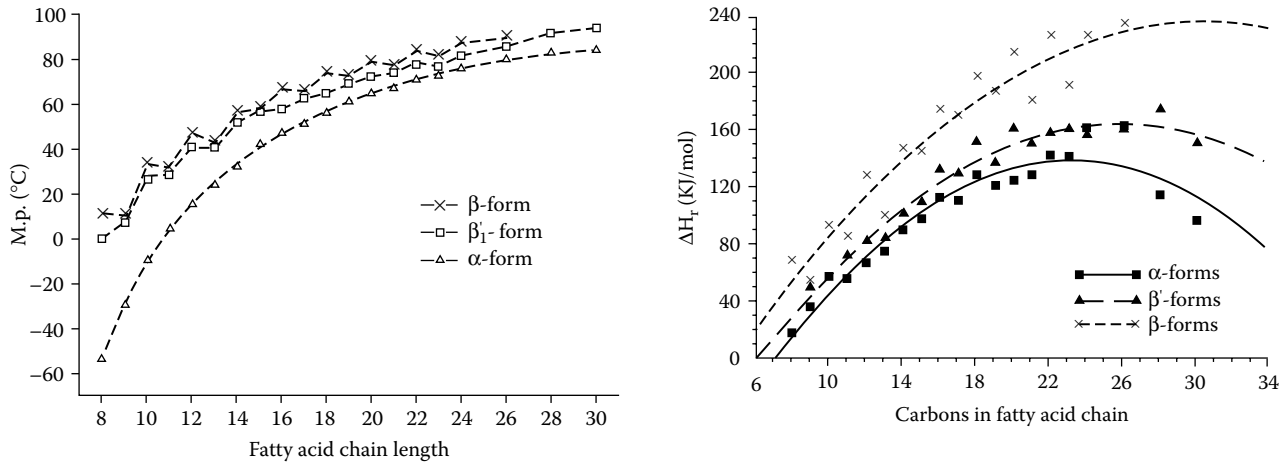

FIGURE 7.12 a/b Melting properties of fatty acids.

demonstrate that predicting the yield properties of a plastic fat is very difficult but that nevertheless some trends can be explained.

7.2.9 Melting behaviour

The melting behaviour of saturated monoacid triglycerides depends largely on the fatty acid chain length. The longer the chains, the higher the melting point. Figure 7.12 shows the evolution of the melting point and the melting enthalpy of the different polymorphs of saturated monoacid triglycerides as a function of the fatty acid chain length. The melting points of the α-form rise smoothly as the chain length increases, while for the β-polymorph, the melting points rise in a zigzag pattern, whereby the triglycerides with even chain lengths exhibit higher melting points. The evolution of the β′-form melting point changes from an alternation between odd and even to a smooth increase as chain length increases.

The melting enthalpies of monoacid saturated triglycerides (Figure 7.12b) can be described by parabolic equations with a maximum at a fatty acid chain length of 22 or more carbon atoms, depending on the polymorph. For unsaturated monoacid triglycerides, both the melting temperature and the melting enthalpy are lower than for the saturated monoacid triglycerides with the same fatty acid chain length. The melting temperature and enthalpy for *cis*-unsaturated monoacid triglycerides is lower than for the *trans* equivalent (Hagemann, 1988).

Since natural fats always contain several different fatty acids and, thus, comprise an enormous number of different triglycerides, a natural fat has a melting range and not a sharp melting point. The melting point of a fat must be defined, for instance, as the temperature at which the fat becomes visually clear and free of crystals (clear point, final melting point). Other commonly employed measurements quantifying certain melting properties of a fat are dropping point, softening point, and slip point. A fat will begin to soften, slip, and drop prior to reaching the final clear point, so the temperatures reported for these measurements are lower than the actual clear point of the fat (Kaylegian and Lindsay, 1995).

The capillary tube melting point, sometimes referred to as the clear point (AOCS Official Method Cc 1-25, latest revision. 1997) corresponds to the melting point as ordinarily determined by an organic chemist. It is defined as the temperature at which the sample becomes completely clear and liquid when heated (at a rate of 0.5°C/min) in a capillary tube of 1 mm internal diameter. The filled tubes must be quickly chilled and held at 4 to 10°C overnight before the determination is made. This means that for some fats this method will give the melting point of an unstable polymorph.

The softening point or open tube melting point (AOCS Official Method Cc 3-25, latest revision 1997, or rather the ISO Equivalent Cc 3b-92 revised in 2002) should be determined exactly as the capillary tube melting point, except that the lower end of the capillary tube is left unsealed. It is defined as the temperature at which the fat becomes sufficiently softened for the fat column to rise in the tube. This method is only satisfactory for relatively hard fats.

The slip point (AOCS Official Method Cc 4-25, revised in 1989) determines the melting point of the sample in its finished state, unlike the preceding methods, in the course of which the material is melted and resolidified. Cylindrical brass rings (2 mm wall thickness, 9 to 11 mm inside diameter, 10 mm height) are filled by being forced into the sample and are then suspended in a bath with a saturated sodium chloride solution. The slip point is determined as the point at which the fat rises from the cylinder.

The Wiley melting point (AOCS Official Method Cc 2-38, latest revision 1991) is quite commonly used in the U.S. as an indication of the temperature at which a fat becomes substantially liquid. Discs of the fat 3/8 inch in diameter and 1/8 inch thick are solidified and chilled in

metal forms for 2 hours or more, after which the disc is suspended in an alcohol–water bath having the same density as the fat sample, and slowly heated. The Wiley melting point is defined as the temperature at which the fat disc becomes completely spherical.

The dropping point (AOCS Official Method Cc 18-80, latest revision 2001) may be used as an alternate method. A sample cup with a drip hole is filled with the fat to be examined, crystallised in the freezer for 15 minutes and then heated at a constant rate. The dropping point is the temperature at which a drop of fat falls through the hole in the bottom of the cup.

However, the melting point of a fat does not tell us everything we need to know. Cocoa butter and milk fat, for example, have about the same capillary tube melting point, but they have very different melting profiles. Cocoa butter consists largely of closely related, high melting triglycerides and, thus, exhibits a narrow melting range. Milk fat has a far wider compositional range. Consequently, its melting range spans about 80°C and its properties change more slowly as the temperature changes. These fats represent two extremes. Many animal carcass fats have a melting pattern more or less comparable to that of milk fat, while some vegetable fats, like coconut oil, behave more like cocoa butter (Walstra, 2003).

References

Aquilano, D. and Sgualdino, G. (2001), Fundamental aspects of equilibrium and crystallization kinetics, in *Crystallization Processes in Fats and Lipid Systems*, Garti, N. and Sato, K., Eds., Marcel Dekker, Inc., New York, Basel, 1–51.

Avrami, M. (1939), Kinetics of phase change. I. General theory, *J. Chem. Phys.*, **7**, 1103–1112.

Avrami, M. (1940), Kinetics of phase change. II. Transformation-time relations for random distribution of nuclei, *J. Chem. Phys.*, **8**, 212–224.

Baur, F.J. et al. (1949), The polymorphism of saturated 1,3-diglycerides, *J. Am. Chem. Soc.*, **71**, 3363–3366.

Bennema, P. et al. (2001), Morphological connected net-roughening transition theory: Application to β crystals of triacylglycerols, in *Crystallization Processes in Fats and Lipid Systems*, Garti, N. and Sato, K., Eds., Marcel Dekker, Inc., New York, 99–150.

Boistelle, R. (1988), Fundamentals of nucleation and crystal growth, in *Crystallization and Polymorphism of Fats and Fatty Acids*, Garti, N. and Sato, K., Eds., Marcel Dekker, New York, 189–276.

Di, L. and Small, D.M. (1993), Physical behavior of the mixed chain diacylglycerol, 1-stearoyl-2-oleoyl-*sn*-glycerol: difficulties in chain packing produce marked polymorphism, *J. Lipid Res.*, **34**, 1611–1623.

Di, L. and Small, D.M. (1995), Physical behavior of the hydrophobic care of membranes: properties of 1-stearoyl-2-linoleoyl-*sn*-glycerol, *Biochemistry*, **34**, 16672–16677.

Duynhoven, J. van et al. (1999), Solid fat determination by NMR, *inform*, **10**, 479–484.

Enden, J.C. van den et al. (1978), A method for the determination of the solid phase content of fats using pulse Nuclear Magnetic Resonance, *Fette Seifen Anstrichm.*, **80**, 180–186.

Enden, J.C. van den et al. (1982), Determination of the Solid Fat Content of hard confectionary butters, *J. Am. Oil Chem. Soc.*, **59**, 433–439.

Evans, U.R. (1945), The law of expanding circles and spheres in relation to lateral growth of surface films and the grain-size of metals, *Trans. Faraday Soc.*, **41**, 365–374.

Foubert, I. et al. (2002), Dynamic mathematical model of the crystallization kinetics of fats, *Food Res. Int.*, **35**, 945–956.

Foubert, I. et al. (2005), Insight in model parameters by studying temperature influence on isothermal cocoa butter crystallisation, *Eur. J. Lipid Sci. Technol.*, **107**, 660–672.

Foubert, I. et al. (2006), Modelling two-step isothermal fat crystallization, *J. Food Eng.*, **75**, 551–559.

Garside, J. (1987), General principles of crystallization, in *Food Structure and Behaviour*, Blanshard, V.M.V. and Lillford, P., Eds., Academic Press, London, 35–49.

Hagemann, J.W. (1988), Thermal behavior and polymorphism of acylglycerides, in *Crystallization and Polymorphism of Fats and Fatty Acids*, Garti, N. and Sato, K., Eds., Marcel Dekker, New York, 9–95.

Hartel, R.W. (1992), Solid-liquid equilibrium in foods, in *Physical Chemistry of Foods*, Schwartzberg, H.G. and Hartel, R.W., Eds., Marcel Dekker, New York, 47–81.

Johansson, D. (1994), *Colloids in fats: the fat crystal as a functional particle*, Ph.D. thesis, Lund University, Sweden.

Kaylegian, K.E. and Lindsay, R.C. (1995), *Handbook of Milkfat Fractionation Technology and Applications*, AOCS Press, Champaign, IL.

Kellens, M.J. (1991), *Polymorphism of saturated monoacid triglycerides*, Ph.D. thesis, K.U. Leuven, Belgium.

Khanna, Y.P. and Taylor, T.J. (1988), Comments and recommendation on the use of the Avrami equation for physico-chemical kinetics, *Poly. Eng. Sci*. **28**, 1042–1045.

Kloek, W. (1998), *Mechanical properties of fats in their relation to their crystallisation*, Ph.D. thesis, Landbouwuniversiteit Wageningen, The Netherlands.

Krog, N. (2001), Crystallization properties and lyotropic phase behavior of food emulsifiers: relation to technical applications, in *Crystallization Processes in Fats and Lipid Systems*, Garti, N. and Sato, K., Eds., Marcel Dekker, New York, 505–526.

Krog, N. and Sparso, F.V. (2004), Food emulsifiers: their chemical and physical properties, in *Food Emulsions*, Friberg, S.E., Larsson, K., and Sjöblom, J., Eds., Marcel Dekker, New York, 45–91.

Lambelet, P. (1983), Comparison of NMR and DSC methods for determining solid fat content of fats, application to milk fat and its fractions, *Food Sci. Technol. (Int.)*, **16**, 90–95.

Larsson, K. (1966), Classification of glyceride crystal forms, *Acta Chem. Scand.*, **20**, 2255–2260.

Larsson, K. (1972), Molecular arrangement in glycerides, *Fette Seifen Anstrichm.*, **74**, 136–142.

Loisel, C. (1996), *Physico-chimie du chocolat: crystallisation du beurre de cacao et propriétés structurales*, Ph.D. thesis, École Nationale Supérieure des Industies Agricoles et Alimentaires, Paris.

Loisel, C. (1998), Phase transitions and polymorphism of cocoa butter, *J. Am. Oil Chem. Soc.*, **75**, 425–439.
Long, Y. et al. (1995), Kinetics of polymer crystallisation, *Prog. Polymer Sci.*, **20**, 651–701.
Malkin, T. (1954), *Progress in the Chemistry of Fats and other Lipids, Vol. II*, Pergamon Press, Oxford, 1–50.
Marangoni, A.G. (1998), On the use and misuse of the Avrami equation in characterization of the kinetics of fat crystallization, *J. Am. Oil Chem. Soc.*, **75**, 1465–1467.
Marangoni, A.G. et al. (2000), Comment on the use of direct pulsed nuclear magnetic resonance solid fat content measurements in phase behavior studies of lipid mixtures, *J. Am. Oil Chem. Soc.*, **77**, 565–567.
Mazzanti, G. et al. (2005), Modelling phase transitions during the crystallization of a multicomponent fat under shear, *Phys. Rev. E*, **71**, 041607-1 – 041607-12.
Mulder, H. and Walstra, P. (1974), *The Milk Fat Globule, Emulsion Science as Applied to Milk Products and Comparable Foods*, Pudoc, Wageningen and CAB, Farnham Royal.
Narine, S.S. and Marangoni, A.G. (1999), Relating structure of fat crystal networks to mechanical properties: A review, *Food Res. Int.*, **32**, 227–248.
Nawar, W.W. (1996), Lipids, in *Food Chemistry*, Fennema, O.R., Ed., Marcel Dekker, New York, 239–319.
Ng, W.L. (1975), Thermal decomposition in the solid state, *Aust. J. Chem.*, **28**, 1169–1178.
Povey, M.J.W. (1995), Ultrasound studies of shelf-life and crystallization, in *New Physico-Chemical Techniques for the Characterization of Complex Food Systems*, Dickenson, E., Ed., Chapman & Hall, London, 196–212.
Rousset, Ph. (1997), *Étude expérimentale et modélisation de la crystallisation de triacylglycérols et du beurre de cacao*, Ph.D. thesis, École Polytechnique Fédérale de Lausanne, Switzerland.
Sato, K. and Kuroda, T. (1987), Kinetics of melt crystallization and transformation of tripalmitin polymorphs, *J. Am. Oil Chem. Soc.*, **64**, 124–127.
Sato, K. et al. (1999), Molecular interactions and kinetic properties of fats, *Prog. Lipid Res.*, **38**, 91–116.
Shannon, R.J. et al. (1992), The polymorphism of diglycerides, *J. Sci. Food Agric.*, **60**, 405–417.
Shellhammer, T.H. et al. (1997), Viscoelastic properties of edible lipids, *J. Food Eng.*, **33**, 305–320.
Smith, P.R. (2005), Healthy high-quality fats from a food science perspective, *Lipid Techn.*, **17**, 177–181.
Supaphol, P. and Spruiell, J.E. (2001), Isothermal melt- and cold-crystallisation kinetics and subsequent melting behavior in syndiotactic polypropylene: a differential scanning calorimetry study, *Polymer*, **42**, 699–712.
Takahashi, H. et al. (1999), Simultaneous x-ray diffraction and calorimetric study of metastable-tostable solid phase transformation of 1,2-dipalmitoyl-*sn*-glycerol, *Biophys. Chem.*, **77**, 173.
Timms, R.E. (1984), Phase behaviour of fats and their mixtures, *Prog. Lipid Res.*, **23**, 1–38.
Timms, R.E. (1997), Fractionation, in *Lipid Technologies and Applications*, Gunstone, F.D. and Padley, F.B., Eds., Marcel Dekker, New York, 199–222.
Timms, R.E. (2003), *Confectionary Fats Handbook. Properties, Production and Application*, The Oily Press, Bridgewater, U.K..
Todt, H. et al. (2001), Quality control with time-domain NMR, *Eur. J. Lipid Sci. Technol.*, **103**, 835–840.
Vanhoutte, B. (2002), *Milk fat crystallisation: fractionation and texturisation*, Ph.D. thesis, Universiteit Gent, Ghent, Belgium.
Walker, R.C. and Bosin, W.A. (1971), Comparison of SFI, DSC and NMR-methods for determining solid-liquid ratios in fats, *J. Am. Oil Chem. Soc.*, **48**, 50–53.
Walstra, P. (1987), Fat crystallization, in *Food Structure and Behaviour*, Blanshard, V.M.V. and Lillford, P., Eds., Academic Press, London, 67–85.
Walstra, P. (1998), Secondary nucleation in triglyceride crystallization, *Progr. Colloid Polym. Sci.*, **108**, 4–8.
Walstra, P. (2003), *Physical Chemistry of Foods*, Marcel Dekker, New York.
Walstra, P. and Beresteyn, E.C.H. van (1975), Crystallization of milk fat in the emulsified state, *Neth. Milk Dairy J.*, **29**, 35–65.
Walstra, P. et al. (2001), Fat crystal networks, in *Crystallization Processes in Fats and Lipid Systems*, Garti, N. and Sato, K., Eds., Marcel Dekker, New York, 289–328.
Zwietering, M.H. et al. (1990), Modelling of the bacterial growth curve, *Appl. Env. Microbiol.*, **56**, 1875–1881.

7.3 Phase behaviour

7.3.1 General principles

The physical properties (e.g., macroscopic and mechanical properties) of a fat are determined by its phase behaviour and this concept was first explained clearly by Mulder (1953). A natural fat is a mixture of many triglycerides and although each triglyceride has its own polymorphism and melting behaviour, natural fats cannot be considered in terms of their individual component triglycerides, but only in terms of their different phases (Timms, 2003).

The phase behaviour of triglyceride mixtures has critical implications in fat blending and separation of component triglycerides from natural fats and oil resources as during fractionation processes. Therefore, the complex melting, crystallisation, and transformation behaviour of natural fats must be elucidated by examining the mixing behaviour of binary, ternary, or more multiple phases of specific triglyceride components (Sato, 2001b).

7.3.1.1 The concept of a phase

A phase is a state of matter (e.g., a triglyceride mixture) that is homogeneous and separated from another phase by a definite physical boundary. A phase can be defined in full by its composition, temperature, and pressure. In food products, the pressure can be ignored for most practical purposes. Natural fats always contain at least two phases: a liquid and a solid phase. There is usually only one liquid phase, but several solid phases may be present at the same time. Different solid phases in fats are usually only distinguishable under a microscope, although their presence may affect macroscopic properties (Timms, 2003).

In the liquid state, the miscibility of triglycerides is almost "ideal", i.e., no heat or volume changes occur on mixing and the ideal or Hildebrand solubility equation applies (Hannewijk et al., 1964; Knoester et al., 1972).

Measurable deviations from ideal behaviour occur only when the triglycerides differ appreciably in molecular weight (and, hence, also volume) (Timms, 1978; 2003). Wesdorp (1990) confirmed this ideal behaviour for triglycerides that do not deviate from the average carbon number of the fat mixture by more than ten carbon numbers.

In practice, the triglycerides in real fats do mix in the solid state to form solid solutions (also called mixed crystals). A solid solution is an intimate mixture of two or more components in the solid state such that neither component can be easily distinguished (Timms, 2003).

7.3.1.2 The concept of a phase diagram

Since (at constant pressure) a phase is defined by its temperature and composition, a diagram with the temperature along one axis and the composition along the other is sufficient to define all the phases. Such a diagram is called a phase diagram and describes the phase behaviour of a mixture of triglycerides (usually at equilibrium) (Timms, 1984; 2003). One of the main purposes of drawing up phase diagrams is to obtain information about the solid phase behaviour, especially with respect to the miscibility of the components in the solid state.

To this end, the mixing behaviour in the liquid state must be known. It is generally accepted that the liquid of a triglyceride mixed system may be treated as a close approximation to an ideal mixture. For such systems the equilibrium solid phase properties can be derived from the phase diagrams (Knoester and Dejong, 1975). A considerable number of phase diagrams of glyceride mixtures have been reported in literature. Rossell (1967) compiled a survey of the available data, and this is still very useful today.

Although most practical fat systems comprise mixtures of at least 10 major triglycerides, most studies have investigated the phase behaviour of binary mixtures. For binary mixtures of triglycerides (A and B), five types of phase diagrams have been observed as shown in Figure 7.13 (Timms, 1984; 2003).

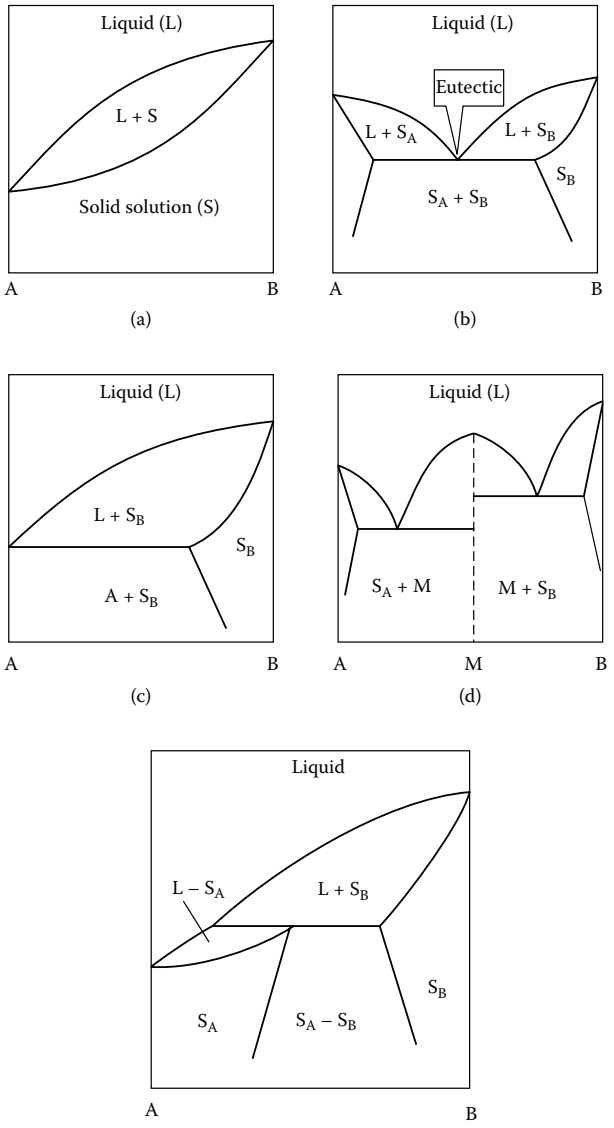

FIGURE 7.13 Phase diagrams of binary mixtures.

- In diagram (a) of Figure 7.13, the triglycerides A and B have similar properties (melting point, molecular volume, and polymorph) and mix to form a continuous solid solution. Noteworthy mixtures of this type are POSt/StOSt and StStSt/StStE (P stands for palmitic acid, O for oleic acid, St for stearic acid, and E for elaidic acid).
- In diagram (b), triglycerides A and B are less similar and the solubility of one in the other is limited, leading to a mixture of solid solutions and a sharp dip and interruption to the liquidus line at the eutectic point. The eutectic system is the most common, noteworthy examples being PPP/StStSt, POSt/POP. Eutectic systems tend to occur when the components differ in molecular volume, shape, or polymorph, but not greatly in melting point. Diagrams (a) and (b) are the two types commonly found in many binary mixtures of triglycerides.
- In diagram (c) of Figure 7.13, the eutectic system tends to shift to a monotectic system, as differences in the melting points of the component triglycerides increase. For example the LLL/PPP (with L standing for linoleic acid) system (melting point difference = 20°C) shows a slight eutectic at 3% PPP, but the LLL/StStSt system (melting point difference = 27°C) shows either a monotectic or a eutectic at 1% StStSt. Similarly, the PPP/POP system (melting point difference = 28°C) shows a monotectic, whereas the PPP/StOSt system (melting point difference = 22°C) shows a slight eutectic at 5.5% PPP. In all such cases, the solid high melting component dissolves a substantial quantity, usually 20 to 30%, of the low melting component.
- In diagram (d), A and B combine to form a special mixture called a molecular compound (M) that behaves like a new, pure triglyceride with unique

properties differing from those of its component triglycerides. Hence, the diagram resembles two eutectic phase diagrams of the (b) type placed side by side. In the system StOSt/StStO, there is clear evidence for the formation of a compound. This system is then really two eutectic systems, StOSt/M and M/StStO, side by side.

- Peritectic systems (e) have been observed to occur only in mixed saturated/unsaturated systems where at least one triglyceride has two unsaturated acids. Noteworthy examples are StOSt/StOO and POP/POO.

Sato et al. performed major research on the phase behaviour and the thermodynamic and kinetic phase properties of binary mixtures of pure component triglycerides (Minato et al., 1996; Takeuchi et al., 2002; Takeuchi et al., 2003). The same group wrote extensive reviews on this matter (Sato et al., 1999; Sato and Ueno, 2001; Sato, 2001a).

Lee (1978) presented a mathematical approach, which can simulate phase diagrams for binary mixtures of lipid molecules showing close agreement with experimental data and using a single parameter to describe the nonideal mixing in each phase. An index of lipid phase diagrams was composed by Koynova and Caffrey (2002).

7.3.2 Phase behaviour of real fats

Binary phase diagrams can display all the properties of a two-component mixture. To display the properties of a real fat, a multicomponent phase diagram with extra axes for each component triglyceride above two is necessary. An individual fat may be considered as a composition point in this multicomponent phase diagram. Small compositional changes in the diagram would reflect the natural variations in the properties of the fat; larger compositional changes would indicate a move to a completely different fat (Timms, 2003).

It is not possible to depict such multicomponent phase diagrams, even if the data were available. Figure 7.14 shows the three types of phase diagrams that Timms (1984) deduced to be possible for binary mixtures of fats. Fp(AB), Fq(CD), Fs(CD), and Fr(CD) are to be considered as different types of fat formed by the mixture of just two triglycerides, A and B, or C and D. Essential differences between mixtures of fats and of pure component triglycerides are as follows:

- There is no unique temperature (the melting point) for a real fat at 0 or 100% composition, where liquidus and solidus or solindex lines meet.
- There is usually no precise eutectic point for mixtures of real fats, although there is often a eutectic minimum in the liquidus curve and, therefore, in the melting point of Clear Point curves.
- When the two components are not pure triglycerides, tie lines, i.e., lines drawn horizontallyin a phase diagram, do not define the ratio of solid to liquid phase present in between the solidus and liquidus lines.

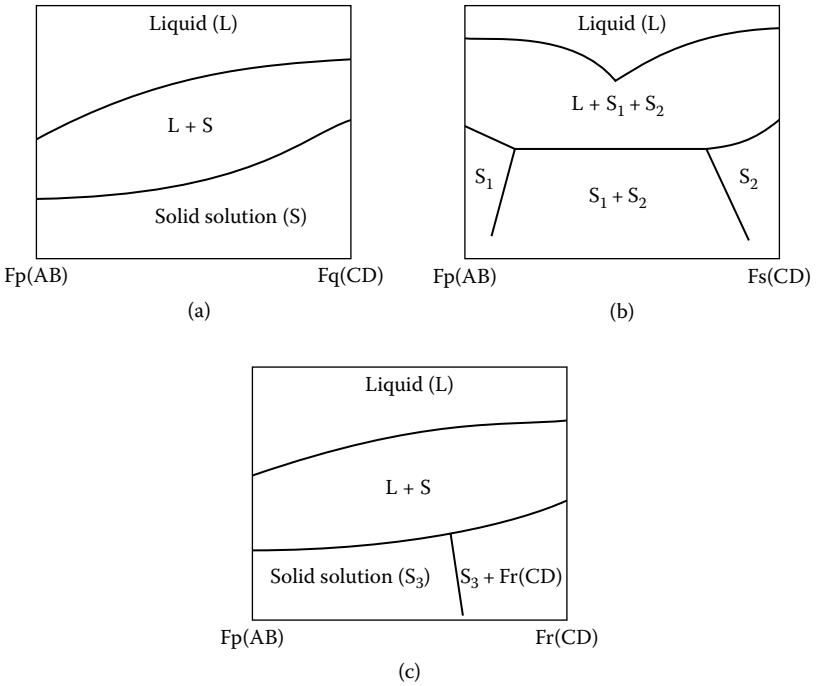

FIGURE 7.14 Phase diagrams of fats. (Adapted from Timms, R.E. (2003), *Confectionary Fats Handbook, Properties, Production and Application*, The Oily Press, Bridgwater, U.K.)

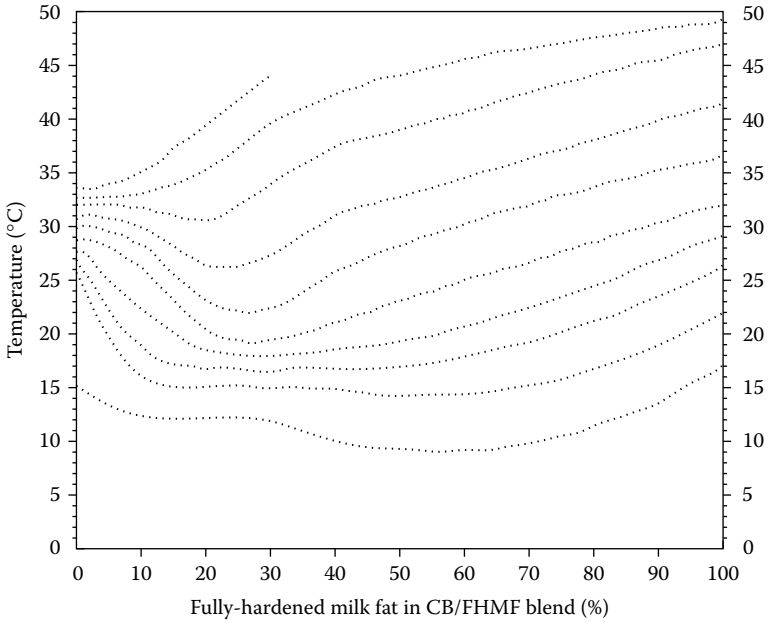

FIGURE 7.15 Isosolid diagram for blends of cocoa butter (CB) and a fully hardened milk fat (FHMF). Isosolid lines are plotted at 5, 10, 20, 30, 40, 60, and 80% solids from the top downwards. (Adapted from Timms, R.E. (1979), *Chem. Ind.*, 257–258.)

Moreover, phase diagrams essentially describe fat systems at equilibrium. They usually depict fats and their mixtures in their most stable polymorphs. In practice it takes time to reach equilibrium in the solid state, so that an individual phase diagram may be considered as a snapshot of the situation at a given time. Additionally, the phase behaviour of two fats may be different for different polymorphs (Kellens and Reynaers, 1992).

Because the tie lines on a phase diagram of mixtures of two fats do not define the ratio of solid-to-liquid phases, the phase diagram alone is insufficient for practical purposes. Therefore, isosolid diagrams (Figure 7.15) should also be drawn up on the basis of solid fat contents (SFCs) to be determined by means of pulse NMR (Timms, 1979).

Paulicka (1970; 1973) was the first to describe the construction and usefulness of phase diagrams of mixtures of cocoa butter and confectionery fats. A more up-to-date typical step-by-step procedure for constructing phase and isosolid diagrams of fats has been outlined by (Timms, 2003).

7.3.3 Phase behaviour of mixtures of triglycerides and partial glycerides

Few studies of the phase behaviour of mixtures of triglycerides and partial glycerides have been reported in literature. Lutton and Jackson (1966) studied a number of binary systems, each involving a 1-monoglyceride and mono-, di-, and triglycerides. A general principle running through the behaviour observed is that a greater chemical similarity between the components leads to a greater interaction or an increased tendency to form a solid solution. Knoester and De Jong (1975) determined deviations from ideality for several systems of mono- or diglycerides with triglycerides by measuring solubility temperatures and heats of mixing. The nonideal phase behaviour of glyceride systems reported in literature was predicted satisfactorily.

References

Hannewijk, J. et al. (1964), Dilatometry of fats, in *Analysis and Characterisation of Oils, Fats and Fat Products, Vol. 1*, Boekenoogen, H.A., Ed., Interscience, London, 119–182.

Kellens, M.J. and Reynaers, H. (1992), Study of the polymorphism of saturated monoacid triglycerides II: Polymorphic behaviour of a 50/50 mixture ot tripalmitin and tristearin, *Fat Sci. Technol.*, **94**, 286–293.

Knoester, M. et al. (1972), The solid-liquid equilibrium of binary mixtures of triglycerides with palmitic and stearic chains, *Chem. Phys. Lipids*, **9**, 309–319.

Knoester, M. and Dejong, D.J. (1975), Non-ideal phase behaviour of binary mixtures of triglycerides with mono-and diglycerides, *Chem. Phys. Lipids*, **14**, 41–48.

Koynova, R. and Caffrey, M. (2002), An index of lipid phase diagrams, *Chem. Phys. Lipids*, **115**, 107–219.

Lee, A.G. (1978), Calculation of phase diagrams for non-ideal mixtures of lipids, and a possible non-random distribution of lipids in lipid mixtures in the liquid crystalline phase, *Biochim. Biophys. Acta*, 507, **433**–443.

Lutton, E.S. and Jackson, F.L. (1966), Binary systems with monoglycerides, *J. Am. Oil Chem. Soc.*, **43**, 357–358.

Minato, A. et al. (1996), Synchrotron radiation x-ray diffraction study on phase behavior of PPP-POP binary mixtures, *J. Am. Oil Chem. Soc.*, **73**, 1567–1572.

Mulder, H. (1953), Melting and solidification of milk fat, *Neth. Milk Dairy J.*, **7**, 149–174.

Paulicka, F.R. (1970), Phase behavior of fats in confectionary coatings, *The Manufacturing Confectioner*, **50**, 73–74,76, 78.

Paulicka, F.R. (1973), Phase behaviour of cocoa butter extenders, *Chem. Ind.*, **17**, 835–839.

Rossell, J.B. (1967), Phase diagrams of triglyceride systems, *Adv. Lipid Res.*, **5**, 353–408.

Sato, K. (2001a), Crystallization behaviour of fats and lipids — a review, *Chem. Eng. Sci.*, **56**, 2255–2265.

Sato, K. (2001b), Uncovering the structures of β′ fat crystals: what do the molecules tell us? *Lipid Technol.*, **13**, 36–40.

Sato, K. et al. (1999), Molecular interactions and kinetic properties of fats, *Prog. Lipid Res.*, **38**, 91–116.

Sato, K. and Ueno, S. (2001), Molecular interactions and phase behavior of polymorphic fats, in *Crystallization Processes in Fats and Lipid Systems*, Garti, N. and Sato, K., Eds., Marcel Dekker, New York, 177–209.

Takeuchi, M. et al. (2002), Crystallization kinetics of polymorphic forms of a molecular compound constructed by SOS (1,3-distearoyl-2-oleoyl-*sn*-glycerol) and SSO (1,2-distearoyl-3-oleoyl-*sn*-glycerol), *Food Res. Int.*, **35**, 919–926.

Takeuchi, M. et al. (2003), Synchroton SAXS/WAXS study of polymorph-dependent phase behaviour of binary mixtures of saturated monoacid triacylglycerol, *Cryst. Growth Des.*, **3**, 373–374.

Timms, R.E. (1978), The solubility of milk fat, fully hardened milk fat and milk fat hard fraction in liquid oils, *Aust. J. Dairy Tech,* **33**, 130–135.

Timms, R.E. (1979), Computer program to construct isosolid diagrams for fat blends, *Chem. Ind.*, 257–258.

Timms, R.E. (1984), Phase behaviour of fats and their mixtures, *Prog. Lipid Res.*, **23**, 1–38.

Timms, R.E. (2003), *Confectionary Fats Handbook. Properties, Production and Application*, The Oily Press, Bridgwater, U.K.

Wesdorp, L.H. (1990), *Liquid-multiple solid phase equilibria in fats-theory and experiments*, Ph.D. thesis, Technical University Delft, The Netherlands.

7.4 Lipid/water interactions

7.4.1 Introduction

Crystalline lipids are packed in a highly ordered, repeating pattern extending in all three spatial dimensions. In the liquid state, the molecules acquire freedom of movement and assume a state of disorder. Phases with intermediate properties between those of the crystalline and the liquid states are also known to occur. These so-called mesomorphic phases consist of liquid crystals (Nawar, 1996). A milestone in the elucidation of the liquid crystalline structures was the introduction of the liquid chain concept by Chapman (1958). By using IR spectroscopy he showed that a high temperature phase transition in anhydrous soap was caused by the melting of the hydrocarbon chains. A few years later, the structure of the most common liquid crystalline phases was revealed by Luzzati et al. (1960).

To obtain liquid crystalline phases, amphiphilic compounds should be present. These compounds exhibit both hydrophilic (polar) and hydrophobic (nonpolar) groups within the molecule (Larsson and Lundström, 1976). They orient on contact with polar solvent molecules, giving rise to polar and nonpolar regions that are separated by the polar end groups. The nonpolar hydrocarbon region may melt before the final melting point is reached. This occurs because the van der Waals' forces among hydrocarbon chains are weaker than the hydrogen bonding between polar groups (Nawar, 1996).

The term lyotropic mesomorphism is used to describe the formation of liquid crystalline systems by the penetration of a solvent in between the amphiphilic molecules. In contrast, liquid crystals formed without the aid of a solvent are said to be thermotropic. So far, lyotropic mesomorphism has been observed almost exclusively in lipid systems containing water. Lipids showing lyotropic mesomorphism frequently form thermotropic mesophases without any additions at high temperatures.

7.4.2 General aspects

7.4.2.1 Liquid crystalline phases

The liquid crystalline structure is determined primarily by the polar groups, while the nonpolar parts of the molecules form liquid-like associations (Söderberg and Ljusberg-Wahren, 1990). The water in such systems cannot be treated as a continuous free medium, but should be considered as part of the structural units. The most common mesomorphic structures are lamellar (termed "neat" in old literature), hexagonal (termed "middle" in old literature), and cubic phases.

The lamellar liquid crystal structure (L_α) corresponds to that existing in biological bilayer membranes and is illustrated in Figure 7.16. It is made up of double layers of lipid molecules separated by water layers (Nawar, 1996). The polar groups are on the outside of the layers in contact with the water. Structures of this kind are usually less viscous and less transparent than the other mesomorphic structures. The capacity of a lamellar phase to retain water

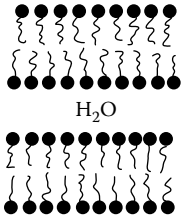

FIGURE 7.16 The lamellar phase consists of double layers of lipid molecules separated by water layers. (From Larsson, K. (1986), in *The Lipid Handbook*, 1st ed., Gunstone, F.D., Padley, F.B., Harwood, J.L., Eds., Chapman & Hall, London, 321–384.)

depends on the nature of the lipid molecules. If the water content is increased above the swelling limit of the lamellar phase, a dispersion of spherical or cylindrical aggregates consisting of concentric, alternating layers of amphiphilic molecules and water gradually forms. This phase is called the lamellar dispersion (Krog and Larsson, 1968). The mechanism behind this change in structure is that the lamellar phase cannot exist in equilibrium with water, as there would be direct contact between water molecules in different states: bulk water and water that forms part of the lipid bilayers. By the formation of closed vesicles preserving the lamellar structure, such contacts are avoided (Larsson and Krog, 1973).

In the hexagonal phase, the amphiphilic molecules form infinite cylinders arranged in a hexagonal array (Nawar, 1996) as shown in Figure 7.17a. The liquid hydrocarbon chains fill the interior of the cylinders, and the space between the cylinders is taken up by water. This type of liquid crystal is referred to as H_I. In the reversed hexagonal structure (H_{II}), water fills the interior of the cylinders and is surrounded by the polar groups of the amphiphile lipid (Figure 7.17b). The hydrocarbon chains make up the continuous phase between the cylinders.

However, Clunie et al. (1965) concluded from calculations based on x-ray and density data that the average diameter of the cylinders is smaller than would be expected with this structure. They suggested that the hexagonal phase could be made up of linear chains of spherical micelles instead of cylinders. When the H_I-phase is diluted with water, spherical micelles form. In contrast, dilution of the H_{II}-phase with water is not possible.

The phases described so far are two-dimensional mesophase structures. Three-dimensional phases of various types are also known and most of them have some form of cubic symmetry (Lis and Quinn, 1991). Because of its three-dimensional periodicity, such a phase is not a true liquid crystal, but all physical properties are closely related to those of the lamellar and hexagonal mesomorphic phases. The cubic phase is very viscous and could not be characterized by ordinary optical observations because it is isotropic. Hence, this phase is sometimes called viscous isotropic (V). A unique property is the presence of two media, one polar and one nonpolar, both continuous throughout the structure (Luzzati et al., 1968). The lattice is body-centred and the structure consists of networks of rods; in some cases, the rods are filled by the hydrocarbon chains (V_I), in others by the polar moieties (V_{II}). This rod system was considered to be the general structure for the cubic lipid-water phases (Figure 7.18). Cubic liquid crystals are stable in an excess of water.

Lindblom et al. (1979) indicated that the cubic monoglyceride–water phase was closely related to the lamellar phase. The structure they proposed consisted of hexagonal lamellar bilayer units. This cubic structure is related to "Schwarz's primitive cubic minimal surface" and consists of a three-dimensional continuous bilayer system separating two separate water channel systems (Figure 7.19).

7.4.2.2 Characterization methods

Polarisation microscopy is the most frequently used method permitting a definite distinction between lamellar and hexagonal phases (Morley and Tiddy, 1993). Both phases show their typical anisotropy when observed through crossed polarizers (D'Antona et al., 2000).

The liquid crystalline phases can be identified unambiguously from their x-ray diffraction patterns (Luzzati et al., 1960). The arrangement of the hydrocarbon chains corresponds to that in a liquid hydrocarbon as shown by a weak and relatively diffuse x-ray diffraction band at wide angles corresponding to a Bragg spacing of about 4.5 Å (Kelker et al., 1980). At small angles, sharp diffraction bands are observed, which are characteristic of the mesomorphic phase. In the lamellar phase several orders are observed giving rise to calculated Bragg spacings in the ratio of 1:2:3:4. From these data, it is possible to determine the bilayer and water layer thickness and the cross section per polar head group (Larsson and Krog, 1973).

A series of Bragg spacings in the ratio of $1{:}\sqrt{3}{:}\sqrt{4}{:}\sqrt{7}$ is characteristic for the hexagonal phase, indicating a two-dimensional hexagonal array of parallel cylindrical micelles. In the same way the diameter of the cylinders in

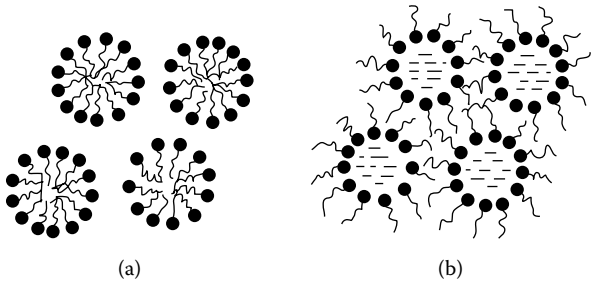

FIGURE 7.17 The hexagonal phase consists of cylinders of liquid hydrocarbon chains (a) or water (b) arranged in a hexagonal array. (From Larsson, K. (1986), in *The Lipid Handbook,* 1st ed., Gunstone, F.D., Padley, F.B., Harwood, J.L., Eds., Chapman & Hall, London, 321–384.)

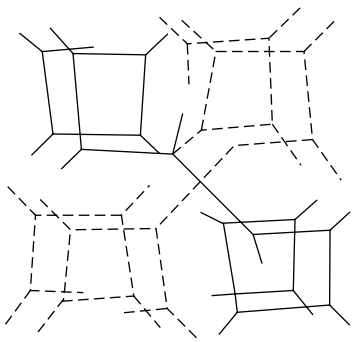

FIGURE 7.18 The general cubic lipid-water system. (Adapted from Luzzati, V., et al., (1968), *Nature,* **220**, 485–488.)

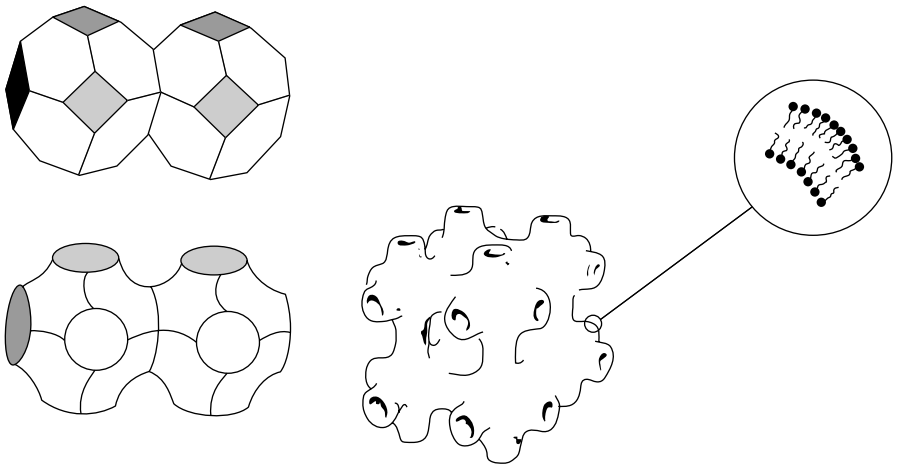

FIGURE 7.19 The cubic monoglyceride-water phase. (Adapted from Lindblom, G., et al., (1979), *J. Am. Oil Chem. Soc.*, **101**, 5465–5470.)

the H_I- and H_{II}-phase can be determined as well as the area of the cross section per polar head group and the distance between adjacent cylinders. As it has often been noted, the analysis of the x-ray diagrams of the cubic phase is generally hindered by a heavily spotted appearance, a consequence of the easy growth of fairly large crystals (Luzzati et al., 1960).

7.4.3 Monoglyceride/water interactions

7.4.3.1 Pure monoglycerides

Although monoglycerides are virtually insoluble in water, they swell to form different liquid crystalline phases (Kelker et al., 1980). The mesomorphic behaviour of monoglyceride/water systems is represented by a phase diagram. In a binary system, it is possible to define where in a single diagram the different liquid crystalline phases exist by using composition and temperature as the parameters. The lipid organisation in these systems depends on the chemical structure of the compounds and on environmental conditions, such as temperature, water, and salt content (Nawar, 1996). This provides an opportunity to shift the transition temperature and drive the system towards any desired mesophase (Chupin et al., 2001).

In the presence of water and above the Krafft temperature, at which the hydrocarbon chains of 1-monoglycerides "melt" and adopt a disordered state, water penetrates among the ordered polar groups. 1-Monoglyceride/water systems form up to three classes of liquid crystalline phases, namely the lamellar (L_α), reversed hexagonal (H_{II}), and cubic (V_{II}) phase. Hyde et al. (1984) have shown that the cubic phase can consist of two forms with different geometries. Two separate H_{II}-phases have also been reported (Larsson, 1988). At low water concentrations an isotropic fluid phase (L_2) is formed (Larsson, 1979). It was suggested that this structure consisted of water lamellae separated by lipid bilayers (Figure 7.20).

FIGURE 7.20 Proposed structure of L_2-phase in aqueous monoglyceride systems. (Adapted from Larsson, K. (1979), *J. Colloid Interface Sci.*, **72**, 152–153.)

At very short chain length (C_6), an ordinary micellar solution is formed in the water-rich region of the phase diagram. No other mesophase is formed; crystals + water transforms directly into an isotropic fluid above the Krafft point (Krog and Larsson, 1968). The lamellar phase and dispersion are the only mesomorphic phases of 1-monocaprylin, 1-monocaprin, and 1-monolaurin. The influence of chain length and unsaturation of the fatty acids on the phase behaviour of 1-monoglycerides is shown in Figure 7.21. In 1-monomyristin, the cubic phase is formed when the lamellar phase is heated (Lutton, 1965). Similarly, the lamellar dispersion phase breaks into a cubic phase and water (Pezron et al., 1990). In 1-monopalmitin and 1-monostearin, the lamellar region has shrunk considerably

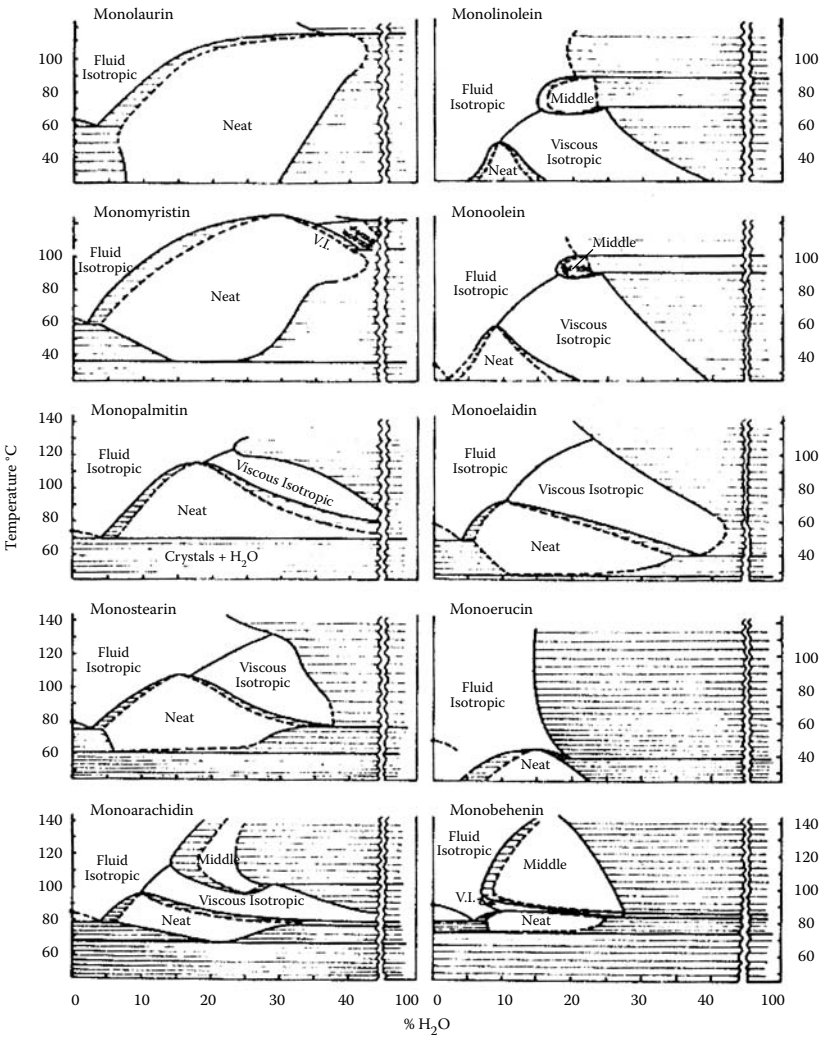

FIGURE 7.21 Aqueous 1-monoglyceride systems. (Adapted from Lutton, E.S. (1965), *J. Am. Oil Chem. Soc.*, **42**, 1068–1070.)

and the cubic phase has grown. In 1-monoarachidin, the cubic phase transforms into the hexagonal structure with closed water cylinders on further heating. The long hydrocarbon tails favour the formation of the H_{II} phase. This is evidenced by the fact that the addition of nonpolar hydrocarbon-type matter to 1-monostearin can produce a hexagonal phase.

The phase diagrams for systems of unsaturated 1-monoglycerides resemble those for systems of saturated compounds, but with the corresponding phase regions occurring at lower temperatures. Unlike the mesophases of saturated monoglycerides, the mesophases of unsaturated monoglycerides can exist at room temperature (Lutton, 1965). The diagram of *trans*-unsaturated 1-monoelaidin is much like that of 1-monostearin, but with the temperature scale shifted some 30°C downwards.

1-Monoolein shows a further drop in the lamellar region and an outcropping of the hexagonal phase near 100°C. 1-Monolinolein shows similar behaviour, but at slightly lower temperatures and with an expanded hexagonal region. The strong cubic and hexagonal phase forming tendency shown with increasing chain length and with unsaturation is not the case for 1-monoerucin. Neither the cubic nor the hexagonal phase was observed. A maximum lamellar phase temperature of 45°C, and a liquid immiscibility line for melted monoerucin and water running between 15 and 20% water characterize this phase diagram.

The polar head group of 2-monoglycerides requires a larger cross section area than that of the 1-isomer, which results in different phase properties (Larsson, 1986).

7.4.3.2 Distilled monoglycerides

Industrially produced distilled monoglycerides usually comprise a major component, although homologous members with longer and shorter chains are present (Krog and Larsson, 1968) as well as some diglycerides, free fatty acids, and glycerol (Brokaw and Lyman, 1958). Due to these differences in composition compared with pure monoglycerides, the phase diagram of distilled monoglycerides shows some specific features.

Temperature-programmed x-ray diffraction analysis has shown that when a mixture of distilled monoglycerides and water is heated a gel phase is formed a few degrees below the region of the lamellar region. In aqueous systems of pure 1-monoglycerides the transition of crystals + water into the lamellar phase always takes place directly, i.e., without formation of an intermediate gel phase (Krog and Borup, 1973). The structure of the gel phase is similar to that of the lamellar phase, with water layers alternating with lipid bilayers. The water penetrates into the crystalline lattice between the polar groups before the hydrocarbons are transformed into a liquid state. The exact temperature of gel formation depends on the chain length of the fatty acid and on the purity of the monoglyceride (Moonen and Bas, 2004).

When water is added to the lamellar phase, it swells to a critical repeat distance. The swelling of the lamellar phase is limited by the long-range van der Waals' forces between the lipid bilayers, which balance the osmotic pressure at an interlayer spacing of about 20Å (Larsson and Krog, 1973). Consequently, pure monoglycerides show limited swelling. At higher water concentration, a lamellar dispersion is formed.

Distilled monoglycerides contain approximately 0.5 to 1.5% free fatty acids. When these fatty acids are neutralised by adding a base, negatively charged groups ($-COO^-$) are formed on the surface of the lipid bilayer. Due to repulsion forces between these electric double layers, the swelling capacity of the lamellar system is greatly increased, in principle to infinite dilution. Neutralisation of the free fatty acids in the distilled monoglycerides, thus, expands the area of the lamellar phase into the higher water content region.

The presence of salts in water decreases the swelling capacity of monoglycerides. The electric repulsion forces created by the ion-active surfactant in the bilayer are shielded, resulting in a decrease in the water layer thickness. This shielding effect supports the theory that the effect of ion-active surfactants in the bilayer is due to electric repulsion (Krog and Borup, 1973). In addition, the transition lamellar phase or dispersion to cubic or cubic + water phase is shifted to higher temperatures if the free fatty acids are neutralized in distilled monoglycerides.

7.4.3.3 The gel and coagel phase

If the lamellar phase is cooled, a metastable gel is formed. In the phase diagram as shown in Figure 7.22, this is expressed by two lower boundaries of the lamellar phase. The upper one corresponds to transformation of β-crystals + water to the lamellar phase on heating, and the lower one corresponds to the transformation of the lamellar phase into the metastable gel on cooling (Krog and Larsson, 1968).

The structure of the gel phase is still lamellar, but the hydrocarbon chains are in the crystalline state. The hydrocarbon chain length is longer in the gel phase than for the

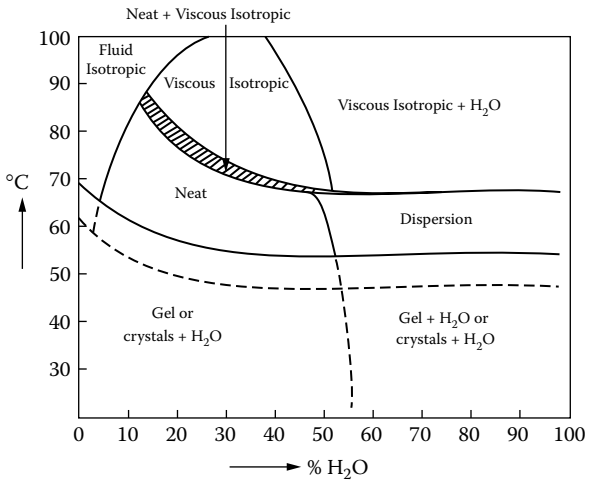

FIGURE 7.22 The phase diagram of the monoglyceride/water system shows two lower boundaries of the lamellar phase. (Adapted from Krog, N. and Larsson, K. (1968), *Chem. Phys. Lipids*, **2**, 129–143.)

lamellar phase, but the water is less ordered. This is caused by a reduced hydration of the gel phase (Morley and Tiddy, 1993). The occurrence of a single x-ray short spacing at 4,15Å shows that the lateral packing of the chains can be described as a hexagonal subcell. This indicates that the chains possess rotational or oscillational freedom (Krog and Larsson, 1968). The gel phase of monoglycerides is called the α-gel, as it is the hydrated form of the α-polymorph (Morley and Tiddy, 1993). The fatty acid chains are tilted about 54° towards the water layers in pure monoglycerides. In a neutralized, distilled monoglyceride–water system, the angle of tilt of the hydrocarbon chains towards the water layers is higher (Krog and Borup, 1973).

The gel phase is stable just below the lamellar region and this stability can be related to a high molecular motion of the polar head groups (Cassin et al., 1998). This is indicated by a small region of α-gel stability just below the lamellar phase in the phase diagram on Figure 7.22.

During extended holding at lower temperature, the water is expelled and the gel phase transforms into a microcrystalline suspension in water, called a coagel. The coagel phase consists of a three-dimensional network of plate-like crystals entrapping water domains. The molecular arrangement within the coagel phase is identical to that found in the bulk β-crystal of monoglycerides.

1-Monoglycerides are a racemic mixture of the stereoisomers D and L; the middle carbon atom of the glycerol moiety is optically active. In the lamellar phase the molecules undergo many gauche-*trans* transitions and have high lateral mobility. The two stereoisomers of monoglycerides are intimately mixed and the bilayer halves will be racemic. When the α-gel phase is formed, the molecular mobility is drastically reduced, but the racemic state of the bilayer is retained. The molecules can no longer undergo gauche-*trans* transitions, although they can still

rotate around their (long) axis. Through chiral discrimination the D and L isomers rearrange within bilayers, after which the transition from α- to β-crystal can start (Sein et al., 2002). Figure 7.23 shows a diagram of the molecular processes during the phase transitions from lamellar phase to α-gel to coagel.

The melting enthalpy of α-crystals is about twice the value obtained for the melting of α-crystals. The α-gel and coagel phase both convert into the lamellar phase when the Krafft temperature is reached. However, when cooled down, the lamellar phase converts only to the α-gel phase. The difference in melting enthalpy of the first heating (α-gel and/or coagel phase to lamellar phase) and the enthalpy of the second heating (α-gel phase to lamellar phase) can be employed to monitor the kinetics of the gel to coagel phase transition (Cassin et al., 1998). To do so, a parameter called the coagel index (CI) is defined according to:

$$CI = \frac{\Delta H(\text{first heat})}{\Delta H(\text{second heat})} \tag{7.50}$$

A CI of 1 indicates that the sample is still in the gel state, a value of 2 means that the sample is in the coagel state. Depending on composition, processing and temperature this transition may take minutes or many months (Heertje et al., 1998).

It is not possible to prepare completely stable α-gels from monoglycerides. Surfactants that are not polymorphic and which are stable in the α-crystalline state, form α-gels with water that are very stable. Some esters of monoglycerides, for example, show this behaviour (Heertje et al., 1998). These compounds are called α-tending because of the absence of a closely packed β-crystalline phase. Consequently, there is no driving force for phase transition. The concentration of the added co-emulsifiers is critical as it may prevent the formation of the lamellar phase. Mesomorphic phases with reversed hexagonal structures are formed instead.

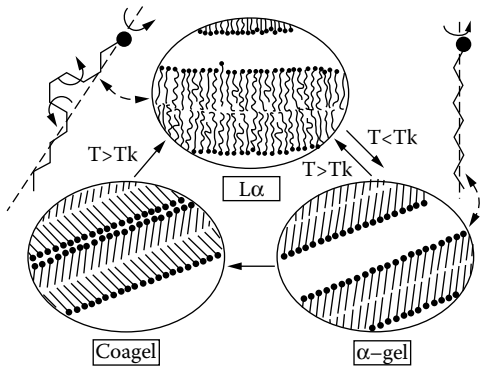

FIGURE 7.23 Schematic representation of the molecular processes during the phase transitions from lamellar phase to α-gel to coagel. (Adapted from Sein, A. et al. (2002), *J. Colloid Interf. Sci.*, **249**, 412–422.)

7.4.4 Phospholipid/water interactions

Like monoglycerides, phospholipids are substances that form highly ordered liquid crystal mesophases in water (Bueschelberger, 2004). Phospholipids, which have a large polar group and two hydrocarbon chains, have phase properties that are closely related to those of monoglycerides because of packing constraints (Pezron et al., 1990). This illustrates that lipids that have hydrophobic and hydrophilic regions of similar relative sizes (expressed for example by the HLB value) give the same type of water interaction (Larsson and Krog, 1973). As with monoglycerides, the ability of phospholipids to disperse in water occurs only above the Krafft temperature, which is nearly constant over a wide range of concentration.

Most phospholipids form a lamellar phase that shows a high degree of swelling (Chupin et al., 2001). Their swelling pressure is higher than that of monoglycerides, demonstrating the decisive role of the polar head group (Pezron et al., 1990). Highly unsaturated phospholipids swell at room temperature while completely saturated phospholipids require higher temperatures to do so (Kelker et al., 1980). In the absence of ionic groups, the lamellar phase is transformed into a reversed hexagonal phase as the water content increases (Larsson and Lundström, 1976). As more water enters the layers, the area per head group increases, forcing the tails to spread out over larger areas. At some point, the tails must become too spread out for a flat layer to be stable. In the reversed hexagonal phase, the chains will have more space than in the lamellar structure. The lamellar and H_{II}-phase can definitely be recognized in, for example, the phosphatidylethanolamine/water system.

Lysolecithin is obtained from lecithin by removing one fatty acid moiety. This compound does not form a lamellar phase (Kelker et al., 1980). Because its polar group is large in comparison with the hydrocarbon fatty acid chain, this molecule preferentially forms a hexagonal phase (H_I). In this phase, the heads take up more area than the tails (Matthew and Finn, 1988).

In addition to lyotropic mesomorphism, phospholipids also exhibit thermotropic mesomorphism. Liquid crystalline phases are formed many tens of degrees below the published capillary melting points. Pure egg-lecithin at 40°C is in a form that is at least partially crystalline, and it transforms into a wax-like phase at about 80°C. It is in a cubic phase between 88 and 109°C. At higher temperatures up to the melting point at 231°C, it is in a lamellar phase (Kelker et al., 1980). These mesophases also exist in mixtures with water in the ranges indicated in the phase diagram in Figure 7.24.

7.4.5 Triglycerides-diglycerides/water interactions

Due to their insolubility in water, tri- and diglycerides can form aqueous mesophases only in the presence of other components. Appreciable amounts can be solubilized in

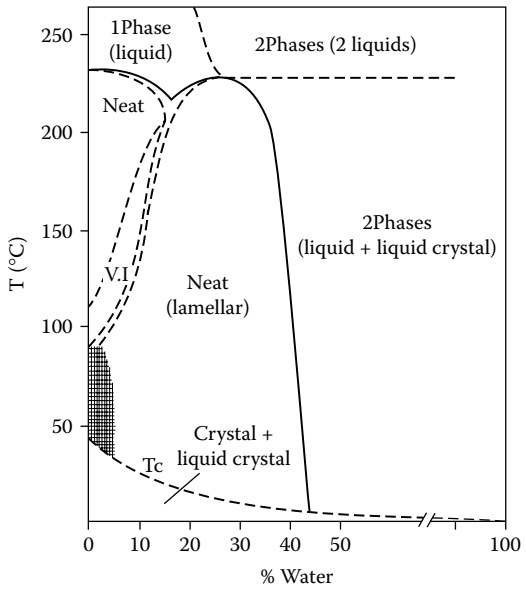

FIGURE 7.24 Phase diagram of egg lecithin/water system. (Adapted from Kelker, H. and Hatz., and with a contribution by Schuman, C. (1980), in *Handbook of Liquid Crystals*, Verlag Chemie, Weinheim, 512–591.)

monoglyceride-water mesophases (Kelker et al., 1980). Little information is available about the influence of water on the crystallisation of triglycerides, although anhydrous milk fat (AMF) in particular, but also other commercial fats, may contain some water as a minor component.

The role of water and phospholipids in vegetable oils has been investigated by Sambuc et al. (1980). When 4% lecithin and 16% water were incorporated, a delay in the onset of crystallisation was noticed for all the fats, while the final solid: liquid ratio was not affected. Savage and Dimick (1995) suggested that water forms micelles or inverse hexagonal mesophases with amphiphilic compounds, such as monoglycerides and phospholipids (see Section 7.4.3 and Section 7.4.4) and that such structures would then serve as templates for the crystallisation of the fat.

Vanhoutte et al. (2002a) investigated the effect of low concentrations of water (up to 0.7%) and phospholipids (0.01-0.06%) on the isothermal crystallisation kinetics of anhydrous milk fat (AMF). The crystallisation was monitored by DSC and pNMR and described by means of the Gompertz model. Higher concentrations of water seemed to decrease the induction time, but no interaction effects between phospholipids and water were observed. In a second study (Vanhoutte et al., 2002b), even lower concentrations of phospholipids (up to 0.035%) were added while the concentration range of water remained the same. No significant effect could then be observed due to the water.

7.4.6 Soap/water interactions

Soaps exhibit a good water-solubility above the melting temperature of the fatty acid chains (the so-called Krafft point), giving rise to micelles, but the solubility is low at just a few degrees below this temperature (McBain and Lee, 1943). Reiss-Husson and Luzzati (1969) revealed the structure of potassium soaps in the micellar region of the phase diagram using x-ray diffraction. A common feature in soaps of saturated fatty acids is that spherical micelles exist at low concentrations, while at increased concentrations a transition into rod micelles occurs. Sodium oleate, however, was found to give rod-shaped micelles at all concentrations.

The short-chain soaps are more soluble than the high-chain soaps at low temperatures and low soap concentrations while at higher temperatures and higher soap concentrations the opposite is true (Vold et al., 1941).

The existence of the liquid crystalline phases mentioned in Section 7.4.2.1 in certain areas of concentration is also a common feature of aqueous soap solutions. Soaps of different chain lengths show a lamellar phase at low water content and at higher water content there is a large region where the H_I-phase exists. With increasing water demand to hydrate K^+, Na^+, and Li^+ ions, the minimum water content required for the existence of the different phases increases in the isotropic and the mesomorphous phase (Ekwall and Mandell, 1968). As an example, the phase diagram of sodium myristate is given in Figure 7.25. In spite of a substantially higher water content, the H_I-phase is much stiffer than the L_α-phase (Kelker et al., 1980). Between

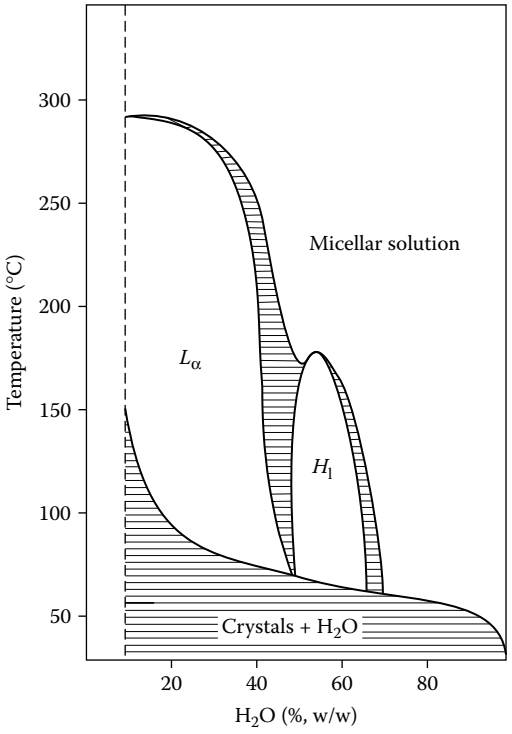

FIGURE 7.25 Phase diagram of sodium myristate. (From Larsson, K. (1986), in *The Lipid Handbook*, 1st ed., Gunstone, F.D., Padley, F.B., Harwood, J.L., Eds., Chapman & Hall, London, 321–384.)

the H_I and the L_α-phase Luzzati et al. (1960) described two-phase mixtures also involving cubic phases.

Acknowledgment

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References

Brokaw, G.Y. and Lyman, W.C. (1958), The behavior of distilled monoglycerides in the presence of water, *J. Am. Oil Chem. Soc.*, **35**, 49–52.

Bueschelberger, H.G. (2004), Lecithins, in *Emulsifiers in Food Technology*, Whitehurst, R.J., Ed., Blackwell Publishing Ltd., Oxford, 1–39.

Cassin, G. et al. (1998), Investigation of the gel to coagel phase transition in monoglyceride-water systems, *Langmuir*, **14**, 5757–5763.

Chapman, D. (1958), An infrared spectroscopic examination of some anhydrous sodium soaps, *J. Chem. Soc.*, **33**, 784–788.

Chupin, V. et al. (2001), Lipid organization and dynamics of the monostearoylglycerol-water system, *Chem. Phys. Lipids*, **109**, 15–28.

Clunie, J.S. et al. (1965), The structure of lyotropic mesomorphic phases, *Proc. R. Soc*, **285**, 520–532.

D'Antona, P.D. et al. (2000), Rheological and NMR characterization of monoglyceride-based formulations, *J. Biomed. Mater. Res.*, **52**, 40–52.

Ekwall, P. and Mandell, L. (1968), Minimum water content of a number of reversed micellar and mesomorphous structures, *Acta Chem. Scand.*, **22**, 699–702.

Heertje, I. et al. (1998), Liquid crystalline phases in the structuring of food products, *Food Sci. Technol. (Int.)*, **31**, 387–396.

Hyde, S.T. et al. (1984), A cubic structure consisting of a lipid bilayer forming an infinite periodic minimum surface of the gyroid type in a glycerolmonooleate-water system, *Z. Kristallogr.*, **168**, 213–219.

Kelker, H., Hatz, R., and with a contribution by Schuman, C. (1980), Lyotropic mesomorphism, in *Handbook of Liquid Crystals*, Verlag Chemie, Weinheim, 512–591.

Krog, N. and Borup, A.P. (1973), Swelling behaviour of lamellar phases of saturated monoglycerides in aqueous systems, *J. Sci. Food Agric.*, **24**, 691–701.

Krog, N. and Larsson, K. (1968), Phase behaviour and rheological properties of aqeous systems of industrial distilled monoglycerides, *Chem. Phys. Lipids*, **2**, 129–143.

Larsson, K. (1979), X-ray scattering study of the L2-phase in monoglyceride-water system, *J. Colloid Interf. Sci.*, **72**, 152–153.

Larsson, K. (1986), Physical properties–Structural and physical characteristics, in *The Lipid Handbook*, 1st ed., Gunstone, F.D., Padley, F.B., Harwood, J.L., Eds., Chapman & Hall, London, 321–384.

Larsson, K. (1988), 2 H-II types of phases in the same monoglyceride-water system, *J. Colloid Interf. Sci.*, **122**, 298.

Larsson, K. and Krog, N. (1973), Structural properties of the lipid-water gel phase, *Chem. Phys. Lipids*, **10**, 177–180.

Larsson, K. and Lundström, I. (1976), Liquid crystalline phases in biological model systems, in *Lyotropic Liquid Crystals and the Structure of Biomembranes: A Symposium Based on the Fifth International Liquid Crystal Conference Proceedings, Stockholm, Sweden, June 17-21*, 1974, Friberg, S.E., Ed., American Chemical Society, Washington, D.C., 43–70.

Lindblom, G. et al. (1979), Cubic phase of monoglyceride-water systems–arguments for a structure based upon lamellar bilayer units, *J. Am. Oil Chem. Soc.*, **101**, 5465–5470.

Lis, L.J. and Quinn, P.J. (1991), The application of synchroton x-radiation for the study of phase transitions in lipid model membrane systems, *J. Appl. Crystallogr.*, **24**, 48–60.

Lutton, E.S. (1965), Phase behavior of aqueous systems of monoglycerides, *J. Am. Oil Chem. Soc.*, **42**, 1068–1070.

Luzzati, V. et al. (1960), La structure des colloïdes d'association. I. Les phases liquide-crystalline des systèmes amphiphile-eau, *Acta Crystallogr.*, **13**, 660–667.

Luzzati, V. et al. (1968), Structure of the cubic phases of lipid-water systems, *Nature*, **220**, 485–488.

McBain, J.W. and Lee, W.W. (1943), Vapor pressure data and phase diagrams of some concentrated soap/water systems above room temperature, *Oil & Soap*, **20**, 17–22.

Moonen, H. and Bas, H. (2004), Mono- and diglycerides, in *Emulsifiers in Food Technology*, Whitehurst, R.J., Ed., Blackwell Publishing Ltd., Oxford, 4058.

Morley, W.G. and Tiddy, G.J.T. (1993), Phase behaviour of monoglyceride/water systems, *J. Chem. Soc. Faraday Trans.*, **89**, 2823–2831.

Nawar, W.W. (1996), Lipids, in *Food Chemistry*, Fennema, O.R., Ed., Marcel Dekker, New York, 239–319.

Pezron, I. et al. (1990), Repulsive pressure between monoglyceride bilayers in the lamellar gel states, *J. Phys. Chem.*, **94**, 8255–8261.

Reiss-Husson, F. and Luzzati, V. (1969), The structure of the micellar solutions of some amphiphilic compounds in pure water as determined by Small-Angle-X-ray Scattering techniques, *J. Phys. Chem.*, **68**, 3504–3511.

Sambuc, E. et al. (1980), Étude de la cristallisation des corps gras plastiques VI — Influence des glycérides partiels et des phosphatides en absence et présence d'eau. B. Cas des lécithines de soja, *Rev. Franç. Corps Gras*, **28**, 13–19.

Savage, C.M. and Dimick, P.S. (1995), Influence of phospholipids during crystallization of hard and soft cocoa butters, *The Manufacturing Confectioner*, 127–132.

Sein, A. et al. (2002), Rheological characterization, crystallization, and gelation behavior of monoglyceride gels, *J. Colloid Interf. Sci.*, **249**, 412–422.

Söderberg, I. and Ljusberg-Wahren, H. (1990), Phase properties and structure of a monoglyceride/sucrose/water system, *Chem. Phys. Lipids*, **55**, 97–101.

Vanhoutte, B. et al. (2002a), The effect of phospholipids and water on the isothermal crystallisation of milk fat, *Eur. J. Lipid Sci. Technol.*, **104**, 490–495.

Vanhoutte, B. et al. (2002b), Effect of phospholipids on isothermal crystallisation and fractionation of milk fat, *Eur. J. Lipid Sci. Technol.*, **104**, 738–744.

Vold, R.D. et al. (1941), Phase rule study of the system sodium myristate/water, *J. Am. Chem. Soc.*, **63**, 1293–1296.

7.5 Interaction between lipids and proteins

7.5.1 Principles of lipid protein interactions

The basis for the interaction between lipids and proteins is related to their amphiphilic nature and is due to their influence on water structure, the so-called hydrophobic effect (Tanford, 1980). In general terms, four alternative types of phases can occur in lipid-protein-water systems. An aqueous lipid solution can coexist with a protein in the same solution; alternatively a solution of molecular lipoprotein complexes can be formed. It is also possible that the lipid forms a liquid-crystalline phase with water; such a phase can either solubilize a protein or coexist with a protein solution. The first two interaction alternatives in water solution have been thoroughly discussed (Tanford, 1980). When the lipid concentration is below the critical micelle concentration there is no interaction besides the eventual association of a few lipid molecules to the protein at certain high-affinity binding sites. At lipid concentrations above the critical micelle concentration there is a mass cooperative binding of numerous lipid molecules to each protein molecule and this may lead to unfolding of the protein.

The interaction between proteins and surfactants has been characterized by a variety of biophysical methods, including light scattering, spectroscopic probe methods, surface tension measurements, equilibrium dialysis, surface-sensitive electrodes, and calorimetry. Interaction isotherms obtained from calorimetric titration to high surfactant concentration of proteins, such as bovine serum albumin, lysozyme, gelatin, and cellulase yield characteristic thermodynamic fingerprints, but the interpretations of the particular enthalpy changes so far defy detailed molecular interpretation. Thus, it is difficult to distinguish contributions to the changes in enthalpy due to surfactant binding, conformational changes in the protein and micellization.

The way proteins fold depends largely on the local forces experienced by the different side chains and the need for the polymer chain to adopt a conformation with relatively high entropy. The forces at play are those due to the solvent environment as well as the proximity of other residues of the protein or residues of proteins with which it interacts. In the presence of lipids the hydrophobic residues of the protein may rearrange to produce a complex of greater entropy. With certain membrane proteins the "solvent" may include the lipid matrix of the membrane and interactions of this type may be required to fold the protein into its native configuration. On the other hand, there is evidence that the interaction of the proteins with membrane lipids is *required* to impose a bilayer conformation on the surrounding membrane lipids and is, therefore, an essential factor in preserving the structure and properties of the membrane itself.

In general, water-soluble proteins interact only weakly if at all with nonionic surfactants, but intrinsic membrane proteins may be solubilized by such surfactants because they are able to interact with the hydrophobic domains that otherwise render them insoluble in aqueous media. Ionic surfactants, by contrast, interact strongly with all proteins and modify their functions and properties (Nielsen et al., 2005). One of the most studied of this type of interaction is between sodium dodecyl sulfate and proteins because of the importance in analysis of protein mixtures. The nonspecific cooperative binding of sodium dodecyl sulphate to soluble proteins results in unfolding of the polypeptide chain. After reduction of any disulfide bridges in proteins, sodium dodecyl sulfate, above the critical micellar concentration, interacts with the polypeptide in a stoichiometry of 1.4 g detergent per g protein. The interaction imposes a regular helical structure on the polypeptide chain, which becomes bent in the shape of a hairpin. The length of the resulting complex is a function of the length of the polypeptide chain and, because of the predictable conformation combined with a constant charge to mass ratio, differences in hydrodynamic and electrophoresis properties can be exploited in separation strategies of complex mixtures. The most notable system is the separation of proteins by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE), which separates polypeptides on the basis of size. Another application of protein–surfactant complexes is in controlling colloidal stability in processed food products and in the stabilization of enzymes performing reactions under nonphysiological conditions.

Where proteins interact with biological membranes at the aqueous-lipid interface the charges of the acidic membrane lipids provide a particular environment capable of interacting with basic amino acids of the protein. In the case of proteins that are interpolated into the hydrophobic interior of the membrane, the environment is more conducive to the location of amino acids with nonpolar side chains. Clearly, both proteins and membrane lipids have hydrophilic and hydrophobic groups, which interact to determine the structure and conformation of the complex.

7.5.2 Lipidprotein–water phase behaviour

Ternary complexes are formed between lipids in cubic phases with certain proteins and such complexes have been exploited in the crystallization of proteins for x-ray diffraction examination. A partial phase diagram of the ternary system of lysozyme-monoolein-water has been constructed (Ericsson et al., 1983) and is presented in

Figure 7.26. The most remarkable feature of the phase diagram is that a relatively large proportion of the water-soluble protein can be incorporated to form a lipid-protein-water phase without any ionic interactions. It was also found that the protein occupied the aqueous phase of the complex in its native configuration. This discovery was to have considerable implications as is discussed below in the crystallization of both soluble and membrane proteins. The incorporation of protein results in an expansion of the cubic phase lattice formed by monoolein-water. The cubic domain of the phase diagram shown in Figure 7.26 contains all three fundamental cubic structures observed in lipid-water systems of the type found in monoolein-water, namely, the gyroid surface C_G, the diamond surface C_D, and the primitive surface C_P in the order of increasing protein-water ratio.

Complex cubic phases are formed with ternary lipid-water systems like monoolein mixed in proportions of two parts protein solution or dispersion with three parts of lipid. When such mixtures are treated with precipitants such as non-ionic detergents or salts the protein begins to crystallize within hours of incubation at 20°C. The method can be used to grow crystals of soluble as well as membrane proteins and other organic and inorganic molecules.

The precise process of crystallization from these tertiary lipid phases has been examined in some detail (Misquitta et al., 2004). Precipitants like Na^+/K^+ phosphate salts, for example, provoke a reduction in water activity, which favours protein–protein interactions. Three-dimensional structures are created when protein–protein contacts are established between successive layers. The key to successful crystallization of proteins is the action of the precipitant to destabilize the cubic phase of monoolein, which is the principle host in the lipid-protein complex, so that it tends to form a liquid-crystalline lamellar phase. A group of agents used to induce the transition are the alkyl glycosides in which hydrocarbon chains of hexyl, octyl, nonyl or decyl are combined with sugar residues of glucose or maltose.

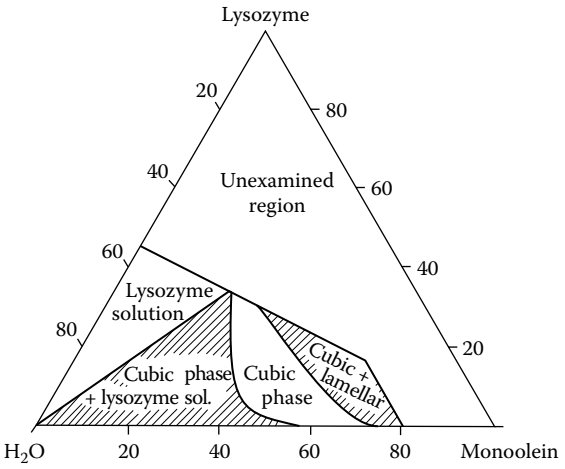


FIGURE 7.26 Phase diagram illustrating the main regions of the monoolein-lysosome-water system. (Adapted from Ericsson, B. et al. (1983), *Biochim. Biophys. Acta*, **729**, 23–27.)

7.5.3 Lipoproteins

Some proteins are adapted to form stable water-soluble complexes with lipids and to function in the mobilization of complex lipids within living organisms. There are two main types of complex; complexes formed between monomeric proteins and lipids and, secondly, large lipoprotein complexes.

One of the most ubiquitous monomeric proteins that bind lipids is albumin. Plasma albumin is a flexible protein that can adopt multiple conformations of approximately equal energy to accommodate the binding of ligands. One of the primary functions of albumin is to bind free fatty acids during mobilization of lipids in the body and transport them in the blood stream as a stable water-soluble complex. The protein has at least five fatty acid binding sites three of which are significantly higher affinity sites than the remainder. The mechanism of binding of the fatty acids and other lipophilic drugs to the protein has been investigated by NMR methods (Lucas et al., 2004). The average residence lifetime of a long chain fatty acid bound to a high-affinity site was found to be greater than 66 ms whereas short-chain fatty acids like octanoic acid have residence times of only a few ms. The lifetimes are relatively short because the fatty acids exchange readily between binding sites on the protein. Dissociation of the fatty acid from the protein, on the other hand, takes place on a time-scale of seconds.

The dissociation of fatty acids from plasma albumin is the rate-limiting step in the delivery of fatty acids to target cells. Their dissociation from the complex at the site of entry into cells is assisted by the presence in the plasma membrane of proteins with a high affinity for fatty acids (McArthur et al., 1999). One such protein, membrane fatty acid-binding protein (FABPpm), binds tightly to free fatty acids and prevents destabilization of the membrane due to the presence of the free fatty acid in the structure. The entry of the free fatty acid into the outer leaflet of the membrane lipid bilayer matrix establishes a transmembrane gradient, which can be dissipated by flip-flop of the uncharged fatty acid from the outer to the inner membrane leaflet. This process can take place spontaneously or may be assisted by another fatty acid binding protein of the membrane, fatty acid translocase (FAT/CD 36). Another soluble fatty acid-binding protein present in the cytoplasm, FABPc, serves to remove free fatty acids present on the cytoplasmic leaflet and to transport them through the cytoplasm. There is also evidence that fatty acids also bind to caveolin-1. This protein is a component of caveolae that are said to function to deliver lipid to the different subcellular organelles. It remains unclear

whether or not intracellular trafficking of fatty acids mediated by FABPc and that by the vesicular-caveolae mechanism act in concert with one another.

Apart from transport to the cell, metabolic mobilization relies on activation of long-chain acyl-CoA. This process is mediated by a family of fatty acid-transport proteins referred to as FATP 1-5/6, which are known to possess acyl-CoA synthetase activity. There is evidence from the variable nature of the N-terminus that these fatty acid-binding proteins may be able to deliver fatty acids to particular membrane sites within the cell. The activation of fatty acids takes place at the highly conserved AMP-binding site located at the cytosolic domain of FATP from where they are primed for metabolism at the appropriate organelle.

Serum lipoproteins are a group of proteins specifically adapted to form structures designed to transport lipids throughout the body. These lipiproteins circulate in the mammalian blood stream to distribute a cargo of lipids from their site of synthesis, usually the liver, to the peripheral tissues. There has been an extensive research effort to characterise lipoproteins because of their association with heart disease and atherosclerosis. The lipids, mainly triacylglycerols, cholesterol, and cholesterol esters, occupy a central core surrounded by a shell of polar lipids and proteins. The proteins act to stabilize the lipid droplet and provide recognition sites for targeting the complex to the appropriate site of delivery. The particles are usually spherical in shape and are classified according to their buoyant density. The characteristics of the different classes of human lipoproteins are presented in Table 7.2. As expected the density increases with increasing protein to lipid ratio. The protein components are synthesized in the liver and small intestine. While many of the 10 major apoproteins found in lipoproteins are common to more than one class of lipoprotein, their combination is distinct in each of them.

Chylomicrons are the least dense of the lipoproteins and are responsible for packaging fats, cholesterol, and other lipids taken up from the diet in the blood stream and conducting them about the body. Because they consist almost entirely of triacylglycerols, they have a buoyant density of < 0.95 g/cm^{-3}. The major protein is apolipoprotein B-48 (apoB-48), which has a molecular weight of 240 kD and forms an amphipathic shell around the spherical fat globule in which the interior surface of the protein is hydrophobic and the interior is hydrophilic.

Triacylglycerols and cholesterol are also synthesized in the liver and the excess of requirements is packaged into very low-density lipoproteins and exported to the peripheral tissues. The proteins stabilizing very low-density lipoproteins include apoB-100 and apoE. ApoB-100 is an extremely large protein comprised of more than 4500 amino acid residues and a molecular weight of 513 kD. ApoB-48 is formed from the first 48% of apoB-100 and arises from the posttranscriptional editing of apoB-100 mRNA in the intestine. The relationship between apoB-100 and B-48 has been the subject of considerable interest (Brodsky et al., 2004).

The intermediate-density lipoproteins result from depletion of the triacylglycerol content of very low-density lipoproteins by the action of lipases associated with capillary surfaces and their consequent enrichment in cholesterol esters. These intermediate-density lipoproteins may be taken up by the liver and further processed or converted into low-density lipoproteins by hydrolysis of more triacylglycerol. Low-density lipoprotein is the major carrier of cholesterol and consists of a core of about 1500 cholesterol molecules esterified mainly to linoleate. The nonpolar lipid is stabilized by a monolayer of phospholipid and apoB-100. High-density lipoprotein is also involved in cholesterol transport, but the source of cholesterol is that scavenged from apoptosing and dying cells and membranes undergoing metabolic turnover. The cholesterol is esterified to a long-chain fatty acid in a reaction catalysed by an acyltransferase intrinsic to the high-density lipoprotein. The cholesterol esters are rapidly transferred to very low- or low-density lipoproteins by a specific transfer protein or targeted to the liver in their high-density lipoprotein vector.

ApoA, apoC and apoE are referred to as exchangeable apolipoproteins and they are responsible for regulating the traffic of lipids into and out of cell by acting as cofactors for plasma enzymes and ligands for cell-surface receptors. The exchangeable apolipoproteins share the same genomic heritage and, therefore, possess structural similarities (Saito et al., 2004). One particular feature is a primary sequence arranged in α-helical motif in which the basic residues are located near the hydrophilic/hydrophobic interface and acidic residues are clustered at the center of the polar face. The helical segments are often interrupted by proline residues. This so-called Class A motif that is exemplified by apoA-1 lipoprotein is illustrated together

TABLE 7.2 The size and composition of mammalian lipoproteins

Lipoprotein	Density	Diameter (nm)	Triglyceride/Cholesterol	Apoprotein
Chylomicrons	< 0.95	1000	10	B-48,C,E
Very low density (VLDL)	0.95-1.006	50	2.3	B-100,C,E
Intermediate density (IDL)	1.006-1.019	30	1.0	B-100,E
Low density (LDL)	1.019-1.063	20	0.2	B-100
High density	1.063-1.210	10	0.03	A

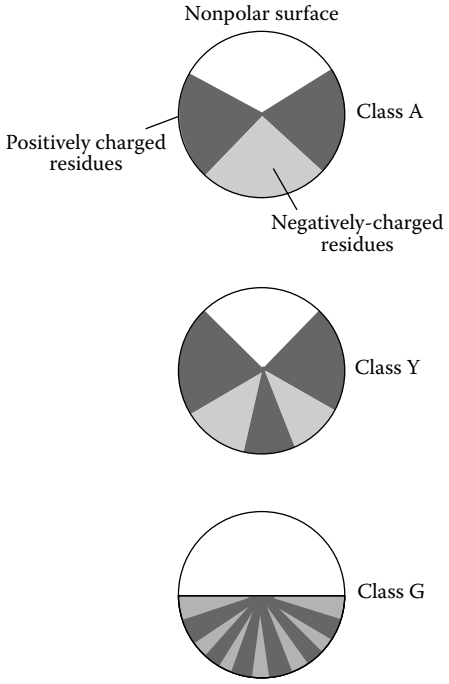


FIGURE 7.27 Helix wheel plots of the three classes of α-helical segments found in apoA-1, apoA-IV, and apoE apolipoproteins. (Adapted from Segrest et al. (1992), *J. Lipid Res.*, **33**, 141–166.)

with Class Y and Class G motifs in Figure 7.27 ApoE isoforms have a predominance of Class G helical motifs and the Class Y helical motif dominates the secondary structure of apoA-IV. The N-terminal amphipathic helices of apoE are bundled into four antiparallel strands forming an elongated globular structure with the hydrophobic faces oriented into the interior. The C-terminal adopts a coiled-coil helix structure that is more exposed to the aqueous phase. In the absence of lipid, apoA-1 adopts a two-domain configuration similar to apoE; an N-terminal helical bundle extending into the central part of the primary structure and a C-terminal helical domain that is less well organized. Both apoA-1 and apoE aggregated into oligomers in the absence of lipid via hydrophobic interactions between residues located at the C-termini of the respective molecules.

The self-association in aqueous media via the C-terminal domains of apoA-1 and apoE is consistent with the role of the C-termini in the interaction of these proteins with lipids. When apoE binds to lipid it has been suggested that the initial binding takes place at the C-terminal, which then induces the 4-helicle bundle of the N-terminus to reorganize so that the hydrophilic faces of the helices open out and become available for binding to the lipid. Similar reorganizations are believed to occur when apoA-1 interacts with lipid. Thus, an initial binding takes place at the C-terminal domain, which is arranged in an elongated hairpin structure. Following this there is a conformational change involving residues 1-43, which serve to unmask a hydrophobic domain in residues 44-65 of the protein.

The driving force for formation of complexes between the apolipoproteins and lipids appears to be a favourable change in enthalpy. A conformational transition from random coil to α-helix on binding of apoA-1 to lipid, for example, is associated with an enthalpy change in the order of -5kJ/α-helical segment. This represents a total enthalpy change of about -11 kJ.mole^{-1} and is additional to the enthalpy change accompanying the interaction of apoA-1 with lipid, which is about -40kJ. mole^{-1}. The change in conformation of the protein, therefore, contributes significantly to the binding affinity between the protein and the lipid.

Two conformers of high-density lipoprotein have been characterised, one discoidal in shape and the other spherical. The discoidal particles are comprised of a phospholipid bilayer disk with two molecules of apoA-1 encircling the edges where the acyl chains are exposed. The size is limited by the length of the apoA-1 molecules that are arranged in a belt of α-helices stacked one on the other in an antiparallel orientation. A similar discoidal particle has also been described for apoE, but, in this structure, four molecules of the protein are arranged at the periphery of the disk. The spherical form of high-density lipoprotein varies in diameter and has more neutral lipid than the discoid form. The amphipathic helices of the apolipoproteins are believed to be interpolated between the phospholipid molecules rather than at the periphery. The extent of interaction of the protein with the lipid is greater as the proportion of protein in the particle decreases.

7.5.4 Enzyme-substrate interactions in lipolysis

A variety of enzymes are required to synthesize lipids and, in turn, hydrolyse them in the normal metabolic turnover of these structural components. Likewise, enzymes play an important role in the dietary uptake and assimilation of lipids in the body. The activity of many lipolytic enzymes share a common feature in that their hydrolytic activity against their normal substrates are greatly influenced by the presence of lipids that are not substrates of the reaction or known to be bound to the enzyme. For hydrolysis to take place, the water-soluble enzyme must be attracted to the substrate located at the lipid–water interface. The electrostatic charges carried by the reactants need to be favourable and the magnitude of the attractive force increases with the size of the opposite charges carried by the protein and the electro-static potential at the substrate interface. In addition, the enzyme must orient about the interface such that the substrate is presented in a favourable manner for the hydrolytic reaction to proceed. Finally, the products of the reaction must diffuse away from the reaction site to be replaced by a fresh substrate molecule. This process is often complicated by the fact that reaction products are often hydrophobic in character and remain concentrated

in the substrate thereby influencing the rate of hydrolysis simply by diluting the substrate and altering the manner in which the enzyme interacts with it at the substrate-water interface.

One of the characteristic features of hydrolysis of lipid substrates by their respective enzymes is the existence of a time delay or lag period before activation of the enzyme is observed. The lag period in activation of phospholipase C from *Bacillus cereus* has been investigated by assay of activity of the enzyme against large unilamellar vesicles composed of different substrate mixtures (Ruiz-Arguello et al., 1998). A lag period in activation of the enzyme was noted in substrate mixtures consisting of phosphatidylcholine (the normal substrate for the enzyme), phosphatidylethanolamine (hydrolysed to a lesser extent), sphingomyelin, and cholesterol (neither are substrates for the enzyme). The duration of the lag period was found to vary between 8 sec and more than 30 min depending on the proportion of the different lipids in the substrate mixture. Furthermore, the maximum rate of hydrolysis varied from 0.4 to more than 55 min^{-1}, again depending on the particular substrate mixture.

The presence of lipids in the substrate that tended to destabilize bilayers and favour hexagonal-II structure, such as phosphatidylethanolamine and cholesterol, were found to enhance activity and shorten the lag phase. Conversely, lipids that are known to stabilize bilayers, such as sphingomyelin, had the opposite effect on enzyme activity. This suggests that, while the substrate is presented in the form of a bilayer, the enzyme is sensitive to the local instability that assists orientation of the substrate about the active site of the enzyme. The interesting feature of all reaction mixtures, however, was that the proportion of the substrate hydrolysed during the lag period was invariably 0.10% of the total substrate present. The explanation for this observation is that the creation of local rafts of bilayer enriched in the diacylglycerol product of the reaction results in aggregation of the vesicular substrate, which, in turn, is responsible for the acceleration in the rate of hydrolysis.

An indication of the effect of reaction products on the physical properties of the substrate has been obtained from studies of the effect of phosphatidic acid in monolayers of phosphatidylcholine subjected to hydrolysis by phospholipase D from *Streptomyces chromofuscus* (El Kirat et al., 2002). The enzyme is a member of a super family that includes endonucleases, helicases, lipid synthetases, and enzymes able to catalyze the formation or hydrolysis of phosphodiester bonds. In its reaction against a substrate of phosphatidylcholine, a phosphatidyl–enzyme intermediate is formed, which is subjected to a nucleophilic substitution by a molecule of water to release phosphatidic acid. The effect of phosphatidic acid in monolayers of substrate at the air–water interface on the surface elasticity modulus and the lag period before accelerated hydrolysis is observed is shown in Figure 7.28 Surface elasticity

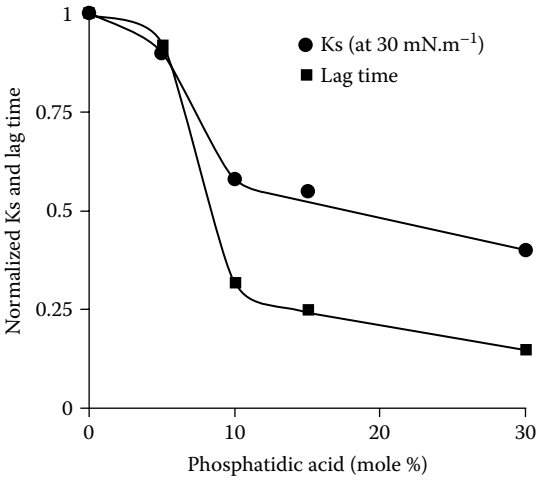


FIGURE 7.28 The value of surface elasticity modulus at 30 mN,m-1 and enzyme activity lag time determined as the time preceding a 5% decrease in monolayer area plotted against the proportion of phosphatidic acid mixed with the phosphatidylcholine substrate. (Adapted from El Kirat, K. et al., (2002), *J. Biol. Chem*, **277**, 22131–21236.)

modulus, Ks, is derived from the surface pressure-area isotherm from the relationship:

$$Ks = -A\,(\partial\pi/\partial A) \qquad (7.51)$$

where A is the molecular area at the corresponding surface pressure, π. The greater the value of Ks for a monolayer the less it is subject to deformation. The correlation between Ks and lag time shown in the figure suggests that deformation of the substrate-water interface is an essential step in orientating the enzyme about the phospholipid substrate and that the product is instrumental in modulating this process.

A number of studies have been reported that examine the effect of substrate presentation and molecular species preferences of secretory phospholipase A_2. The enzyme hydrolyses the fatty acid esterified to the *sn*-2 position of the glycerol backbone of diacylglycerophosphatides. The activity of the different subclasses of this enzyme are known to be modulated by proteins and peptides, such as melittin and phospholipase A_2-activating protein, that are believed to act by modifying the manner of presentation of the substrate (Koumanov et al., 2003). The effects of such proteins in activating the enzymes differs depending on whether the substrate is in the form of a dispersion of pure lipid or is present in a biological membrane. These differences highlight the role of regulatory peptides in the biological function of these phospholipases. In addition to regulation by proteins the presence of nonsubstrate lipids is also found to influence catalytic activity. Type-II secretory phospholipase A_2 can be "activated" by displacement from an interface comprised of susceptible diacylglycerophospholipid substrate by binding to an interface of nonsusceptible phospholipids, such as

sphingomyelin. The sphingomyelin may be presented in the form of a separate dispersion added to the assay mixture or as a phase-separated domain within the bilayer of substrate molecules. The affinity of the enzyme for the sphingomyelin interface may, in turn, be reduced by the association of the sphingomyelin with cholesterol to form a liquid-ordered phase. This effect is observed as an "activation" of the enzyme by cholesterol.

The action of one phospholipase can trigger the activity of another phospholipase with different substrate specificity. Such actions may represent manifestations of the highly complex biochemical homeostatic mechanisms that are present in living cells responsible for maintaining the molecular species composition of cell membranes within relatively narrow limits. One example is the activation of secretory phospholipase A_2 by ceramide, the product of sphingomyelinase on its substrate, sphingomyelin (Koumanov et al., 2002). The activation of secretory phospholipase A_2 by ceramide is additional to "activation" by release of the enzyme bound to sphingomyelin domains. The activation is apparently due to the creation of phase-separated domains of bilayer and hexagonal-II phase in the substrate dispersion. The presentation of diacylglycerophospholipid substrate at the interface between the two-phase structures would appear to be the most plausible explanation for the enhanced activity of the enzyme. This may be related to the observation that substrate molecular species with a polyunsaturated fatty acid, such as arachidonic acid, in the *sn*-2 position of the glycerol are the much-preferred substrates compared with molecular species with relatively saturated fatty acids acylated to the *sn*-2 position.

The substrates for triacylglycerol lipases are usually presented in the form of an emulsion, such as dietary fat droplets or lipoproteins. These particles consist of substrate presented in a bulk phase, which is stabilized at the aqueous interface by a monolayer surface phase composed of phospholipid and other proteins. The enzyme catalysis takes place in the surface phase so both substrate and the enzyme must partition from their respective bulk phases into the interfacial region for reaction to occur. In many cases stabilization of lipid emulsions by phospholipids impedes access of the enzyme to its substrate and this is evidenced by a significant lag period that may be of duration of hours before lipase activity accelerates towards equilibrium. Such lag period can often be reduced or even eliminated by the introduction of reaction products, such as diacylglycerol or fatty acids; some lipases require cofactor proteins to augment their catalytic function (Brockman, 2000).

7.5.5 Technical applications

There are a number of important technical applications of lipid–protein interaction, mainly in the fields of food technology and the pharmaceutical industry. Food emulsions such as margarine and ice cream have polar lipids and proteins at the fat–water interface. Baking performance of cereals depends upon lipid–protein interaction. The properties of starch products can be monitored by formation of an amylase–lipid complex as a direct consequence of hydrophobic interaction.

Among pharmaceutical applications, the use of lipids and surfactants as formulation aids play an essential role in delivery and uptake of a variety of drugs into the body. Liposome vectors are commonly exploited for this purpose. An active component can be entrapped in liposomes or vesicles, giving a controlled-release system. By solubilizing proteins in the lipid bilayer that exposes a "label" on the surface, it may even be possible to achieve drug targeting. Clinical experiences so far, however, appear to be limited mainly because liposomal dispersions are not thermodynamically stable despite claims that phospholipid formulations of water-insoluble drugs achieve desirable versatility (Zhang et al., 2005).

References

Brockman, H.L. (2000), Kinetic behavior of the pancreatic lipase-colipase-lipid system, *Biochimie*, **82**, 987–995.

Brodsky, J.L. et al. (2004), Vesicular trafficking of hepatic apolipoprotein B100 and its maturation to very low-density lipoprotein particles; studies from cells and cell-free systems, *Trends Cardiovacs. Med.*, **14**, 127–132.

El Kirat, K. et al. (2002), Role of calcium and membrane organization on phospholipase D localization and activity. Competition between a soluble and insoluble substrate, *J. Biol. Chem*, **277**, 22131–21236.

Ericsson, B. et al. (1983), A cubic protein-monoolein-water phase, *Biochim. Biophys. Acta*, **729**, 23–27.

Koumanov, K.S. et al. (2002), Ceramides increase the activity of the secretory phospholipase A2 and alter its fatty acid specificity, *Biochem. J.*, **363**, 45–51.

Koumanov, K.S. et al. (2003), Bimodal regulatory effect of melittin and phospholipase A2-activating protein on human type II secretory phospholipase A2, *Cell. Biol. Int.*, **27**, 871–877.

Lucas, L.H. et al. (2004), Epitope mapping and competitive binding of HSA drug site II ligands by NMR diffusion measurements, *J. Am. Chem. Soc.*, **126**, 14258–14266.

McArthur, M.J. et al. (1999), Cellular uptake and intracellular trafficking of long chain fatty acids, *J. Lipid Res.*, **40**, 1371–1383.

Misquitta, Y. et al. (2004), Rational design of lipid for membrane protein crystallization, *J. Struct. Biol.*, **148**, 169–175.

Ruiz-Arguello, M.B. et al. (1998), Phospholipase C hydrolysis of phospholipids in bilayers of mixed lipid compositions, *Biochemistry*, **37**, 11621–11628.

Saito, H. et al. (2004), Contributions of domain structure and lipid interaction to the functionality of exchangeable human apolipoproteins, *Prog. Lipid Res.*, **43**, 350–380.

Segrest, J.P. et al. (1992), The amphipathic helix in the exchangable apolipoproteins: a review of secondary structure and function, *J. Lipid Res.*, **33**, 141–166.

Tanford, C. (1980), *The Hydrophobic Effect*, John Wiley & Sons, New York.

Zhang, J.A. et al. (2005), Development and characterization of a novel Cremophor EL free liposome-based paclitaxel (LEP-ETU) formulation, *Eur. J. Pharm. Biopharm.*, **59**, 177–187.

7.6 Biological membranes

7.6.1 Introduction

The structure of biological membranes has a history spanning more than a century and a chronology of developments of the topic can be sourced from the various models proposed for membrane structure published throughout this period (Gorter and Grendel, 1925; Danielli and Davson, 1935; Singer and Nicolson, 1972; Robertson, 1972; Green and Brucker, 1973). One of the most durable of the models is the so-called fluid mosaic model proposed by Singer and Nicolson in 1972. The model envisaged that membrane lipids form a bilayer configuration, which serves as a matrix for the association of the proteins.

The principal arguments used to support this concept were thermodynamic; the experimental evidence was tenuous and based on assumptions that the arrangement of the lipid was indeed a bilayer. The most convincing evidence, which was not cited in the formulation of the model, is based on electron density calculations obtained from x-ray scattering intensity profiles of oriented films of hydrated egg lecithin, itself a lipid not found in a biological membrane (Levine and Wilkins, 1971). The electron densities were found to be consistent with an osmotically insensitive component assumed to be devoid of water and consisting of amphiphilic lipid molecules oriented with their hydrocarbon chains shielded from the aqueous component by polar groups aligned at the interface.

It is now recognised that the ability of the membrane lipid molecules to associate in an aqueous environment in infinite lipid bilayers represents the basic mechanism underlying the formation of biological membranes. The property of such structures to separate aqueous compartments was also seen as a critical step in the evolution of living organisms. The significance of the ordered, but fluid, state of the lipid molecules, particularly for the interaction with membrane proteins, was revealed by studies of lipid–water systems preceding the formulation of the fluid mosaic model (Chapman et al., 1967).

There is now a consensus that the lipid bilayer represents the matrix of biological membranes. In most cases the lipids are more or less fluid, but in some noteworthy examples they are crystalline. These include the purple membrane of *Halobacterium halobium* and related bacteria in which the lipids of the bilayer are arranged in a crystal structure in patches on the cell surface that support the light-driven proton pump, bacteriorhodopsin. In the overwhelming number of cases the lipid bilayer is thought to be predominantly in a fluid state such that the components are able to diffuse relative to one another subject to a number of important constraints. There is, for example, a lateral heterogeneity in the relative mobility of lipids and proteins, which is due to the creation of domains of more ordered lipid arrangements dispersed amongst more disordered fluid domains.

It is remarkable that membranes form such a stable unified structure considering the diversity of lipid molecular species and different types of proteins associated with each morphologically distinct membrane. It is also implicit that biochemical mechanisms must be responsible for creating and preserving this diversity, including that of the relative proportions of the different lipids and proteins. The nature of these processes, however, is largely unknown.

Many membrane functions can be directly related to the physical properties of the lipid bilayer. The action of membranes as a barrier to the diffusion of water-soluble solutes, especially charged ions, is due to the creation of a hydrocarbon layer by the orientation of the hydrophobic moieties of the constituent lipids into the core of the structure. Variations in curvature and area per unit mass and motion of the constituent molecules can also be ascribed to the relatively fluid nature of the structure. The slow or prohibited translocation of lipids and proteins from one side of the membrane to the other creates functional asymmetries that are essential to the performance of life-sustaining processes. Thus, membrane proteins have a unique orientation with respect to the membrane bilayer, a property that is integral to the biosynthesis of cell membranes. This fact ensures that the functions performed by the proteins are vectorial. Since many membrane proteins are concerned with the selective translocation of solutes across the membrane, their mutual orientation means that transport takes place only in the required direction.

Certain membrane lipids and proteins are glycosylated. The associated carbohydrates are particularly abundant on the outer surface of the plasma membrane where they form the glycocalyx. The function of this layer is to participate in recognition phenomena and as receptor sites for different hormones, toxins, and other ligands. In endothelial tissues the glycocalyx serves to shield the vascular wall from the shear stresses of blood flow, impede leakage of blood constituents across the endothelial lining, and prevent adhesion of leucocytes and platelets to the endothelium (Rehm et al., 2004).

Membrane trafficking between subcellular compartments is a mechanism that operates to redistribute membrane components and their associated ligands within cells. The process is also central to the biogenesis and homeostasis of membranes. Endocytosis and exocytosis are processes that involve the uptake of plasma membrane into the cell and fusion of subcellular membrane vesicles with the plasma membrane, respectively. A model for endocysis to account for the participation of cytoplasmic proteins has been proposed recently (Hommelgaard et al., 2005). According to this model, small lipid domains

consisting of ordered phase lipids and associated proteins associate laterally in the plasma membrane to form larger, endocytotic invaginations, which are stabilized by interaction with the cytoplasmic protein, caveolin. This interaction reduces the endocytic activity of these structures and, in addition, binding to the actin cytoskeleton via filamin makes the caveolae largely immobile. Nevertheless, a few caveolae may circumvent the restraints and become internalized. Short-range caveolar motility including membrane fission and fusion are also thought to take place so that clathrin-coated vesicles that bud off from the plasma membrane may fuse to become contiguous with the plasma membrane again. Internalization of caveolae can be specifically induced by complete reorganization of the actin cytoskeleton and removal of plasma membrane caveolae in a coordinated wave.

In the budding and fusion of membrane vesicles from particular compartments, only membrane components that are destined to be translocated are included in the vesicle; resident membrane constituents that define the compartment are excluded from membrane forming the vesicle. The mechanism that operates to segregate membrane proteins involves short peptide sequences on the membrane proteins that represent recruitment signals.

In addition to this segregation process, a recovery system is known to act to retrieve resident proteins that leak into the vesicular compartment and return them to their original location. Protein–protein interactions responsible for the targeting and docking of vesicles between appropriate membranes are mediated by the Rab family of GTPases. About 60 different Rab proteins, which are tethered to the membrane by covalently bound prenyl groups, have been found to be expressed in mammals and these serve to control the diversity of vesicle traffic in different cell types. They act to assemble soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complexes on the vesicle (v-SNARE) and respective target (t-SNARE) membrane surface (Hong, 2005). The SNARE proteins are largely anchored to the membrane by a short C-terminal sequence of hydrophobic amino acids. Docking between complimentary v- and t-SNARES ensures interaction takes place between the appropriate membranes. There is evidence that some SNAREs are involved in the subsequent fusion between docked membranes (Chen et al., 2005).

Fusion and fission are fundamental processes in membranes. The fusion between membranes can be regarded as a local phase transition at adjacent lipid bilayers where the action takes place. Whereas the choline phosphatides form the lamellar L_α structure over a wide range of pH, in the presence of various ions, other phospholipid classes in the membrane favour nonlamellar arrangements. Furthermore, certain polar lipids can form domains that favour the formation of nonlamellar structures at the growth temperature (Sot et al., 2005). Nonbilayer structures can also be induced in membranes containing certain negatively charged lipids by screening the electrostatic charges on the lipid molecules with cations.

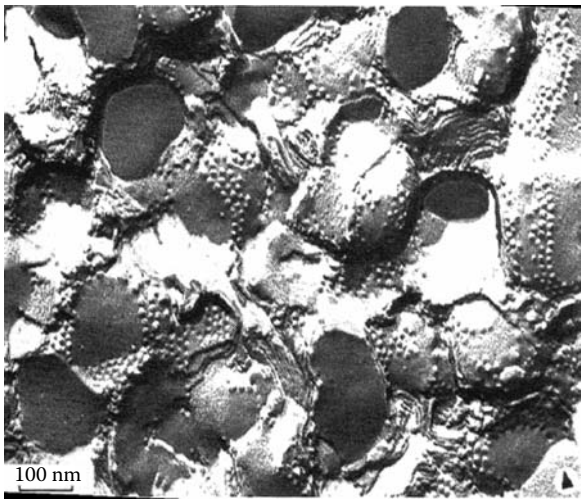
The precise rearrangement of lipids to bring about fusion between adjacent membranes has been the subject of considerable interest. One model of the sequence of structures leading to fusion is the stalk hypothesis (Haque and Lentz, 2004). An initial intermediate structure (stalk) is said to form by the rearrangement of lipids in the outer leaflets of closely contacting bilayers in a manner that alters the normal topology of the bilayer. Evolution of the stalk into a trans-monolayer contact involves rearrangements in nonbilayer structures without a change in membrane topology. The free energy of such non-bilayer lipidic structures is thought to be dominated by bending of the monolayer and hydrophobic mismatch or "void" energies. The fusion process is facilitated by the strategic location of lipid molecular species that are intrinsically positively or negatively curved or long-chain lipids that favour a stable bilayer arrangement. Stable fusion pores can form only to the extent that the unfavorable free energy of these intermediates can be overcome by thermal energy.

That molecular species of lipids present in cell membranes exhibit complex thermotropic and lyotropic mesomorphism has been well established from biophysical studies of lipid–water systems over many years. What is less clear is the manner in which these properties influence the creation and stability of local membrane lipid domains, the function of the different membrane proteins and the mechanisms operating to preserve membrane lipid composition. The overwhelming evidence supports the notion that the lipids of biological membranes are arranged in a bilayer configuration despite the fact that a considerable proportion of the molecular species of lipid present in a cell membrane may assume a nonbilayer arrangement if dispersed alone under physiological conditions.

It is clear that the lipids of cell membranes play a crucial role in the function and properties of cell membranes. The properties of membrane lipids dispersed in aqueous systems have been examined in great detail by a range of biophysical techniques. The properties of lipid–water systems using such techniques have been extensively reviewed elsewhere in this volume and the results of such studies form a useful basis upon which their behaviour in biological membranes may be inferred. Some of the structural features of membrane proteins will be referred to below so that the factors that govern the interaction between the membrane proteins and lipids may be examined in an informed context.

7.6.2 Lipid arrangement in biological membranes

The principal lines of evidence for the arrangement of lipids in biological membranes are based on studies of certain classes of lipid that form lamellar structures in aqueous dispersions and are supported by arguments



(a)

(b)

FIGURE 7.29 Electron micrographs of freeze-fracture replicas prepared from (a) total polar lipids extracted from higher plant chloroplast membranes dispersed in chloroplast assay medium. (b) Intact chloroplast membranes from which the lipid extract was prepared. The scale bar and direction of shadowing is shown in (a).

along the lines of the predicted behaviour of amphiphiles when hydrated in aqueous systems. This approach, however, ignores the presence, often in very significant proportions, of lipids that do not form lamellar structures when dispersed in aqueous media at temperatures approximating to that of the growth temperature of the organism from which they are extracted. Indeed, it is commonly observed that dispersions of total polar lipid extracts of many membranes do not form pure lamellar phases, but exhibit a mixture of different phases consisting of both lamellar and nonlamellar structures. This is illustrated in Figure 7.29, which shows freeze-fracture electron microscopic images of replicas prepared from an aqueous dispersion of total polar lipids extracted from chloroplast membranes compared to the intact membrane. Clearly there are bilayer as well as nonbilayer structures formed in the polar lipid dispersion. No such structures are observed in the intact membrane in which the membrane-associated particles are believed to represent the intrinsic proteins of the photosynthetic apparatus distributed in smooth regions said to be the central domain of a lipid bilayer.

The implications of these observations is that either the arrangement of the polar lipid in the membrane is, in part, lamellar and coexisting with nonlamellar structures or that interaction with other membrane components, such as the protein, imposes a lamellar arrangement on those molecular species of lipid that would otherwise form a nonlamellar structure. It remains a possibility, however, that the particles themselves represent lipoprotein complexes, which may comprise lipids that are not components of the bilayer matrix.

The methods used to determine the arrangement of lipids in biological membranes include diffraction and spectroscopic measurements as well as freeze-fracture electron microscopy and monomolecular film area determinations. Some of this evidence will be examined in the following sections.

7.6.2.1 Monomolecular film area measurements

The first demonstration that lipids form a monolayer at the air–water interface was reported by the polymath and statesman, Benjamin Franklin. In a letter to Doctor Brownrigg published in the *Philosophical Transactions* (1774), he stated:

> In a diſh of water, if the ſmalleſt drop of oil be let fall in the middle, the whole ſurface is preſently covered with a thin greaſy film proceeding from the drop; but as ſoon as that film has reached the ſides of the diſh, no more will iſſue from the drop, but it remains in the form of oil, the ſides of the diſh putting a ſtop to its diſſipation by prohibiting the farther expanſion of the film.

This work largely lay fallow until taken up by Langmuir (1917) who received the 1932 Nobel Prize for chemistry for his work on surfactant films. This foundation of surface chemistry together with Overton's earlier studies (1895) showing that hydrophobic molecules penetrate membranes more readily than hydrophilic solutes led to the experiments by Gorter and Grendel (1925) upon which the first model of membrane structure was formulated. Their experiment consisted of comparing the area occupied at an air–water interface by the lipids extracted from a known area of membrane; they examined erythrocyte membranes from six species, including man.

The experimental methods used in these experiments, however, were found to be flawed; the use of acetone solvent resulted in incomplete extraction of the lipid, but this was compensated to some extent by inaccuracy in

measurement of membrane area. Furthermore, there is also uncertainty about the packing density of lipid molecules in the surface film at which the area should be measured; in fact, the measurements were made on films with a surface pressure of only $2mN.m^{-1}$. Under these conditions, it was found that the area occupied by the lipid at the air–water interface was about twice that of the area of membrane from which it was extracted. This led to the conclusion that the structure of cell membrane was composed of a lipid bilayer.

It is now known that the density of phospholipids in a bilayer configuration is somewhat greater (equivalent to a monomolecular film pressure of $30–35mN.m^{-1}$) than employed in the original experiments. Moreover, the Gorter and Grendel experiment has been repeated since by other workers to ensure complete lipid extraction and with accurate measurements of membrane area. The results invariably show that the area occupied by the lipids in a surface film is less than that required to form two layers over the entire membrane area. Table 7.3 shows the maximum proportion of membrane area that could be accounted for as lipid in bilayer arrangement. It can be seen that as the proportion of protein to lipid increases in a membrane the amount of lipid that could be accounted for as lipid bilayer decreases.

It is important to recognise that no conclusions can be drawn about the arrangement of lipids in biological membranes using this approach. If, however, a bilayer configuration is *assumed* for the lipid then the remaining area could be accounted for by intrinsic proteins. This is the assumption used in calculating the surface area occupied by lipids in the human erythrocyte membrane (Engelman, 1969). Accordingly, the total volume of membrane occupied by the hydrocarbon chains of the phospholipids was calculated using an average chain length of 16.5 carbon atoms with 1.26 unsaturated bonds per chain. To this volume was added that occupied by the cholesterol, obtained from density measurements, assuming that the entire molecule resides in the hydrophobic region of the membrane. The value obtained was too large for a membrane consisting of a single layer of lipid molecules, but insufficient for one covering the entire red cell surface in a bilayer. It was suggested that the discrepancy could be accounted for by nonlipid components if it was assumed that the lipid formed a bilayer.

TABLE 7.3 Theoretical area of membranes consisting of lipids in bilayer arrangement calculated from area occupied by total lipid extracts at the air/water interface

Membrane	Protein:Lipid (wt/wt)	Lipid Bilayer (%)
Plasma membrane		
Erythrocyte	1.50	67
Myelin	0.28	103
Acholeplasma laidlawii	1.78	62
Mitochondria		
Inner membrane	3.55	40
Outer membrane	1.22	72
Endoplasmic reticulum	0.90	83

7.6.2.2 Diffraction studies

The principle methods of diffraction analysis rely on examination of assemblies of molecules that are arranged in a regular repeating lattice. This is necessary because scattering occurs only weakly and amplification can be achieved most conveniently with a large ensemble, e.g., crystals. As we have noted above, membranes are said to be fluid, which implies that the molecules are in a state of disorder. The notable exception is the purple membrane, referred to above, which exists in a rigid, two-dimensional crystalline array. The purple membrane has proved to be amenable to detailed diffraction studies and the arrangement of the constituent bacteriorhodopsin and lipids has been determined to a resolution of 0.3 nm (Mitsuoka et al., 1999).

In these studies, the two-dimensional order in the membrane has been examined using electron diffraction methods since the dose of x-rays required to detect a diffraction pattern from such a small assembly would cause radiation damage to the membrane. This problem is avoided in transmission electron microscopic methods, which have been modified to enable the detection of electron scattering intensities and their subsequent conversion into structural coordinates of the two-dimensional protein-lipid crystals. The intensity with which electrons are scattered varies according to the electrical potential within the structure, which, for practical purposes, is roughly proportional to electron density and, in turn, to atomic number. In this sense the electron diffraction method is analogous to x-ray diffraction, since the Fourier transform of the electron scattering yields the profile of atomic density through the structure.

Apart from the usual problems of phase determination, the advantage of the electron scattering method is that very low doses of electrons can be employed (less than 1 electron per unit cell) and, in order to reconstruct the image, information is combined from a large number of unit cells. This avoids radiation damage to the sample, which would otherwise destroy the structure. The strategy for constructing a 3-dimensional image of a 2-dimensional array of molecules is achieved by analysing specimens tilted with respect to the angle of incidence of the electron beam. This method relies on the fact that the Fourier transform of a transmission electron micrograph of a 2-dimensional crystal is a central section to a 3-dimensional Fourier transform of the crystal, that is, through a lattice of line in reciprocal space perpendicular to the plane of the crystal. Thus, the amplitudes and phases of Fourier terms along each lattice line can be obtained by combining the Fourier transforms from an appropriate number of different, tilted views of the 2-dimensional array. As in the x-ray diffraction analysis and inverse Fourier transform

produces a 3-dimensional map of the density distribution within the monomolecular lattice.

The picture that emerges from these studies is that of a protein that consists of 7 rods, each of about 4 nm long and 1 nm apart. The rods span the lipophilic portion of the membrane, three approximately vertical to the plane of the membrane and four tilted by 10° to 20° to the vertical. Each rod is an α-helix of the polypeptide chain and, since the complete sequence of the protein is known, it has been possible to show that some of the chain connecting the seven rods is on one side of the membrane and some on the other. Most of the amino acid side chains of the rods are neutral. By contrast, those parts of the protein that are near or extend into the water on either side of the membrane contain 19 charged amino acid residues and the parts that protrude into the water on the outside of the membrane contain 6 charged groups. Within the rods there are 9 charged groups, 5 negative, 4 positive, which may be sufficiently close to neutralise each pair of charges in a predominantly nonaqueous environment. The length of the protein in contact with the lipid is approximately equal to the length 2 molecules of lipid. The individual bacteriorhodopsin molecules are aggregated within the structure into trimers, which produce a three-fold axis of symmetry within the membrane.

The lipids of the membrane occupy the interstitial spaces between the protein molecules, including the central region between the protein trimers. The lipids of the purple membrane are typical archibacterial lipids containing phytanyl chains and a major sulphated triglycosyl archiol lipid dominating the composition with minor amounts archiol-based phospholipids reflecting the high salt concentrations of the environment in which these bacteria live. The lipids in the purple membrane, which consist largely of branched-chain phytanylether phospholipids and glycolipid sulphate, are arranged in a bilayer configuration. This is not the arrangement adopted by the lipids when the proteins are removed as revealed by freeze-fracture electron microscopic studies of the type illustrated in Figure 7.29a. Spin label studies have indicated that the hydrocarbon chains of the lipids in the membrane are relatively immobilized while the lipids in aqueous dispersions have hydrocarbon chains, which are comparatively mobile even towards the ether linkage of the phytanyl chains to the glycerol. All these studies indicate that there is a strong interaction between the lipid and the protein in the membrane of the halophilic bacteria, which leads to a considerable modification of the phase behaviour of the lipids observed in isolated dispersions.

Electron diffraction methods have been used to investigate the structure of other membrane proteins including the nicotinic acetylcholine receptor, which is a member of the pentameric "Cys-loop" super family of transmitter-gated ion channels associated with neuronal acetylcholine receptors, $GABA_A$ receptors, $5\text{-}HT_3$ receptors, and glycine receptors. An atomic resolution 0.4 nm was reported to produce a model showing that the two ligand-binding α subunits have a different extended conformation from the three other subunits in the closed channel. Furthermore, the acetylcholine-coordinating amino acid side-chains of the α subunits were found to be far apart in the closed channel, indicating that a localised rearrangement, involving closure of loops B and C around the bound acetylcholine molecule, takes place upon activation (Unwin, 2005).

Conventional crystallographic methods cannot be used to investigate the structure of biological membranes because they are fluid structures with two rather than three-dimensional order. Likewise, the structure of membrane proteins when extracted from the lipids is not usually amenable to diffraction analysis because they are notoriously difficult to crystallize. Dispersions of membrane lipids, however, form well-recognised phases when dispersed in aqueous systems and these can be characterised from powder reflections. Methods have been developed to orient biological membranes into stacks using Langmuir–Blodgett methods or ultracentrifugation from which powder diffraction patterns can be recorded, but the stacking order is generally low and, hence, analyses tend to be of relatively low resolution. Selective deuterium substitution for protons can provide valuable positional information within the profile using neutron diffraction methods.

Electron density profiles through the unit cell of membrane repeats have been constructed from x-ray diffraction data collected from biological membranes that are oriented naturally within the biological tissue, such as myelin and retinal rod outer segment disc membranes, or oriented by processes, such as centrifugation. Studies of a variety of membranes at resolutions of about 1.1 nm have shown the structures to be generally asymmetric with respect to the distribution of electron density. The sarcoplasmic reticulum membrane has been examined in detail by both x-ray and neutron diffraction methods. Electron density profiles for water, lipid, and protein have been constructed from this data that show that the major protein of the membrane, Ca^{2+}ATPase, spans the membrane, and is in contact with water on both surfaces. A major portion of the protein protrudes substantially into the extra vesicular aqueous domain. The extent of this protrusion depends on the functional configuration of the enzyme (Blasie et al., 1990).

By the use of deuterated lipids, it was possible to determine the arrangement of the lipid in the form of a bimolecular structure with the polar groups of the lipids residing at the aqueous interface on either surface of the structure and with the hydrocarbon chains extending into the interior. As with the protein, the membrane lipid and water profiles are asymmetric consistent with the complementary asymmetry in the protein mass distribution within the lipid bilayer domain of the sarcoplasmic reticulum membrane profile structure. A model of the structure of the sarcoplasmic reticulum membrane constructed from the diffraction and other data is illustrated in Figure 7.30.

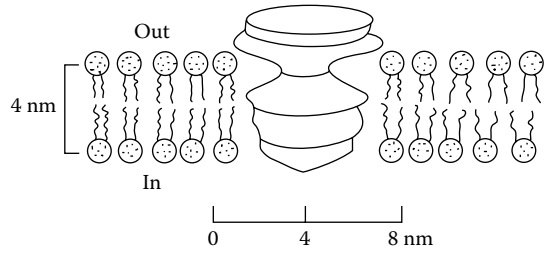


FIGURE 7.30 A model of the sarcoplasmic reticulum membrane constructed from electron density distribution profiles derived from diffraction studies of oriented membranes. (Adapted from Herbette, L. et al. (1985a), *Biochim. Biophys. Acta*, **817**, 103–122.)

The high brilliance of synchrotron radiation has been exploited to provide information on structural changes that take place in membrane proteins and lipids on time-scales appropriate to membrane function. Examination of membranes from halobacterium placed on substrate surfaces have revealed changes in the structure of the bacteriorhodopsin associated with the light-driven pumping of protons that occur on time-scale of ms (Oka et al., 2005). Similarly, synchrotron x-ray diffraction intensities have been recorded through temperature and pressure jumps used to induce structural transitions between phases of hydrated lipid bilayers from which relaxation rates and mechanisms could be determined (Rappolt et al., 2003).

7.6.2.3 Microscopy

With the advent of the electron microscope in the 1950s, the ultrastructure of the cell was visualized in considerable detail and the morphology of cells were defined largely by the various cell membranes. The appearance of cell mem-branes in stained thin cross section was uniform and consisted of two darkly staining bands sandwiching an electron lucent layer. The outer bands were said to represent the membrane proteins in an extended β-sheet configu-ration coating a lipid bilayer. Although this model was inconsistent with hydrophobic contact between the proteins and lipids it did recognize that membranes were asymmetric with different proteins located on opposite sides of the membrane.

The introduction of freeze-fracture electron microscopy shortly afterwards soon highlighted discrepancies in the unit membrane hypothesis current at the time. This method in which fixation of tissues was achieved by rapid thermal quenching techniques avoided the problems of uncertainty of heavy metal stain deposition. The structure of cell membranes is revealed by examination of templates replicating the topography of surfaces created when the frozen specimen is fractured. The replica is formed by shadowing with a thin layer of platinum, which in turn is supported by a layer of electron translucent carbon.

Figure 7.29b shows a replica produced when the fracture plane occurs along the plane of the membrane. The most conspicuous features are the membrane-associated particles in the fracture planes running longitudinally in the membrane. These were originally identified as enzyme complexes, which were supposedly rearranged during conventional fixation and staining required for thin section electron microscopy. Several lines of evidence were used to establish that the fracture plane occurs through a central region of the membrane rather than along the membrane–water interface. One was the observation that when a layer of radioactively labelled fatty acid applied to a glass surface was sandwiched with another layer of unlabelled fatty acid and thermally quenched, all radioactivity remained on the original plate when the plates were prised apart. This experiment is somewhat removed from the situation in biological membranes and brings into question the nature of the forces that anchor the fatty acids to the substrate surface.

More convincing evidence was obtained from etching studies. These showed that by allowing water to sublimate from the fracture surface before shadowing with heavy metal to form the replica different membrane surfaces could be discerned as the water table was lowered. Failure of etching to alter the appearance of the initial fracture surface indicated that this surface was devoid of water and represented a hydrophobic domain within the membrane. Those surfaces of membrane originally in contact with the aqueous medium were exposed by the etching process.

Interpretation of features exposed in lateral fracture planes through biological membranes is still conjectural. Evidence that membrane-associated particles consist, at least in part, of intrinsic membrane proteins is supported by observations that removal of lipid by digestion with phospholipases is found to result in an increase in density of particles suggesting that the surrounding smooth areas of fracture face represent lipid. Nevertheless, there are claims that vertical movement of proteins in the membrane matrix is possible so that domains of intrinsic proteins that are normally present in the hydrophobic domain of the membrane do not appear as membrane-associated particles on the longitudinal fracture planes.

Figure 7.31 illustrates this, showing a freeze-fracture electron micrograph of the plasma membrane of the blue-green alga, *Synecoccus*. The organism had been grown at 38°C at which temperature the distribution of membrane-associated particles was fairly random. The organism from which the replica was made had been cooled to 15°C prior to thermal quenching and this has produced a phase separation of the membrane components. Large regions of smooth fracture plane can be seen that correspond to the creation of gel phase, as judged by calorimetric and x-ray diffraction evidence (Furtado et al., 1979).

The conventional interpretation is that the high melting point lipids phase separate into a gel phase from which intrinsic membrane proteins are excluded. By phase separation it is usually inferred that this is a lateral phase

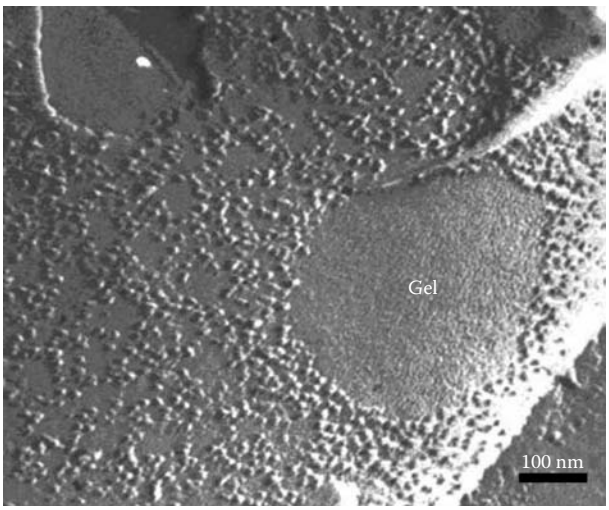


FIGURE 7.31 Freeze fracture of a *Synocuccus* cell membrane showing gel phase separation of membrane-associated particles. The density of particles around the periphery of the phase-separated domain is not sufficient to account for all particles originally present in the membrane.

separation and this is, in part, true as can be seen by an increase in density of particles around the periphery of the smooth regions of the membrane fracture plane. The additional particles in this high-density region cannot, however, account for all the particles originally present in the membrane. This suggests that membrane proteins have been ejected from the hydrophobic interior of the membrane into the aqueous phase or the constituents of the particles have undergone a reorganization that enables them to accommodate into a lamellar gel phase. From this we may conclude that some intrinsic proteins are arranged in oligomeric complexes and it has been argued that non-bilayer forming membrane lipids not only promote protein complexes, but serve to seal the irregular protein–lipid interface to the passage of solutes (Quinn, 1983). It is clear that intrinsic membrane proteins can integrate into gel and liquid-ordered phases, but not in the same manner as in a fluid matrix.

What is perhaps more difficult to explain is that in complementary fracture faces there are no holes or pits corresponding to the particles present in the opposite surface. Plastic deformation of the lipid between the fracture and shadowing operations resulting in obliteration of the pits has been suggested as a possible explanation, but contemporary methods of freeze-fracture make this unlikely. Another problem that is difficult to explain is that the diameter of the particles is often more than twice the thickness of a lipid bilayer (Tsvetkova et al., 1994) and the size of the protein complexes associated with membranes are consistent with these dimensions (Williams et al., 1982). The integration of intrinsic membrane proteins into the bilayer, therefore, is far from clear and we do not yet have an unambiguous interpretation of all the features seen in freeze-fracture replicas of biological membranes. Nevertheless, taken together with other evidence, the current assignments of smooth regions to the internal cleavage along the central plane of lipid bilayer and membrane-associated particles as representing intrinsic membrane protein complexes are consistent with the fluid-mosaic model of membrane structure.

A recent development for visualizing the surface topography of membranes is the atomic force microscope. The principle of the method relies on recording the vertical displacement of a sharp probe tip as it is drawn across the surface of the membrane. Electrostatic and van der Waals' interactions between the probe and the membrane surface produce the forces deflecting the probe in the vertical (z) direction, which is then amplified by a laser optical system. Scans in the x-y direction give a three-dimensional contour map of the membrane surface with a resolution in the vertical direction approaching 0.1 nm and between 0.5 and 1 nm in the lateral dimensions.

Atomic force microscopy has been used to characterise the phase separation of domains in supported lipid bilayer membranes. The resolution is sufficient to demonstrate that bacterial toxins, for example, are targeted specifically to liquid-ordered phase that is organized into rafts in the fluid bilayer membrane (Geisse et al., 2004). The method has also been applied to map the cytoplasmic and periplasmic surfaces of the photosynthetic membrane of the purple bacterium, *Rhodobacter*, to obtain a model for the arrangement of the light-harvesting and reaction centre complexes (Scheuring et al., 2005). The pigment-protein complexes are inserted across the lipid bilayer in an arrangement that allows the two light-harvesting complexes to collect the incident light and funnel the energy to a pair of bacteriochlorophylls located in the reaction center. Electrons are then transferred to two ubiquinone receptors reducing ubiquinone to ubiquinol, which is subsequently oxidised by the cytochrome bc_1 complex. The net result is a cyclic electron transfer coupled to the generation of a protonmotive force, which is utilized to synthesize ATP. The topography of the native membrane is characterised by two features, small rings about 5 nm in diameter, representing the light-harvesting complexes, in contact with larger S-shaped complexes approximately 10×20 nm in size with protruding central proteins which are dimers of the reaction centre complexes. The two surfaces of the membrane can be distinguished by the extent to which the light-harvesting and reaction centre complexes, respectively, protrude from the membrane surface.

7.6.2.4 Magnetic resonance spectroscopy

Magnetic resonance methods have been used to characterise the polymorphism of membrane lipids as well as provide information on the order, motion, and interaction between lipids in bilayer and other arrange-ments. One of the advantages of nuclear magnetic resonance spectroscopy is that the properties of nuclei with nonzero magnetic moments in natural abundance, such as ^{1}H, ^{13}C,

and ^{31}P, can be examined in biological samples. Alternatively, isotopic labelling or enrichment with suitable isotopes, such as ^{2}H or ^{19}F, that introduce minimal perturbation into the system can also be used to probe molecular motion and configuration in lipid assemblies.

The average conformation of the lipid acyl chains and the polar head groups can be deduced from ^{2}H-NMR methods. Furthermore, specific and nonspecific interactions between molecules in the structure can be characterised. An important parameter that can be obtained is the order parameter representing a measure of the angular distribution of molecules about a preferred molecular orientation. NMR methods exploiting quadrupolar splitting of deuterium nuclei have also been widely used to study lipid polymorphism and the structure of lipids in biological membranes. The versatility of the method relies on the fact that it is possible to substantially substitute ^{2}H for ^{1}H in all domains of the lipid without significantly perturbing the physical properties of the membrane. It is also possible to substitute deuterated water for $^{1}H_2O$. When deuterated lipids are in an anisotropic arrangement the deuterium quadrupolar resonance is split to an extent that depends on the size of the lipid structures and decreases as motional averaging tends to isotropic. It is possible to derive an order parameter from the extent of splitting of the deuterium resonances for particular residues of lipids in different polymorphic phases.

An example of the use of deuterium magnetic resonance methods to define the structure of lipids in a biological membrane is illustrated in Figure 7.32. This shows a ^{2}H-NMR spectra, recorded at 30°C, of a fatty acid auxotroph of *Escherichia coli* grown on ^{2}H-oleic acid together with a spectrum of an aqueous dispersion of a total lipid extract of the cells recorded under the same conditions. A rather broad powder pattern is observed for the deuterons incorporated into the cell membranes with two peaks separated by a frequency, $\Delta\upsilon_q$ of 6.7 kHz and a sharp isotropic signal, which can be assigned to a small amount of residual HOD in the sample. The value of $\Delta\upsilon_q$ is related to the segmental order of the C-D bond and the large intrinsic line width is typical of biological membranes and reconstituted membrane systems. The ^{2}H-NMR spectrum obtained from an aqueous dispersion of a total lipid extract of the cells is characterised by two sharp peaks, separated by 9.9 kHz, which is considerably greater than $\Delta\upsilon_q$ of the lipids in the membranes. The spectrum is typical of lipids in a liquid-crystalline bilayer configuration. The general conclusion from studies of this type is that the lipids in biological membranes are arranged in a bilayer with the motion of the lipids constrained by their interaction with other membrane components.

^{31}P-NMR signals from phospholipid assemblies can be used to characterise the arrangement of the constituent molecules and the particular phase that is formed. The information is obtained from the line shapes of proton decoupled spectra, which indicate chemical shielding

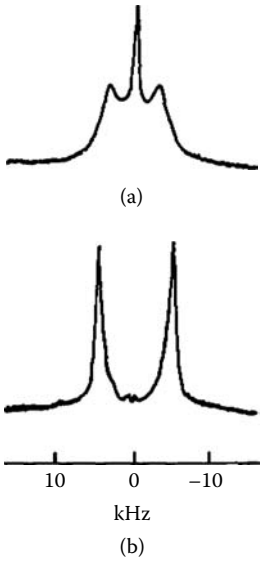


FIGURE 7.32 ^{2}H NMR spectra recorded at 30°C of (a) a suspension of a fatty acid auxotroph of *Escherichia coli* grown on [^{2}H]-oleic acid, and (b) an aqueous dispersion of a total lipid extract from the organism used to record spectrum (a). (Data from Killian, A.J. et al. (1992) *Biochim. Biophys. Acta*, **1105**, 253–262.)

anisotropy of the lipid phosphate moiety. Thus, different orientations of the phosphate segment yield characteristic resonances at different frequencies. In the case of lipids in a bilayer configuration, the ^{31}P-NMR spectrum is typical of a shielding tensor that is axially symmetric around a director axis consisting of a low-field shoulder and high-field peak. Oriented bilayers give rise to individual narrow resonances with an angular-dependent chemical shift. The interpretation of these features is that molecules with their long axis perpendicular to the magnetic field contribute to the high-field peak and molecules with a parallel orientation correspond to resonances at a position in the shoulder region and that the director axis coincides with the bilayer normal about which the phosphate segment rotates.

The rate of reorientation resulting from Brownian motion of lipid molecules in bilayers can also be determined from the line shape. When the rate of reorientation of the phosphate segment is fast, the line shape narrows until eventually, when motion is effectively isotropic on the timescale of the measurement (<10 μs), motional averaging leads to a narrow and symmetric resonance line. Motion of this type is experienced by phospholipid molecules in small, rapidly tumbling bilayer vesicles, micelles, inverted micelles, and a variety of cubic phases. Although there is virtually no difference in the motion of the phosphate segment of phospholipid in bilayer and hexagonal-II arrangement, the macroscopic orientation of the lipid cylinders within the spectrometer magnetic field gives rise to additional averaging of the chemical shift anisotropy that clearly distinguishes this phase from bilayer phase.

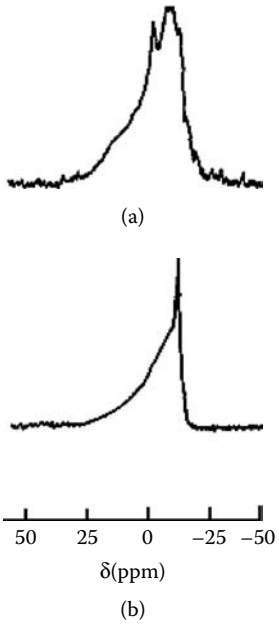


FIGURE 7.33 ^{31}P NMR spectra of (a) a suspension of inner membrane vesicle isolated from a fatty acid auxotroph of *Escherichia coli* recorded at 30°C, and (b) an aqueous dispersion of a total lipid extract from the organism used to obtain spectrum (a) and recorded at 37°C.

The use of ^{31}P-NMR methods to characterise phospholipid arrangement in biological membranes is illustrated in Figure 7.33a. This shows a ^{31}P-NMR spectrum recorded at 30°C of a suspension of inner membrane vesicles isolated from an *Escherischia coli* fatty acid auxotroph. The spectrum is dominated by a single major component characterised by a low-field shoulder and a high-field peak. A minor isotropic component can be seen at zero chemical shift anisotropy. The line shape of the major component is typical of lipids arranged in a liquid-crystalline bilayer configuration in which the chemical shift interaction is averaged by fast, long-axis rotation of the lipids. There is a residual chemical shift anisotropy of 29 ppm. The isotropic component is probably due to the presence of a small population of vesicles in which the lateral diffusion of the lipids and vesicle tumbling result in a complete motional averaging of the chemical shift anisotropy and not from motion of the lipid alone within the membrane. A comparison with an aqueous dispersion of a total lipid extract of the cells is also shown in Figure 7.33b.

This spectrum is characteristic of liquid-crystalline bilayers and indicates a similarity between the phase formed by the lipid in the intact membrane and in the isolated lipid dispersion. The chemical shift anisotropy, however, is increased to 36 ppm. The dispersion, moreover, is not stable and with time or at an elevated temperature a broad isotropic component appears in the signal superimposed on the bilayer signal which gradually disappears altogether with a sharpening of the isotropic signal. This change in phase is likely to be due to the high proportion of nonbilayer forming lipids in the membranes of this organism.

The dynamic properties and composition of phase separated domains created in macroscopically oriented lipid bilayers containing phospholipids and cholesterol have been investigated using ^{1}H, ^{2}H, and ^{19}F pulsed field gradient NMR spectroscopy. In a ternary mixture containing saturated and unsaturated molecular species of phosphatidylcholines and cholesterol, it was found that in a phase separation of liquid-ordered domain the rate of lateral diffusion of the lipids was about fivefold less than those in the surrounding liquid-disordered phase (Oradd et al., 2005).

The affinity of saturated phosphatidylcholines for cholesterol observed in the NMR experiments was said to be less than that for sphingomyelin. This was investigated by electron paramagnetic resonance methods in which a spin label probe was located at different depths within the bilayer (Chachaty et al., 2005). Two distinct environments were reported by the probes in aqueous dispersions of binary mixtures of sphingomyelin and cholesterol; a short-lived nanodomain of condensed complexes and disordered molecular clusters of the two lipids. The disordered clusters are reduced in number with increasing cholesterol in the mixture and eventually disappear when this reaches about 40 mole %. The molecular arrangement of sphingomyelin and cholesterol clusters sensed by the spin-probe located within a leaflet of the bilayer is shown in Figure 7.34. The lines in the diagram refer to the configuration of hydrocarbon chains of sphingomyelin molecules and the shaded disks to cholesterol. The dynamic equilibrium amongst the nanodomains is likely to result from fluctuations in the local concentration of cholesterol which diffuses rapidly in the plane of the membrane.

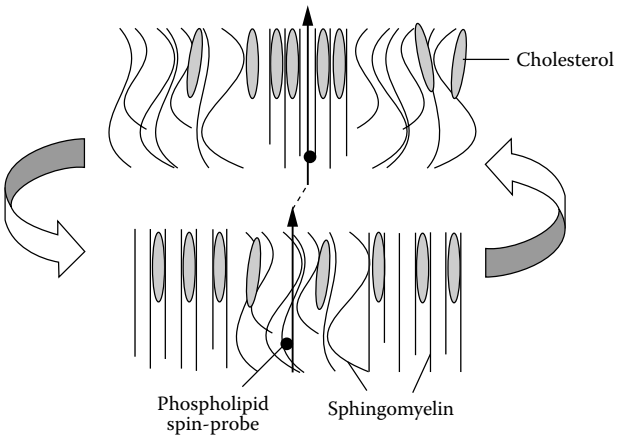


FIGURE 7.34 Molecular model of sphingomyelin-cholesterol bilayer leaflet showing two environments of a phospholipids spin-probe located in an ordered nanodomain (upper leaflet) and disordered nanodomain (lower leaflet). During transition from the highly ordered to poorly ordered environments the probe is displaced by a few nanometers on a time scale of the creation and disruption of the short-lived sphingomyelin-cholesterol condensed complexes.

7.6.3 Transmembrane lipid asymmetry

Biophysical studies have established that the lipid bilayer of biological membranes is fluid and the interior hydrocarbon domain has a viscosity approximating that of olive oil. As detailed in the following section, the polar lipids are able to rotate freely about their axis perpendicular to the plane of the membrane and to diffuse readily in the lateral plane. Movement from one leaflet of the bilayer to the other, however, is severely constrained and is measured in half times of hours or days. The origin of this constraint is the free energy required to move a hydrated polar moiety from the aqueous interface into the hydrocarbon interior of the structure.

As a consequence of this restricted motion, an asymmetric distribution of lipids can be created and maintained across biological membranes. The first evidence that the polar lipids are not randomly distributed between the two leaflets of the membrane lipid bilayer was obtained using reactivity of aminophospholipids to chemical probes, such as formylmethionyl-(sulfonyl)methylphosphate and trinitrobenzenesulfonic acid (TNBS), both of which are impermeant to the membrane. Similar conclusions were drawn from studies of the susceptibility of membrane lipids to hydrolysis by phospholipase A_2 and sphingomyelinase hydrolysis when digestion was restricted to the outer surface of sealed vesicles or when exposed exclusively to the outer surface of the cell membrane. The transmembrane distribution of minor lipid constituents has also been established using lipid-specific antibodies and lipid hydrolases.

The magnitude of the difference can be seen from an analysis of the distribution of lipid classes present in the plasma membrane of cells. Data from the plasma membranes of erythrocytes and platelets, for example, show that sphingomyelin and most of the remaining choline phosphatides are oriented on the outer leaflet of the plasma membrane, whereas the aminophospholipids, mainly phosphatidylserine and phosphatidylethanolamine, are abundant on the inner leaflet (Quinn, 2002). This is illustrated in Figure 7.35, which shows the percentage distribution of the major lipid classes between the cytoplasmic and outer leaflet of the human erythrocyte membrane.

An account of how membrane lipids are distributed throughout the cell and topologically across membranes has been given by Van Meer (2000). An asymmetric distribution of phospholipids is generated during membrane biogenesis. Most of the enzymes responsible for synthesis of phospholipids and their subsequent turnover are located at sites accessible to the cytoplasmic surface of the membrane. Evidence for this has come from various approaches including the action of regulatory factors like c-Fos. c-Fos is an inducible transcription factor that constitutes DNA-binding AP-1 complexes which regulate gene expression responsible for long-lasting cellular changes and act to modulate phospholipid biosynthesis.

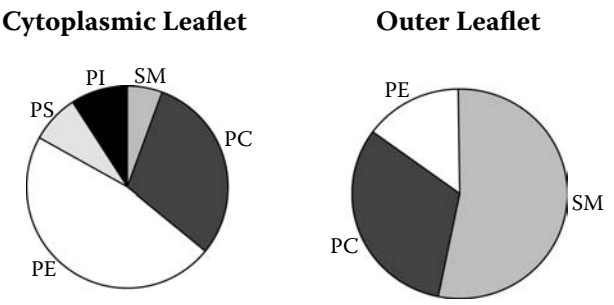


FIGURE 7.35 Percentage distribution of the major phospholipids between the cytoplasmic and outer leaflets of the human erythrocyte membrane. SM, sphingomyelin; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; Pi,phosphatidylinositol.

It has been shown that c-Fos interacts directly with lysophosphatidic acid acyl transferase and phosphatidic acid phosphatase, both key enzymes in phospholipid biosynthesis, on the cytoplasmic surface of the endoplasmic reticulum (Bussolino et al., 2001). The biosynthesis of sphingolipids appears to be an exception to the rule. Thus, sphingomyelin is synthesized on the luminal surface of the membrane in the Golgi phase and glycosidic residues of the glycosphingolipids are also attached to ceramide at this site.

Phospholipid turnover also takes place in an asymmetric manner. The enzymes responsible for phospholipid turnover in response to receptor-mediated phospholipase c activation are active from the cytoplasmic surface of the membrane. Likewise, diacylglycerol kinases converting the product of phospholipase c back into the key intermediate of phospholipid biosynthesis, phosphatidic acid, are also located on the cytoplasmic surface of the membrane (Sanjuan et al., 2001).

Once synthesized several factors influence the particular leaflet of the membrane lipid bilayer where the lipids reside. One is static interactions with intrinsic and extrinsic membrane proteins which, by virtue of their mechanism of biosynthesis, are also asymmetric with respect to the membrane. The interaction of the cytoplasmic protein, spectrin, with the erythrocyte membrane has been the subject of a number of studies. Coupling of spectrin to the transmembrane proteins, band 3, and glycophorin 3 via ankyrin and protein 4.1, respectively, has been well documented (Van Dort et al., 1998).

Interaction of spectrin with membrane lipids is, however, conjectural, but use of a fluorescine derivative of phosphatidylethanolamine has helped to investigate the binding affinity of spectrin to lipid bilayers comprised of phosphatidylcholine or a binary mixture of phosphatidylcholine and phosphatidylserine (O'Toole et al., 2000). It was concluded on the basis of fluorescence intensity measurements that despite possessing a net negative charge at pH>5.6 the surface of the protein interfaced with the lipid

presented a positively charged domain that presumably represented sites of interaction with negatively charged lipids. Nevertheless, the dissociation constants of spectrin for binding to neutral and negatively charged phospholipid interfaces could not be distinguished suggesting a multipoint attachment of the whole protein to the interface with specific domains on the protein preferentially interacting with phosphatidylserine.

Another example of preferential interaction of membrane-associated proteins with acidic phospholipids is dynamin. Dynamin acts at the cytoplasmic surface of the plasma membrane to effect receptor mediated endocytosis. The protein locates at the neck of deeply invaginated coated pits where it induces membrane constriction and the pinching off of endocytotic vesicles. The action is mediated by a conformational change in the protein driven by GTP hydrolysis. Studies of the binding of dynamin to different phospholipid monolayers has revealed that the protein binds very strongly to acidic phospholipids, particularly phosphatidylinositol and phosphatidic acid. The binding to these phospholipids is said to induce penetration of the protein into the hydrophobic domain of the lipid, causing destabilization of the membrane and fission (Burger et al., 2000).

While examples such as these provide evidence of strong interactions of negatively charged membrane lipids with membrane proteins, the role of these interactions in maintaining asymmetric distribution of lipids across biological membranes is unclear. In any event such effects are likely to be of minor importance relative to actively mediated phospholipid translocation processes.

Active translocation of phospholipids across the plasma membrane has been demonstrated both from the inner to the outer leaflet and from the outer to the inner leaflet. The translocation processes specifically transport phosphatidyserine and phosphatidylethanolamine from the outer to the cytoplasmic surface of the membrane, while choline phosphatides are transported from the cytoplasmic to the outer surface. The rate of translocation, in general, is greater for the amino phospholipids compared with the choline phospholipids.

The aminophospholipid translocase is an ATPase II-type enzyme that requires Mg^{2+} and is activated by phosphatidylserine and to a lesser extent by phosphatidylethanolamine and is sensitive to the sulphydryl group reagent, N-ethylmaleimide. Because the enzyme is inhibited by vanadate ions, it is categorised as a P-type ATPase. The properties with respect to substrate specificity, biochemical, and physical properties of the enzyme and strategies for identification and isolation from various sources have recently been reviewed (Daleke, 2003). The protein has a molecular weight of about 116 kDa and has been isolated from the plasma membranes from various sources.

Sequence data of ATPase II from human, mouse, and bovine tissues indicates that the protein has several P-type consensus sequences and a membrane topology that includes 10 putative transmembrane domains. An ATP binding site and a phosphoenzyme formation site are located within the largest cytosolic loop, whereas a sequence implicated in the coupling to transport activity was identified in another hydrophilic loop. The sequence of mammalian ATPase II is homologous to a yeast ATPase encoded by the *drs2* gene, of which the null mutant of a yeast strain lacks a specific phosphatidylserine internalization activity that is otherwise present in wild type yeast strains. Four isoforms of the ATPase II enzyme have been identified in bovine brain. The substrate specificities and selectivities were characterized and the results are summarised in Table 7.4.

This shows relative specificities of phosphatidylcholine and phosphatidylethanolamine compared with phosphatidylserine for activation of the ATPase by different isoforms of recombinant ATPase II. It was found that there was no significant difference between the presence or absence of phosphatidylcholine suggesting that this phospholipid is not an activator of ATPase activity. The ratio of activation phosphatidlyserine/phosphatidylcholine shown in the table is a reliable indication of the relative specific activity of the different ATPase II isoforms. The activity is in the order $\alpha2 > \alpha1 > \beta2 > \beta1$. A different order is observed in relative selectivity of phosphatidylserine compared with phosphatidylethanolamine in which the order is $\alpha2 > \beta2 > \alpha1 > \beta1$.

The involvement of Drs2 protein in the transbilayer movement and distribution of phospholipids in the plasma membrane of the *S. cerevisiae end4*Δ mutant in which both growth and the internalization step of endocytosis are blocked at a restrictive temperature of >34°C has been investigated (Pohl et al., 2005). It is known that several proteins, such as ATP-binding cassette multidrug transporters Pdr5 and Ste6, accumulate in the plasma membrane of the yeast after incubation under nonpermissive conditions and it was thought that this may have consequences for the organization and dynamics of the plasma-membrane lipid phase. It is known that the Ste6 protein transports synthetic alkyl phospholipids, but not whether the protein-mediated transbilayer movement and the transbilayer asymmetry of lipids in the plasma membrane of the *S. cerevisiae end4*Δ mutant are altered. The transbilayer movement of fluorescently labelled analogues of choline and serine phosphatides has been measured in an *S. cerevisiae end4*Δ*drs2*Δ strain, to determine whether

TABLE 7.4 Relative phospholipid specificities for activation of different isoforms of recombinant ATPaseII

Relative Specificity	α1	α2	β3	β4
PS/PC	53.0	68.0	25.0	32.5
PS/PE	2.7	10.9	1.3	3.5

PS, phosphatidylserine; PC, phosphatidylcholine; PE, phosphatidylethanolamine. *Source:* Data from Ding, J., et al. (2000), *J. Biol. Chem.*, **275**, 23378–23386.

internalization of analogues by transbilayer movement depended on a functional *drs2* gene in endocytosis-deficient yeast cells. It has been shown that exposure of endogenous aminophospholipids to the exoplasmic leaflet of the mutant cells is altered with respect to wild-type cells.

There is also a family of membrane P-glycoproteins involved in transmembrane phospholipid movement. The translocase responsible for nonspecific movement of phospholipid from the cytoplasmic leaflet to the cell surface has been shown to be identical to the multidrug resistance protein-1. The proteins appear to translocate particular phospholipids on different cell types (Sugawara et al., 2005). Sphingolipids, for example, are preferentially transported across the plasma membrane of LLC-PK1 kidney epithelial cells transfected with MRP1 cDNA, but phosphatidylcholine was the preferred phospholipid translocated by MRP3 in bile canicular cells where the protein predominates in the plasma membrane of fibroblasts of transgenic mice.

The loss of membrane phospholipid asymmetry is known to be an important signalling mechanism as it results in the appearance of specific phospholipids on opposite sides of the membrane, a situation that triggers a variety of cellular responses (Zwaal et al., 2005). A family of membrane proteins referred to as phospholipid scramblases (PLSCR) have been implicated in dissipating phospholipid asymmetry in a process that depends on elevated cytoplasmic calcium concentration. Four genes have been identified that code for phospholipid scramblases in human and mouse and all have been conserved through evolution. The amino acid sequence deduced for one of the human phospholipid scramblases, HuPLSCR1, indicates that the scramblase is a Type-2 membrane protein of molecular weight 35 kDa (318 amino acid residues) with a transmembrane helical domain located towards the C-terminus (Wiedmer et al., 2000).

The N-terminal cytoplasmic domain was found to possess a putative phosphorylation site and a calcium-binding segment. There is evidence that regulation of scramblase activity may be mediated by phosphorylation in the presence of PKCδ, the action of which results in the surface exposure of phosphatidylserine in apoptosing cells (Frasch et al., 2000). Regulation of scramblase activity and its localization in the nucleus may also occur via palmitolylation at cysteine thiol residues of the protein (Ben-Efraim et al., 2004).

7.6.4 Membrane lipid dynamics

With the notable exception of lipids in the purple membrane of *Halobacterium*, which adopt more or less a crystal structure, the lipid matrix of biological membranes is said to be fluid. The term fluidity is not a precise quantitative parameter, but it implies that the molecules of the structure exhibit motion with respect to one another. The measurement of this motion is often performed by the use of membrane lipid analogues that incorporate para-magnetic, fluorescent, or other probes and it is important to recognise that it is the motion of the probe that is observed and not the native molecules. It is not unknown for probe molecules to exert a considerable perturbation on the motion and structure of the surrounding lipids or to behave in a manner distinctly different from the lipids they are designed to mimic.

Another problem that also has to be addressed is the need to introduce the probe into the membrane without disturbing the structure. The most common method is to incubate cells or membrane suspensions in a medium into which a small aliquot of lipid probe has been injected from a concentrated solution in organic solvent, such as ethanol. Alternatively the probe may be co-dispersed with unlabelled phospholipid or mild detergent. The strategy of choice often depends on whether the probe increases or decreases the hydropathy of the lipid analogue compared to the native lipids, which comprise the membrane. Possibly the least perturbing method is the use of phospholipid transfer proteins to perform the task, but this method relies on close physical resemblance between the analog and the phospholipid for which the protein has affinity.

Nevertheless, with these restrictions in mind the general picture that has emerged is that lipid molecules are relatively mobile within the membrane and constraints on this motion occur by interactions with the proteins and other membrane constituents.

7.6.4.1 Motion of lipids in membranes

The two methods that have been exploited to greatest effect to examine lateral diffusion of lipids in the plane of biological membranes and from one leaflet of the membrane lipid bilayer to the other are electron paramagnetic resonance and fluorescence probe techniques. A range of probe molecules have been synthesized as analogs of membrane lipids. One example is the synthesis of Lucifer yellow derivatives of phospholipids and cholesterol, which have been introduced into human erythrocyte ghost membranes and living melanoma cells to measure lateral diffusion rates. The rates of diffusion were then compared with diffusion in phospholipid bilayer membranes.

Lateral diffusion rates were obtained using a spot fluorescence photo-bleaching recovery method. The method involves bleaching of probe molecules in a defined surface area of membrane using an intense pulse of laser light and monitoring recovery of fluorescence as unbleached probe molecules from the surrounding area of membrane diffuse into the bleached domain. It was found that Lucifer yellow derivatives of cholesterol and phosphatidylethanolamine diffused rapidly with a diffusion coefficient >1 $\mu m^2\ s^{-1}$ in lipid dispersions at temperatures greater than the gel to liquid-crystalline phase transition temperature, but the diffusion rate decreased dramatically for probes in gel

phase lipid (<0.01 $\mu m^2\ s^{-1}$). Intermediate values of diffusion coefficient were observed in erythrocyte ghost membranes and in the plasma membrane of live melanoma cells. In the latter case only approximately half the probe molecules were identified in the mobile fraction indicating that motion of the probe is restricted by interaction with other membrane components. The order of diffusion coefficients recorded for these probes is similar to that of other fluorescent lipid probes in mammalian plasma membranes.

Translational motion of lipid can also be determined from bimolecular collision frequencies that can be measured by perturbation of the signals derived from probe molecules. Such methods include fluorescence quenching or excimer formation for luminescent probes and spin-spin interactions between spin-labelled probes.

It is clear from these observations that the diffusion of lipids does not take place through a homogeneous lipid bilayer matrix and there is evidence from photoactivatible fluorescent probes of a nonhomogeneous distribution of lipids within the plane of the membrane. Studies of lateral distribution of lipids in the bacterium *Micrococcus luteus*, for example, have been undertaken using a reversible photo cross-linker, anthracene phospholipid analog (de Bony et al., 1989). The results showed that the two major polar lipid components, phosphatidylglycerol and dimannosyldiacylglycerol, are not homogeneously distributed in the plane of the membrane. The lateral diffusion coefficient of about 0.1 to 0.2 $\mu m^2\ s^{-1}$, is in line with the above measurements.

In addition to translational motion, membrane lipids undergo rotational motion about their long axes perpendicular to the plane of the membrane as well as motion within the molecule. The latter involves rotational motion of the polar group and *trans-gauche* isomerisations of the hydrocarbon chains. Rotational diffusion rates can be determined from decay of polarisation of fluorescence of probe molecules excited by plane-polarized light. The lifetime of the excited fluorescent state is in the order of a few nsec, which is appropriate to the rotational relaxations of lipids in biological membranes. Both time-resolved and steady-state depolarisation methods have been used in these measurements.

A novel fluorescence method has been reported recently (Benninger et al., 2005) in which fluorescent probes are located on the cell surface or within the membrane and their absorption anisotropy used to provide information on orientational constraints within their local environment. The method is able to detect changes in lipid organization in cell membranes on time scales faster than 1 sec. One of the results obtained showed that the presence of cholesterol in the cell membrane exerts a considerable ordering effect on the surrounding lipids. The depletion of cholesterol from the membrane had little effect on the orientation of the molecules in the surface region, but was associated with a marked transition to a more disordered environment in the hydrocarbon domain.

Information regarding molecular rotational motion can also be derived from motionally induced modulation of the angular anisotropy of the interaction in the magnetic resonance spectra. The time-scale of the motional sensitivity depends on the frequency equivalent of the anisotropic interactions that are being modulated. For spin-probes, this lies in the nsec range and for nuclear magnetic resonance it covers the time-domain from 1 to 100 msec.

7.6.4.2 The motion of membrane proteins

The mode of synthesis of membrane proteins and the way they interpolate into the lipid bilayer matrix ensures that they retain a unique orientation with respect to the membrane. The location of intrinsic proteins within the bilayer or the tethering of lipid-anchored proteins to the surface imposes constraints on their motion. An important consequence of the fluid nature of the membrane lipid matrix is that the intrinsic proteins are able to diffuse laterally in the plane of the membrane and rotate about an axis perpendicular to this plane. This motion is often demonstrated to be crucial to the functions they perform.

While on theoretical grounds the rate of lateral diffusion and rotational motion of proteins should be related inversely to the viscosity of the lipids, this is not generally the case in living cells. Indeed the rate of diffusion for most proteins is of the order of 10 to 100 times slower than predicted solely from the lipid viscosity. The reason for this is thought to be confinement of membrane proteins within membrane microdomains, corrals formed from membrane-cytoskeleton interactions, and protein density-dependent effects.

The rate of rotational diffusion of membrane proteins can be determined by the time-resolved technique of flash photolysis (phosphorescence anisotropy) or time-averaged saturation transfer electron paramagnetic resonance (Cherry, 2005). Solid-state NMR methods have also been used to measure rotational motion of intrinsic membrane proteins incorporated into magnetically aligned lipid bilayers (Park et al., 2005). There is general agreement between all these methods that the factor constraining protein rotational motion is the viscosity of the lipid domain in which the protein is embedded.

One protein that has been examined by both saturation transfer electron spin resonance and flash photolysis is the Ca^{2+}-ATPase of sarcoplasmic reticulum (Squier et al., 1988; Birmachu and Thomas, 1990). The pump protein exists in the membrane in the form of monomers rotating independently about an axis perpendicular to the plane of the membrane in equilibrium with aggregates of more than one pumping unit. Increasing the viscosity of the aqueous medium in which the membrane vesicles were suspended had no effect on rotational relaxation rates of the protein, whereas decreasing the viscosity of the lipid with addition of diethylether markedly increased the rate of rotation of the protein. It was also reported that there

was a tendency of the protein to aggregate as the viscosity of the lipid increases.

Variation of membrane lipid viscosity with temperature (7.7 P at 4°C, cf. 2.6 P at 20°C) was reflected in rotational rates of the Ca^{2+}-ATPase monomers and calculation of the effective radius of rotating protein gave the same value (2.3 nm) at both temperatures. It follows that, in the absence of constraints on rotation of intrinsic membrane proteins by interaction with other proteins such as the cytoskeleton, the rotational motion about the axis perpendicular to the plane of the membrane is directly related to the viscosity of the lipid.

The fluorescence recovery after the photo-bleaching method described above for the measurement of lateral diffusion of lipids can be applied equally to the measurement of protein diffusion. The bleached region of membrane into which unbleached molecules from the surrounding membrane diffuse need not be in the form of a spot and a variety of bleaching patterns have been employed according to the particular aspect of the structure or dynamics of the membrane system under examination. Periodic pattern bleaching, for example, can be used to measure dynamics over large areas of one or more regions. Spot or localized line bleaching on the other hand yields spatially resolved information in which comparisons can be made between the properties of different lateral domains and different locations within the same region. Diffusion rates can also be obtained from single particle tracking methods. Typical lateral diffusion rates for proteins in biological membranes occur in the range of 0.01 to 0.0001 $\mu m^2.s^{-1}$. This rate is some orders of magnitude slower than values recorded for soluble proteins diffusing in aqueous media and again reflects the effect of membrane lipid viscosity on lateral diffusion rates of membrane proteins.

The lateral diffusion rates of both intrinsic membrane proteins and GPI-proteins located on the cell surface have been detected using fluorescently labelled antibodies to the specific protein or chimeric proteins incorporating green fluorescent protein. More recently, the diffusion rates of the small GTPase, Ras, a lipid anchored enzyme on the cytoplasmic surface of the cell membrane, has been investigated (Goodwin et al., 2005). This protein is anchored to the inner cytoplasmic leaflet by attachment to a farnesyl group in conjunction with either an S-acyl group (HRas and NRas) or a polybasic domain (KRas). The function of the lipid anchor is to target the protein to specific membrane microdomains, e.g., KRas to the fluid domains, HRas and NRas to cholesterol-rich domains. The enrichment of the cellmembranes of living cells with cholesterol caused a significant reduction of diffusional rates of all the Ras isoforms, whereas depletion of membrane cholesterol by growth in lipoprotein-deficient medium resulted in increased diffusional rates.

Many proteins are required to traverse membranes in passage from their site of synthesis to their ultimate destination within the cell. Some proteins synthesized in the cytoplasm, for example, are membrane proteins of the mitochondria or chloroplast and must cross the outer membrane or envelope of the respective organelles. Chaperone proteins are often involved, but fluidity of the lipid matrix is an essential factor in the process. Other examples of protein movement with respect to the lipid bilayer matrix include toxins of various types. An analysis of the topography of diphtheria toxin protein has been reported in which changes in the location of the protein in the lipid bilayer are induced by pH. The toxin is secreted by the bacterium *Corynebacterium diphtheriae* and is comprised of two polypeptide chains, A and B, linked by a single disulfide bond. There are three recognizable domains in the protein, the catalytic domain of the A chain and the receptor and transmembrane domains of the B chain. The toxin is taken up into the target cell by receptor-mediated endocytosis and is subsequently exposed to acid conditions in the endosome compartment. This apparently triggers a conformational change in which the protein becomes more hydrophobic allowing the A chain of the toxin to penetrate the lipid bilayer and gain access to the cytosol. This step initiates the catalytic ADP ribosylation of the diphthamide residue of elongation factor 2, thereby arresting protein synthesis and death of the cell. Changes of the topography of the transmembrane domain of the B subunit have been modelled by following the reactivity of different residues on exposure to a pH gradient (Figure 7.36; *cis* upper side pH5; *trans* lower side pH7) (Rosconi et al., 2004).

The model shows that transmembrane domain consists of 9 helical segments, two of which (8 and 9) form a transbilayer helical loop and three segments (5 to 7) are deeply embedded in the hydrophobic region of the lipid bilayer. The catalytic site on the A chain, which is attached to the N-terminus of the transmembrane domain, is translocated across the bilayer in response to exposure to a pH gradient. The catalytic domain is formed from two β-sheet subdomains oriented approximately perpendicular to one another and linked by four extended loops, which allow a partial unfolding of the structure during membrane translocation.

7.6.4.3 Gel-fluid phase separation in membranes

The molecular species of cell membrane lipids separate into different phases as a function of their melting points. At lower temperatures, lipids are quasi-solid, corresponding at the molecular level to a gel in which they are virtually immobile with their chains fully extended and closely packed in hexagonal array. At temperatures above their melting temperature (T_M), the acyl chains of the lipids are fluid, corresponding at the molecular level to being loosely packed; the lipids themselves are relatively mobile, both in terms of the ability of different lipids to diffuse along the plane of the bilayer and flexibility of their hydrocarbon chains.

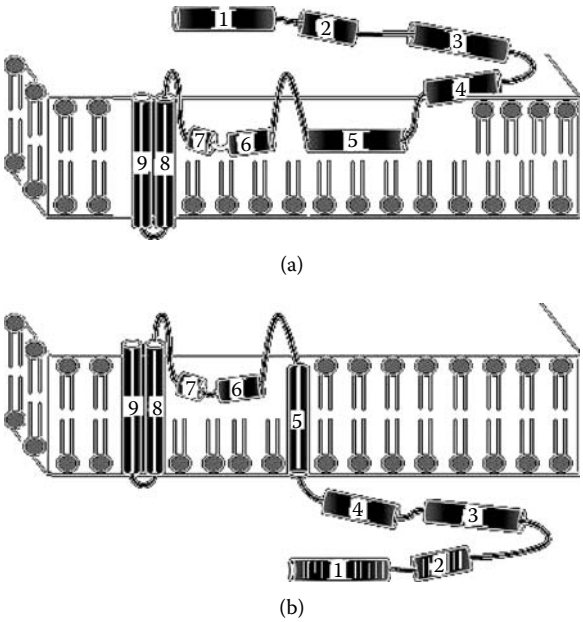


FIGURE 7.36 Molecular models of the transmembrane domain of the A chain of diphtheria toxin during translocation across the lipid bilayer. (a) Helical segments 1–4 are initially located on the *cis* side (low pH) with segments 5–9 associated to different extents with the lipid bilayer. (b) Helical segments 1–4 are translocated across the lipid bilayer to ultimately reside on the *trans* side (pH7) of the membrane.

While all lipids undergo this transition, they do so at very different temperatures: unsaturated lipids remain liquid until near or below 0°C, whereas saturated lipids are in the liquid state only at or above mammalian body temperature (37 to 45°C or, for saturated glycosphingolipids, even higher, at 60 to 70°C). The polar head group also influences T_M. For example, for equivalent hydrocarbon chain composition the T_M of phosphatidylethanolamines are more than 20°C higher than the corresponding phosphatidylcholines. Biological membranes are comprised of an ensemble of molecular species of lipids, some in pure form will be above T_M others below. A consequence of this is that there will be a tendency for the lipids that are below T_M at a particular temperature to phase separately into a gel phase domain leaving the remaining lipids to form a separate fluid phase.

An example of a gel phase separation in biological membranes is illustrated above in Figure 7.31 in which the blue-green alga, *Synecoccus*, was cultured at a temperature of 38°C and thermally equilibrated at 15°C before thermal quenching. Cooling the cells to 15°C caused a lateral phase separation of the membrane-associated particles and the creation of large domains of smooth fracture face, both in the plasma membrane and in the photosynthetic membranes of these organisms. The smooth regions are believed to represent gel-phase lipid domains from which the intrinsic membrane proteins have been excluded. In control experiments, where cells were thermally quenched from the growth temperature and also in cells grown at low temperatures and thermally quenched from 15°C, a random distribution of membrane-associated particles was observed and this correlates with changes in the lipid composition of the membrane. Thus, the membrane lipids possess fatty acyl residues that are considerably less saturated at low growth temperatures compared to when the organism is grown at higher temperature.

The question as to which lipids are located in the phase separated domains and which lipids phase separate together with the proteins has been investigated by differential scanning calorimetry, low-angle x-ray diffraction, fluorescence probe techniques, and freeze-fracture electron microscopy (Mannock et al., 1985). The results of the calorimetric study are illustrated in Figure 7.37. This shows that organisms cultured at 28°C exhibit a broad endotherm in their heating scans and a midpoint temperature of 14°C, which presumably corresponds with the transition of gel phase lipid domains into a liquid-crystalline configuration. A similar endotherm is observed in cells cultured at 38°C although judging from the enthalphy of these transitions the amount of gel-phase lipid separating out in cells cultured at 38°C is greater than in the corresponding membranes of cells cultured at 28°C. There is, however, a dramatic change in the midpoint temperatures of the endotherms observed in heating thermograms of total polar lipid extracts of organisms

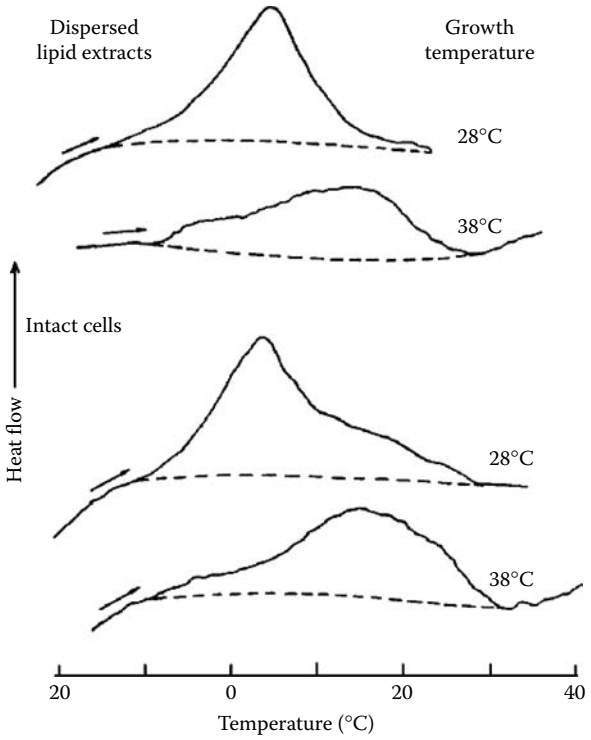


FIGURE 7.37 Differential scanning calorimetric heating curves recorded from *Synococcus* cells grown at two temperatures and total polar lipid extracts of cells dispersed in aqueous medium.

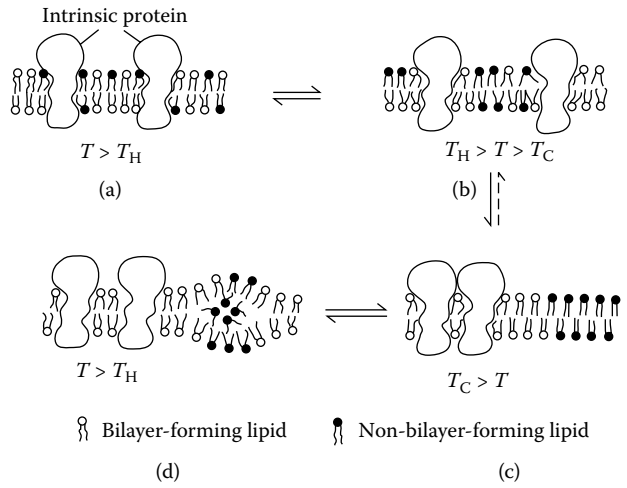


FIGURE 7.38 Illustration of the lipid phase separation model for low temperature to cell membranes. The scheme shows the consequences of cooling from the growth temperature (a) to a temperature below the hexagonal-II to liquid-crystalline phase transition temperature of the nonbilayer forming lipids (b) and subsequently below the gel to liquid-crystalline phase transition of all the membrane lipids (c). The effect of reheating to the growth temperature is shown in (d). (Data from Quinn, P.J. (1985), *Cryobiology*, **22**, 128–146.)

cultured at 28°C and 38°C that occur at 2° and 4°C, respectively. This observation clearly shows that it is the high melting point lipids that phase separate from the membrane proteins in the intact biological membrane. The question remains as to which lipids have higher melting points and which lipids have low melting points. This has important implications with regard to the ability of the membrane to restore a random distribution of components after thermally induced phase separations. It is well known, for example, that the phase separations of the type observed in *Synococcus* result in irreversible changes and loss in viability of the cells, which are unable to repair their leaky membranes.

It is seen that the lipid composition of *Synococcus* is relatively simple, and appreciable proportions of the membrane lipids undergo phase transitions over a relatively narrow range of temperatures. With more complex mixtures, typical of many biological membranes of higher organisms, the transition endotherms observed on heating membranes previously cooled to low temperatures exhibit transitions that are invariably broad and extend over tens of degrees. Attempts have been made to resolve these broad transitions in erythrocyte membranes and lipid mixtures simulating the membrane lipid composition into components contributed to by each of the major lipid classes present in the membrane. The results of such experiments using human erythrocyte phospholipids have shown that the phase separation of the sphingolipids dominated the higher temperature phase behaviour of the outer leaflet of the membrane, and the phosphatidylethanolamines appear to phase separate initially in the inner leaflet upon cooling the membrane.

The T_M of membrane lipids is also a function of hydration and complex lyotropic mesomorphism of these lipids has been well characterised. Such effects in creating phase separations in membranes has been described. For example, particle-free domains in the plasma membrane of rye protoplasts have been shown to be associated with freeze-induced dehydration injury to these cells. It is thought that close apposition of the plasma membrane with the underlying subcellular membranes causes the exclusion of intrinsic membrane proteins. Irreversible injury to the cells is sustained when the phase-separated lipid forms a nonlamellar phase consisting of aggregates of tubular inverted micelles of lipid.

The phase separation of nonbilayer forming lipids also forms the basis of a lipid-phase separation model to account for the irreversible damage to membranes when cells are exposed to low temperatures. The model provides a molecular explanation for the consequences of phase separation of components within the membrane and is illustrated in Figure 7.38. It shows that when membranes are cooled from the growth temperature, the first transition that the membrane lipids will be subjected to will be a hexagonal-II to liquid-crystalline lamellar phase transition of those lipids that tend to form nonbilayer phases at the growth temperature. It is believed that intrinsic membrane proteins impose a lamellar configuration on these lipids at the growth temperature consistent with the role of these lipids in packaging of proteins and sealing them into the lipid bilayer matrix. When this constraint is no longer imposed and in the absence of any specific interactions between the proteins and the nonlamellar lipids, it is suggested that the lipids diffuse to form a homogeneous fluid bilayer of lipids in the absence of any small component of lipid, which is below the gel to liquid-crystalline phase transition temperature. Such lipids may form small domains of gel-phase lipid in the fluid membrane matrix.

There is, as yet, no direct information on the extent to which bilayer and nonbilayer-forming lipids may be heterogeneously distributed within membranes, but where this is due to a tendency of the particular lipids to form nonbilayer structures this constraint would be expected to be removed on cooling below the hexagonal-II to lamellar-phase transition temperature. With the possible exception of membranes subjected to chilling damage, such as those described above for *Synococcus*, this type of phase separation is not likely to cause irreversible damage to membranes and any heterogeneity may be restored on reheating to the growth temperature. Further cooling results in transitions from liquid-crystalline to gel phase beginning first with the higher melting point lipids, which, as we have seen, will segregate into domains of pure gel-phase lipid from which the intrinsic membrane proteins and low melting point lipids are excluded.

The central argument of the lipid phase separation model of irreversible damage to biological membranes is

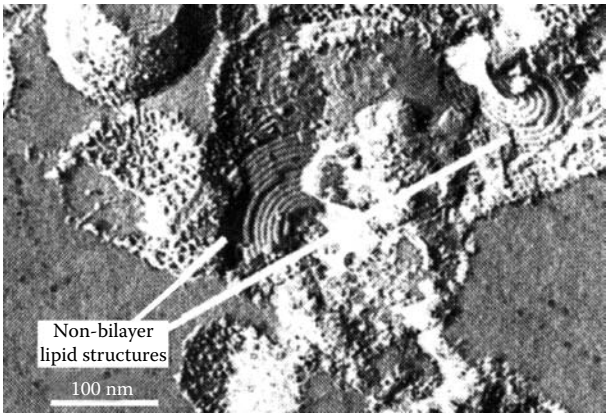


FIGURE 7.39 An electron micrograph of a freeze-fracture replica prepared from bean chloroplasts heated for 5 min at 55°C before thermal quenching.

that the higher melting point lipids present in gel-phase domains will be rich in the hexagonal-II-forming lipids. This assumption is based on the fact that, with equivalent hydrocarbon substituents, lipids that tend to form hexagonal-II structures have liquid-crystalline to gel-phase transitions at temperatures that are considerably higher than those corresponding to bilayer-forming lipids. Where phase separations between lipid classes of this type are created, the changes are not likely to be reversed on reheating to temperatures above the hexagonal-II to lamellar-phase transition temperature. In practice, it would be improbable for there to be a single temperature where all the nonlamellar lipids would co-exist together with the bilayer-forming lipids in the fluid phase, allowing the original distribution of the lipids and the proteins to be restored. Damage to the membrane in these circumstances would be expected to result when the membrane is reheated to temperatures where the domains of the phase-separated hexagonal-II-rich lipids tend to form nonbilayer structures. The creation of nonbilayer structures, such as inverted lipid micelles, unless suppressed or dealt with by normal homeostatic mechanisms operating within the membrane, would serve to destroy the permeability barrier properties of the membrane. If this breakdown of membrane barrier is of sufficient duration to permit loss of essential components or irreversible alterations in the intracellular compartmentation, loss of cell viability will result.

Phase separations of the constituents of biological membranes can also be driven by exposure of membranes to salts or pH that screen charges on the acidic phospholipids as well as to high temperatures. The structural changes in thylakoid membranes of higher plant chloroplasts subjected to thermal stress is illustrated in Figure 7.39. Chloroplasts maintain a normal morphology during a brief exposure (5 min) to temperatures up to 35°C. Incubation at temperatures of between 35° and 45°C causes complete destacking of the grana and incubation at higher temperatures causes a phase separation of nonbilayer lipids into stable aggregates of cylindrical inverted micelles. Interpretation of the effects of temperatures greater than 45°C is based on phase conditions that result in a release of the constraints imposed by interaction of the major nonbilayer-forming lipid of the membrane, monogalactosyldiacylglycerol, with other membrane components and its segregation into domains of nonbilayer lipid structure. Gross phase separations of this type require that the shift in thermal stability of the stacked membrane is relatively large because three-dimensional aggregates of lipid are not observed if the chloroplast membrane is destacked by manipulation of the ionic environment before heat treatment.

It has been suggested that a shift in the phase of the nonbilayer lipid, monogalactosyldiacylglycerol, underlies these structural changes and that the functional role of this nonbilayer lipid may be to package the light-harvesting chlorophyll a/b-protein complexes together with the photosystem-II core protein complex into an efficient functional unit localised within the grana stack.

All membranes contain molecular species of lipid that at physiological temperatures do not form bilayer structures. These include phosphatidylethanolamine, monoglycosyldiacylglycerol, and cardiolipin. While it is generally assumed that such lipids are constrained into a bilayer arrangement by interaction with other membrane constituents, their phase separation to form inverted micelles is an event that is thought to be associated with membrane fusion. Recent NMR evidence, however, has identified lipids undergoing isotropic motion in intact cell membranes that are said to be associated with raft domains (Ferretti et al., 2003). Motion of this type may be a feature of the boundary region separating fluid from liquid-ordered domains where packing faults and molecular mismatches are expected to occur.

7.6.4.4 Domain creation by lipid–lipid interactions

Great emphasis has more recently been placed on lipid phase separations that are brought about by the association between particular membrane lipids to create lateral domains within the bilayer. The phase separation in these instances is driven by the order created in the bilayer by the formation of complexes. Complexes have been described between phosphatidylcholine and diacylglycerol, phosphatidylcholine and α-tocopherol, phosphatidylethanolamine and glucosylceramide, and choline phosphatides and cholesterol. The ordered phase in each case depends subtly on the molecular species of lipid involved in the complex and the extent of order created within the phase-separated domains again depends on the molecules involved.

Diacylglycerol accumulates transiently in cell membranes as a consequence of phospholipase C-type enzymes activated by a variety of hormones, growth factors, and neurotransmitters. The amount of diacylglycerol formed

can reach 2 mol% in some physiological situations and the resulting change in membrane lipid composition represents a molecular signal transducing mechanism responding to the interaction of agonists with their respective cell surface receptors. Structural changes in the lipid bilayer have been detected in phospholipids containing diacylglycerol and these physical perturbations may be responsible for the molecular signal, such as activation of protein kinase C or to enhance membrane fusion processes.

Diacylglycerols when codispersed with disaturated phosphatidylcholines in molar ratios of up to 30 mol% create lateral phase separated domains of a stoichiometric 1:1 complex of diacylglycerol and phosphatidylcholine within bilayers of pure phospholipid (Quinn et al., 1995). It may be expected that physiological proportions of diacylglycerol may result in lateral phase separations in small domains within membranes. The domains created by the demixing process tend to destabilize the phospholipid bilayer. The presence of much higher, and unphysiological, proportions of diacylglycerols (greater than 30 mol%) is known to induce nonbilayer phases such as hexagonal-II (H_{II}) and cubic phases in disaturated phosphatidylcholines.

The transient nature of lamellar phase complexes between diacylglycerol and membrane phospholipids is necessary in the process of switching off the signal generated by the formation of diacylglycerol. This is accomplished by diacylglycerol kinase, which converts the substrate into phosphatidic acid. Control is presumably exercised by the molecular species of diacylglycerol that are generated from the parent phospholipid. Studies on the effect of acyl chain length of diacylglycerol on activation of protein kinase C has shown that diacylglycerol with shorter hydrocarbon chains (C6 to C10) are more effective than molecular species containing longer acyl chains. The miscibility of the short-chain diacylglycerols with the phospholipid in liquid-crystalline bilayers could be the explanation for the high activity of the short-chain diacylglycerol. By contrast, longer chain diacylglycerols form complexes with the phospholipids, which exist in gel phase at temperatures higher than the fluid phase transition of the phospholipid and are unable to activate protein kinase C. Complex formation has been characterised in binary mixtures of both dipalmitoylphosphatidylcholine/dipalmitoylglycerol and dimyristoylphosphatidylcholine/dimyristoylglycerol.

In terms of the effect of diacylglycerol on domain formation in membranes it is clear that this is highly dependent on the molecular species of diacylglycerol and the degree of unsaturation of the polar lipids comprising the membrane lipid bilayer matrix. Interaction and complex formation between saturated molecular species may generate liquid-ordered domains, whereas interaction between unsaturated molecular species tends to destabilize the bilayer in favour of the formation of inverted phases that result in repackaging of intrinsic proteins or membrane fusion.

Diacylglycerol generation from proteolipid complexes containing polyphosphoinositol lipids by intranuclear phospholipase C is a recognised signalling pathway in the control of gene expression (Martelli et al., 2003). These complexes have been localized to interchromatin granule clusters by immunostaining with monoclonal antibodies in preparations that have been extracted with cold Triton-X100. On this basis it is said that the complexes do not originate from the membrane bilayer lipid matrix of the nuclear envelope; however, the possibility that they represent detergent-resistant membrane domains of the envelope cannot be discounted on the present evidence.

The consequence of the presence of ceramide in membranes is dominated by its tendency to self assemble into rigid bilayer structures especially in the presence of sphingomyelin and cholesterol. This has led to the suggestion that ceramides play an important role in membrane signaling processes, membrane fusion, and permeability of solutes through bilayers (Goni et al., 2005). Evidence that is consistent with these functions is that ceramides are recovered in significant amounts in detergent-resistant membrane fractions even from cells in an unstimulated state. Ceramide domains are more hydrophobic than the parent sphingolipid domains, promoting membrane fusion. In relatively minor proportions (3 to 6 mol%), ceramide synergises with cholesterol in stabilising ordered lipid domains. Ceramide alone is able to promote order in the lipid chains of phosphatidylcholine, even with monounsaturation in the *sn-2* chain, and it partitions into liquid-ordered domains with an affinity markedly higher than that of other (fatty acid-matched) sphingolipids (Wang, 2003).

The generation of ceramide in membranes takes place at different subcellular sites. *De novo* biosynthesis occurs at the endoplasmic reticulum, whereas at the plasma membrane ceramide is derived by hydrolysis of sphingomyelin and complex sphingoglycolipids. The hydrolytic enzymes responsible for production of ceramide at the plasma membrane are designated by their pH optima into acid and neutral sphingomyelinases. Cellular modulators of ceramide production include diacylglycerol and other protein kinase C activators and serine proteases. Degradation of ceramide by ceramidases is enhanced by a variety of agents including cytokines, cell differentiating agents, death receptor ligands, cancer chemotherapeutic agents, and ionizing radiation (Jaffrezou et al., 2002).

It has been proposed that the cellular response to ceramide elevation or depletion depends directly on where the ceramide is located (Blitterswijk et al., 2003). According to this view ceramide in the Golgi influences biosynthetic pathways in formation of sphingolipids and their transport in vesicles to the plasma membrane where they promote domain formation. Clustering of receptors on the cell surface, promoted by rafts, induces endocytosis in

response to transient ceramide formation. Finally, changes in membrane permeability associated with the action of ceramidases on mitochondrial and plasma membrane are said to mediate apoptotic events.

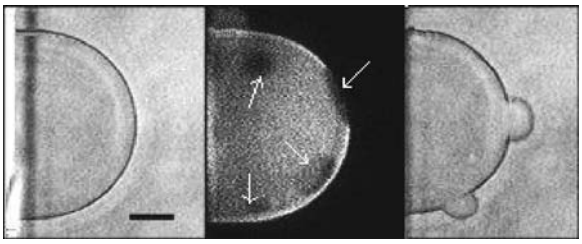
Ceramides are known to be one of the main mediators of apoptosis in cells. Increasing the levels of ceramide accumulating during inhibition of ceramidases, which convert ceramide to sphingosine and free fatty acid, results in cell death. Likewise, inhibition of ceramide production by blocking the *de novo* synthesis pathway or inhibiting neutral sphingomyelinase activity slows down apoptosis in response to a variety of factors including chemotherapeutic agents, tumor necrosis factor-ß, angiotensin-II, and B-cell activation (El Bawab et al., 2002).

Perhaps the most studied of the complexes is that between choline phospholipids and cholesterol. Cholesterol interacts with lipids containing long-chain ($\geq$C14) saturated fatty acids in a manner dominated by van der Waals interactions. In order for van der Waals' forces to operate, the sterol and lipid must fit closely. This steric constraint requires of the sterol that it have the 3-OH head group, the sterol rings must be planar, the hydrocarbon tail must be of the appropriate length, and it should be the natural enantiomer (Miao et al., 2002). On the phospholipid side, the primary requirement is for long, saturated lipid chains; in particular, *cis* double bonds are poorly tolerated in that part of the fatty acid chain (approximately the first 12 carbons) that interacts with the planar sterol rings. Glycerolipids, made in the endoplasmic reticulum, often have 1 to 4 double bonds in this region of their *sn*-2 acyl chain, whereas sphingolipids (made in the Golgi) are either fully saturated or have a double bond at the C15 position where it is accommodated by the flexible hydrocarbon tail of cholesterol. However, this requirement for saturation applies strictly only to the fatty acid in the *sn*-1 position for a glycerolipid; a single double bond, even in a relatively superficial position abutting the sterol rings of cholesterol, is tolerated nearly as well as a fully saturated chain in this position. It is the presence of multiple double bonds in one chain, or unsaturation in both chains, that strongly prevents condensation.

The range of membrane lipids that can condense with cholesterol and partition into a liquid-ordered (L_O) phase is much greater than is commonly assumed. This includes, in particular, the glycerolipids of the inner leaflet of the plasma membrane, and of internal membranes within the cell, provided they meet the condition of saturation in the *sn-1* chain and no more than one double bond in the *sn-2* chain. The fatty acid attached at *sn-1* to glycerolipids is normally fully saturated except in certain tissues, such as brain and spermatozoa, and sphingolipids have a linear hydrocarbon chain at this position as part of the sphingosine base. Interactions between cholesterol and phospholipids are also believed to take place through hydrogen bonds. Sphingomyelin contributes both hydrogen bond acceptors (esteric O) and donors (-OH and -NH) to the membrane surface, whereas phosphatidylcholine has only acceptors (esteric O). The principal hydrogen bond site appears to be with the -NH group of the sphingomyelin, which can be detected indirectly by effects, such as the accessibility of 3-OH group of cholesterol to cholesterol oxidase, the extractability of cholesterol from the membrane, or the effect of cholesterol on interfacial elasticity. The multiple hydrogen-bonding propensity of the sphingomyelin molecule results in the formation of relatively large hydrogen-bonded clusters (4 to 8 molecules) of sphingomyelin in the membrane (Mombelli et al., 2003). Cholesterol can only form a single hydrogen bond and so acts as a terminator of the hydrogen bonding networks. The gain to the membrane of the single hydrogen bond formed with cholesterol is at the expense of larger clusters of hydrogen-bonded sphingolipids, and so makes a relatively minor contribution to membrane properties.

The condensing effect of cholesterol on phospholipids to create a liquid-ordered phase can also be viewed from the simple action of the sterol in spacing out the phospholipid molecules at the bilayer interface. The polar groups of the phospholipids tend to repel each other via charge–charge interactions and this effect is reduced by the interposition of uncharged cholesterol molecules. Computer modeling of the interacting molecules shows that cholesterol makes closer contacts with the *sn*-1 rather than *sn*-2 fatty acid chain of glycerolipids, which explains why unsaturation is allowed in the *sn*-2 chain. Moreover, such studies show that the main interaction is not that of cholesterol with the lipid chains, but of the tightly packed lipid chains with each other (Rog and Pasenkiewicz-Gierula, 2001). Cholesterol acts as a hydrophobic spacer that allows the saturated lipids to pack tightly and thereby interact more with each other along their entire length. Presumably constraint upon the flexibility of the first C12 atoms of the fatty acid chains by the presence of cholesterol promotes van der Waals' interactions between the chains. The small -OH head group of cholesterol is probably of key importance in allowing room for tighter packing of the bulky phosphocholine head group, which can orient on the bilayer surface to prevent exposure of the hydrophobic region of the bilayer to water.

One of the material advances in understanding the role of liquid-ordered phases in cell biology has been the isolation of these phase-separated domains from living cells. This is achieved by exploiting the relatively low solubility of the liquid-ordered phase in weak nonionic detergents. The fraction of membrane that survives solubility can be separated by density gradient centrifugation from the remaining solubilized membrane components. The isolation of detergent-resistant membrane has been modeled using giant unilamellar vesicles containing a lipid composition in which liquid-ordered phase is created in a matrix of fluid lipid. An example is shown in Figure 7.40. Giant unilamellar vesicles formed from a mixture of phosphatidycholine, sphingomyelin, and cholesterol (mole ratio



(a) (b) (c)

FIGURE 7.40 Detergent-induced vesicle formation from liquid-ordered phase. (a) Phase contrast image of a giant unilamellar vescile formed from mixture of phosphatidylcholines:sphingomyelin:cholesterol in mole proportions 60:20:20 containing a fluorescent phospholipid analog. (b) Fluorescence image of (a) in which arrows indicate liquid-ordered domains from which the fluorescence probe has been excluded. (c) A phase contrast image recorded 2 sec after an aliquot of Brij 98 surfactant had been delivered to the medium adjacent to the vesicle surface. The domains of liquid-ordered phase bleb and eventually bud off as small unilamellar vesicles. Bar = 20 μm. (From Staneva, G., Seigneuret, M., et al., (2005), *Chem. Phys. Lipids*, **136**, 55–66.)

60:20:20) containing a fluorescent phospholipid that is excluded from liquid-ordered phase show domains of phase separated liquid-ordered phase surrounded by a bilayer of fluid phospholipids. When a small volume of concentrated detergent is delivered close to the membrane, the domains of liquid-ordered phase bleb from the surface and eventually bud off to form a population of small vesicles. Fluorescence microscopy reveals that all the domains of liquid-ordered phase are removed from the parent vesicle. The remaining fluid bilayer is solubilized by the detergent, but the small vesicles remain intact. It is thought that a similar process is involved in the fractionation of detergent-resistant membrane fractions from living cells.

Membrane preparations resistant to solubilization by nonionic detergents have been isolated from a variety of cells and tissues and are referred to as detergent-resistant membrane fractions or membrane rafts. The functions that have been proposed for membrane rafts include the transduction of molecular signals across membranes (Simons and Toomre, 2000), regulation of exocytosis (Salaun et al., 2004), endocytosis (Parton and Richards, 2003), and apoptosis (Garcia et al., 2003), organization of the actin cytoskeleton (Falk et al., 2004) and involvement in the entry of pathogens (Lafont et al., 2004). They are reputed to perform these functions by action as selective filters for the assembly of appropriate receptor and effector components required to mediate the function.

The cholesterol content of lipid rafts isolated from biological membranes is in the order of about 30 mol% of total lipids (Pike et al., 2002). Monomolecular film experiments to examine the condensing effect of cholesterol on sphingomyelin indicate a maximum effect in a stoichiometry of 1:2; cholesterol:sphingomyelin although the evidence is rather indirect and this value must be regarded as indicative only (Radhakrishnan et al., 2001). Cholesterol produces a marked effect on membrane characteristics as its mol% in a monolayer or bilayer of saturated lipids rises above 20 mol% to a maximum of 50 mol% after which it forms a separate cholesterol-only phase. Normal homeostatic processes operate to ensure that the level of cholesterol in the plasma membrane is strictly regulated and can only be manipulated experimentally by removing cholesterol (typically with methyl ß-cyclodextrin or cholesterol oxidase). However, red blood cells have only one membrane, which is exposed continuously to a rich supply of cholesterol in the blood plasma. In membrane rafts isolated from both human and goat red blood cells (ruminant cells have twice as much sphingolipid as human red cells), there is a strict 1:1 stoichiometry between cholesterol and sphingomyelin, although in these cells the cholesterol level is higher in the solubilised (disordered) membrane than in the rafts (Koumanov et al., 2005). This suggests that lipid-ordered microdomains impose an absolute equimolar stoichiometry between cholesterol and the saturated lipid, and as readily exclude excess sterol as they include it when needed.

Although the condensing effect of cholesterol on lipids becomes marked at >20 mol% cholesterol, other effects occur at concentrations too low (≤5%) to form a liquid-ordered phase. It is seen that cholesterol molecules in disordered bilayers of phospholipid align opposite each other with their tails in contact in the respective leaflets, thereby coupling the two sides of the bilayer (Hildenbrand and Bayerl, 2005). This coupling requires the membrane to be planar and is lost when a high degree of curvature (e.g., in coated pits or caveolae) is introduced.

While cholesterol is the major sterol in mammalian cells, in other species a range of different sterols predominate. It has been argued that the fine tuning of sterol structure during evolution results in (and, therefore, may be driven by) greater ability to condense lipids into the liquid-ordered phase (Miao et al., 2002). However, the fungal sterol ergosterol, and 7-dehydrocholesterol, which are characterized by a second double bond in the B ring promoting planarity of the rings, appear to enhance liquid-ordered phase formation more strongly than cholesterol. The advantage to the lower phyla of the better condensing sterols may be the need in poikliotherms to maintain membrane fluidity at low temperatures; the advantage of cholesterol for homeotherms may be its enhanced ability to couple the inner and outer leaflets of the bilayer.

Other conspicuous components of membrane lipid rafts are glycosphingolipids and ethanolamine phosphatides. Biophysical studies of binary mixtures of glucosylceramide extracted from the spleen of patients with Gaucher's disease and phosphatidylethanolamines have revealed that a complex is formed with a stoichiometry of 2:1 phospholipid:sphingolipid (Feng et al., 2004). The glucosylceramide is characterised by very long chain amide-linked fatty acids, which impart a high melting point to these lipids. From the point of view of their participation in the

creation of lipid rafts in biolocical membranes, they may be responsible for coupling the liquid-ordered domains on the cell surface with an ordered cytoplasmic domain due to interdigitation of the amide-linked fatty acid with the cholesterol-rich phase of the opposite bilayer leaflet (Morris et al., 2004).

7.6.5 The functions of lipids in membranes

Some of the functions of the lipid component of biological membranes have been mentioned in previous sections. These include the formation of the matrix in which the various functional membrane proteins are supported, the creation of a passive barrier to the diffusion of solutes, and the packaging of intrinsic proteins into oligomeric complexes. Additional functions are the involvement in membrane fusion processes, transmembrane signal transduction, and a reservoir of precursors of mediator agents, such as platelet activating factor, prostaglandins, and related compounds.

7.6.5.1 Membrane fusion

The fusion between membranes is required in secretion of products from cells and is associated with the biogenesis and differentiation of membranes during cell growth. The fusion between membranes requires that the bilayer arrangement be disturbed. This process can be achieved in several ways all or some of which may be involved in particular fusion events. The most obvious way of disrupting a bilayer is to segregate lipids that will form nonbilayer arrangements so that they are free of the constraints that normally impose a bilayer arrangement on them. Secondly, modification or rearrangement of membrane components that normally interact with the nonbilayer lipids takes place so as to induce them to form no-bilayer structures. Finally, instability can result from the introduction of external agents into one or both membranes in close proximity that cause disruption of the lipid bilayer structure.

All membrane fusions associated with secretion and membrane biogenesis involve cytoplasmic membrane surfaces. Another type of fusion is that between cells or cells and enveloped viruses where initial contact is established on the outer surface of the plasma membrane. The process of endocytosis where plasma membrane is internalized within the cell also falls into this category of membrane fusion. The barriers to fusion in these two cases are different and, hence, so are the strategies required to bring about the instability necessary to mediate fusion.

The process of membrane fusion involving cytoplasmic surfaces differs because the presence of carbohydrates, representing the so-called glycocalyx on the outer plasma membrane surfaces, impedes close apposition of the fusing membranes. Nevertheless, a perturbation of the bilayer arrangement of lipids is believed to be associated with all fusion events irrespective of the surfaces involved. Model systems consisting of liposomes have been extensively employed to simulate the fusion process. Using these methods, the sequence of fusion has been shown to begin with the approach of membrane surfaces, which establish close contact in the region where fusion takes place. This is associated with a partial dehydration of the surface and, in multicomponent systems, there may be phase separations in which certain membrane constituents are segregated into the domains of close contact. These constituents may be "fusogenic molecules," including lipids that prefer nonlamellar configurations. Charged membrane constituents, on the other hand, may be excluded from such domains by electrostatic repulsion.

Studies combining assays of leakage of vesicle contents and mixing of contents of two vesicle populations with biophysical methods able to detect structural changes in the vesicle membrane have been undertaken to characterise the fusion process. Such studies have shown that fusion between the vesicles is correlated with the appearance of a small component of the membrane phospholipid that experiences isotropic motion in an overall transition from a bilayer membrane to an hexagonal phase in which all the contents of the original vesicles leaks to the extravesicular medium. This isotropic component is believed to represent molecules participating in the intermembrane attachment at the fusion site and is associated with the arrangement of the phospholipids molecules into a cubic phase. The incorporation of transmembrane peptides into lipid bilayer membranes has also been used to demonstrate that intrinsic membrane proteins may also introduce instability into bilayers that result in fusion (Siegel et al., 2006).

The entry of certain viruses into cells involves fusion between the viral envelope and the plasma membrane of the target cell. Fusion in this case is known to be mediated by specific, membrane-spanning fusion proteins. Of these fusion proteins the influenza virus haemagglutinins have been most extensively characterised. Several haemagglutinins have been sequenced and the crystal structure of the major component of one haemagglutinin has been determined. The way in which haemagglutinin causes lipid bilayers to fuse is not yet understood despite a detailed knowledge of the conditions required to achieve fusion and the rearrangements in the haemagglutinin that accompany the fusion event (Borrego-Diaz et al., 2003). Patch clamp studies of fusion between erythrocyte ghosts and fusion-competent fibroblasts together with fluorescence microscopic assay of cytoplasmic mixing have shown that the earliest recorded event is the sudden opening of an aqueous pore connecting the cytoplasm of the two cells. In the initial stages, a fluctuation in pore conductance indicates that the pore is undergoing cycles of conductance and occlusion. The size of the pore was estimated to be about the same as proteinaceous channels formed by gap junction complexes between cells or that formed

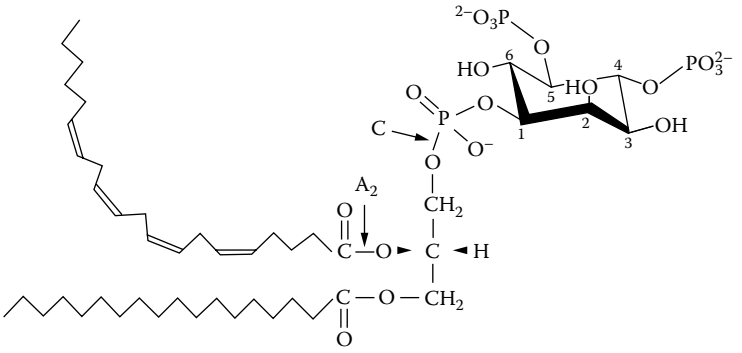


FIGURE 7.41 Structure of phosphatidylinositol-4,5-bisphosphate, showing the sites of phospholipase A_2 and C hydrolysis. The structure shown is the 1-stearoyl, 2-arachidoyl molecular species.

by the terminal components of complement factors. The pore eventually dilates over a period of tens of seconds; the constraints on expansion were thought to be due to the interaction of the proteins of the erythrocyte membrane with the underlying cytoskeleton.

Another intervention that can promote nonbilayer lipid phases is the action of lipolytic enzymes. Lysophospholipids, for example, generated by the hydrolysis of diacylphosphatides by phospholipases A_1 or A_2 cause heamolysis because of their detergent-like action on lipid bilayers. Phospholipase C and sphingomyelinases produce water-soluble products and diglycerides and ceramide, respectively, both of which are known to promote fusion (Boukh-Viner et al., 2005).

7.6.5.2 Receptor-linked phospholipid hydrolysis

The hydrolysis of membrane lipids can also release products capable of generating or transmitting molecular signals in cells. A variety of mechanisms are known to be involved in the transduction of signals across cell membranes. These include the facilitated diffusion of solutes and ions through gated channels, modulation of adenylate cyclase activity, activation of protein kinases, and activation of phospholipases to enhance turnover of specific membrane phospholipids. The hydrolysis of a relatively minor phospholipid, phosphatidylinositol-4,5-bisphosphate, is known to be directly linked to binding of a variety of hormones and neurotransmitters to receptors located in the plasma membrane (Blazer-Yost and Nofziger, 2005).

Indeed, about half of all known agonists act in this way (see Table 7.5). The enzyme responsible is phospholipase C activated by a specific G-protein. The two hydrolytic products are a water-soluble inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol, which remains associated with the membrane. Both products have second messenger functions. The structure of the phospholipid and cleavage locations are illustrated in Figure 7.41.

The second messenger function of IP_3 is to release Ca^{2+} from subcellular storage compartments, such as the endoplasmic reticulum. It does so by binding with high affinity to a calcium channel causing facilitated diffusion of Ca^{2+} down a concentration gradient maintained by a calmodulin-sensitive Ca^{2+}-ATPase. IP_3 concentrations are subsequently reduced by a stepwise dephosphorylation mediated by specific phosphatases, although removal of the first phosphate at position 5 by a phosphatase partly associated with the plasma membrane is sufficient to abolish its channel-opening role. An alternate pathway is the further phosphorylation of IP_3 catalyzed by an ATP-dependent kinase, which forms inositol-1,3,4,5-tetrakisphosphate. It has been shown that this compound can facilitate diffusion of Ca^{2+} across the plasma membrane resulting in a further increase in cytoplasmic calcium concentration. Some of the metabolic transformations of inositol phosphates are illustrated in Figure 7.42. It can be seen that eventually complete dephosphorylation of inositol phosphates provides *myo*inositol that can be resynthesised to inositol phospholipids.

The diacylglycerol product of inositol phospholipid hydrolysis by phospholipase C causes a change in the physical properties of the lipid domain of the plasma membrane resulting in activation of protein kinase C. These changes in membrane lipid phase behaviour are also known to promote fusion between membranes. The action of diacylglycerol is switched off by further metabolism. One pathway is catalysed by a diacylglycerol kinase employing a substrate of ATP yielding phosphatidic acid, which is a key phospholipid involved in phospholipid biosynthesis including resynthesis of inositol phospholipids. Phosphatidic acid also effectively reverses the change induced by diacylglycerol on membrane lipid phase behaviour. An alternative pathway is mediated by a lipolysis reaction in which arachidonic acid, present in high proportions at the carbon-2 position of the diacylglycerol, is released.

Many agonists that stimulate turnover of inositol phospholipids also promote enhanced turnover of the more ubiquitous choline phospholipids. Phosphatidylcholine turnover cycles can also generate second messengers, such

TABLE 7.5 Receptor-stimulated inositol phosphatide turnover agonists

Agonist/Receptor	Responsive Cell Types
α-Adrenergic	Liver, muscle, central nervous system, fat cells
Muscarinic—cholinergic	Smooth muscle, central nervous system, heart, pancreatic islets, sympathetic ganglia, chromaffin cells, avian salt gland
Histaminergic	Central nervous system, astrocytoma cells, chromaffin cells
Serotoninergic	Central nervous system, platelets
Platelet-activating factor	Platelets, liver, exocrine glands
Glutaminergic	Oocytes
Purinergic/ATP	Liver, endothelial cells
Glucose	Pancreatic islets, yeast
Hypothalamic-releasing hormone	Pituitary
Intestinal peptide hormones	Pancreas
Peptide hormones:	
Vasopressin	Liver, vascular smooth muscle, sympathetic ganglia, platelets, fibroblasts
Angiotensin II	Liver, vascular smooth muscle, anterior pituitary, kidney
Bradykinin	Fibroblasts, carcinoma cells
Substance P	Smooth muscle, nerve cells, parotid
Growth factors	3T3 tissue culture cells
Thrombin/collagen	Platelets
Antigens	Mast cells, macrophages
Sperm	Oocytes
Light	Photoreceptors

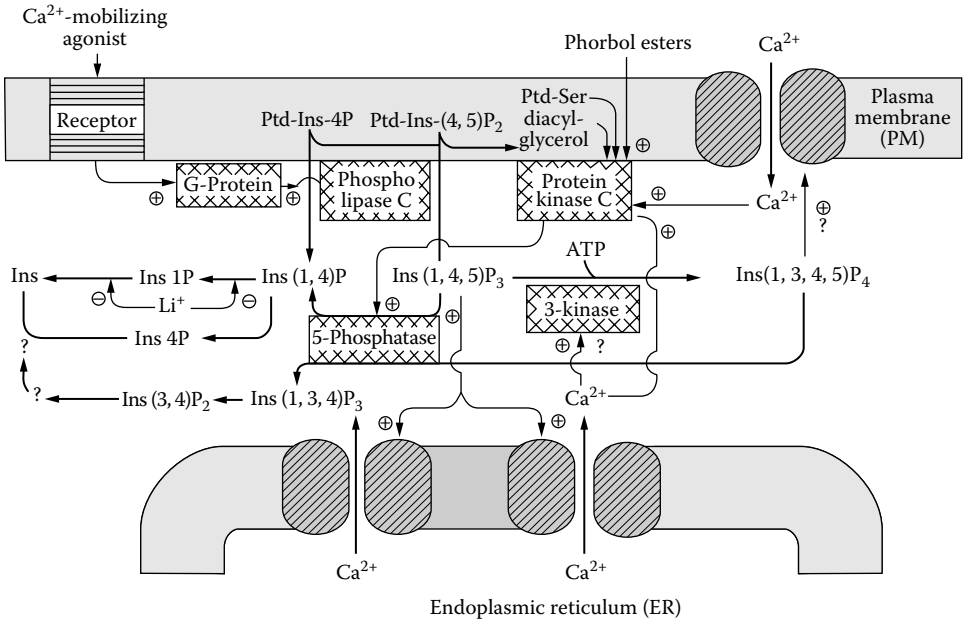


FIGURE 7.42 Schematic diagram of cellular responses triggered by receptor-mediated hydrolysis of phosphatidylinositol-4,5-bisphosphate in the plasma membrane.

as diacylglycerol and arachidonic acid. GTP-binding proteins have been implicated in ligand-receptor activation of phospholipases A_2, C, and D, which catalyse the degradation of phospholipid. Many agonists also appear to stimulate pathways of phosphatidylcholine biosynthesis via an effect on CDP-choline synthesis. It is now evident that turnover of membrane glycerophospholipids and sphingolipids are integrated within cells to modulate the activity of effector enzymes including protein kinase c (Becker and Hannun, 2005).

7.6.5.3 Lipid mediators, hormones, and receptors

As noted above, essential fatty acids, such as arachidonic acid, are constituents of membrane lipids where they serve as precursors for synthesis of a variety of lipid mediators. Other lipid precursors that have been shown to be released as a consequence of stimulation by specific agonists include platelet activating factor, lysophospholipids, and diacylglycerols (Deo et al., 2004; Baumruker et al., 2005). Further metabolism of arachidonic acid released from complex membrane lipids by the action of

phospholipase A_2 enzymes takes place in an integrated series of reactions to produce the eiconsanoid group of compounds, including the prostaglandins, thromboxane, and leucotrienes. These compounds all have potent effects on white blood cells and are involved in inflammatory and hypersensitivity reactions.

Gangliosides displayed on the outer surface of the plasma membrane act as receptors for certain toxins including cholera toxin. The B-subunit of the toxin binds to the ganglioside resulting in dissociation of the B-subunit from the A-subunit, which then locates in the membrane. Activation of the A-subunit takes place by formation of A_1 and A_2 components. The A_1 component catalyses the ADP-ribosylation by NAD of the GTP-bound α-subunit of G-protein bringing about an irreversible dissociation from the ß-complex and uncontrolled activation of adenylate cyclase. This leads to enhanced water and salt secretion into the gut and dehydration, which is the classical manifestation of infection. Similar interactions are observed in the action of diphtheria toxin on membranes as seen in Figure 7.35.

Membrane lipids can act as targets for certain toxins. Many venoms, for example, contain phospholipases and agents that increase the susceptibility of membrane phospholipids to attack by these phospholipases. Sterols may also represent targets for toxins. These include thiol-activated cytolysins from streptococci (e.g., streptolysin O); antifungal polyeonic agents, such as filipin, ampotericin, etc.; and plant alkaloids like the saponins. It appears that in each case there is formation of toxin-sterol complexes, which create water-filled channels in the membrane leading to a breakdown in permeability barrier properties.

Membrane lipids also act as cell surface antigens. The most notable of these is the blood group antigens. Gangliosides present in the erythrocyte membrane contain the determinants for ABO blood groups although the specific oligosaccharide representing the determinant is also a component of a glycoprotein.

References

Baumruker, T. et al. (2005), The role of sphingosine and ceramide kinases in inflammatory responses, *Immunol. Lett.*, **96**, 175–185.

Becker, K.P. and Hannun, Y.A. (2005), Protein kinase C and phospholipase D: intimate interactions in intracellular signaling, *Cell. Mol. Life Sci.*, **62**, 1448–1461.

Ben-Efraim, I. et al. (2004), Phospholipid scramblase 1 is imported into the nucleus by a receptor-mediated pathway and interacts with DNA, *Biochemistry*, **43**, 3518–3626.

Benninger, R.K. et al. (2005), Fluorescence imaging of two-photon linear dichroism: cholesterol depletion disrupts molecular orientation in cell membranes, *Biophys. J.*, **88**, 609–622.

Birmachu, W. and Thomas, D.D. (1990), Rotational dynamics of the Ca-ATPase in sarcoplasmic reticulum studied by time-resolved phosphorescence anisotropy, *Biochemistry*, **29**, 3904–3914.

Blasie, J.K. et al. (1990), Large-scale structural changes in the sarcoplasmic reticulum ATPase appear essential for calcium transport, *Biophys. J.*, **58**, 687–693.

Blazer-Yost, B.L. and Nofziger, C. (2005), Phosphoinositide lipid second messengers: new paradigms for transepithelial signal transduction, *Pflugers Arch.*, **450**, 75–82.

Blitterswijk, W.J.v. et al. (2003), Ceramide: second messenger or modulator of membrane structure and dynamics? *Biochem. J.*, **369**, 199–211.

Borrego-Diaz, E. et al. (2003), Completion of trimeric hairpin formation of influenza virus hemagglutinin promotes fusion pore opening and enlargement, *Virology*, **316**, 234–244.

Boukh-Viner, T. et al. (2005), Dynamic ergosterol- and ceramide-rich domains in the peroxisomal membrane serve as an organizing platform for peroxisome fusion, *J. Cell. Biol.*, **168**, 761–773.

Burger, K.N. et al. (2000), Dynamin is membrane-active: lipid insertion is induced by phosphoinositides and phosphatidic acid, *Biochemistry*, **39**, 12485–12493.

Bussolino, D.F. et al. (2001), c-Fos associates with the endoplasmic reticulum and activates phospholipid metabolism, *Faseb. J.*, **15**, 556–558.

Chachaty, C. et al. (2005), Building up of the liquid-ordered phase formed by sphingomyelin and cholesterol, *Biophys. J.*, **88**, 4032–4044.

Chapman, D. et al. (1967), Physical studies of phospholipids. VI Thermotropic and lyotropic mesomorphism of some 1,2-diacyl phosphatidylcholines (lecithins), *Chem. Phys. Lipids*, **1**, 445–475.

Chen, Y. et al. (2005), YKT6 is a core constituent of membrane fusion machineries at the Arabidopsis trans-Golgi network, *J. Mol. Biol.*, **350**, 92–101.

Cherry, R.J. (2005), Membrane protein dynamics: rotational dynamics, in *The Structure of Biological Membranes*, Yeagle, P.L., Ed., CRC Press, Boca Raton, FL, 389–410.

Daleke, D.L. (2003), Regulation of transbilayer plasma membrane phospholipid asymmetry, *J. Lipid Res.*, **44**, 233–242.

Danielli, J.F. and Davson, H. (1935), A contribution to the theory of permeability of thin films, *J. Cell. Comp. Physiol.*, **5**, 495–508.

de Bony, J. et al. (1989), Transverse and lateral distribution of phospholipids and glycolipids in the membrane of the bacterium *Micrococcus luteus*, *Biochemistry*, **28**, 3728–3737.

Deo, D.D. et al. (2004), Activation of platelet-activating factor receptor-coupled G alpha q leads to stimulation of Src and focal adhesion kinase via two separate pathways in human umbilical vein endothelial cells, *J. Biol. Chem.*, **279**, 3497–3508.

Ding, J. et al. (2000), Identification and functional expression of four isoforms of ATPase II, the putative aminophospholipid translocase. Effect of isoform variation on the ATPase activity and phospholipid specificity, *J. Biol. Chem.*, **275**, 23378–23386.

El Bawab, S. et al. (2002), Ceramidases in the regulation of ceramide levels and function, *Subcell. Biochem.*, **36**, 187–205.

Engelman, D.M. (1969), Surface area per lipid molecule in the intact membrane of the human red cell, *Nature*, **223**, 1279–1280.

Falk, J. et al. (2004), NrCAM coupling to the cytoskeleton depends on multiple protein domains and partitioning into lipid rafts, *Mol. Biol. Cell.*, **15**, 4695–4709.

Feng, Y. et al. (2004), Characterization of a quasicrystalline phase in codispersions of phosphatidylethanolamine and glucocerebroside, *Biophys. J.*, **86**, 2208–2217.

Ferretti, A. et al. (2003), High-resolution proton NMR measures mobile lipids associated with Triton-resistant membrane domains in haematopoietic K562 cells lacking or expressing caveolin-1, *Eur. Biophys. J.*, **32**, 83–95.

Franklin, B. et al. (1774), Of the stilling of waves by means of oil extracted from sundry letters between Benjamin Franklin, William Brownrigg and the Reverend Mr Farish, *Philosoph. Trans.*, **64**, 445–460.

Frasch, S.C. et al. (2000), Regulation of phospholipid scramblase activity during apoptosis and cell activation by protein kinase Cdelta, *J. Biol. Chem.*, **275**, 23065–23073.

Furtado, D. et al. (1979), Phase separations in membranes of *Anacystis nidulans* grown at different temperatures, *Biochim. Biophys. Acta*, **555**, 352–357.

Garcia, A. et al. (2003), Rafts: a simple way to control apoptosis by subcellular redistribution, *Biochimie*, **85**, 727–731.

Geisse, N.A. et al. (2004), Targeting of *Helicobacter pylori* vacuolating toxin to lipid raft membrane domains analysed by atomic force microscopy, *Biochem. J.*, **381**, 911–917.

Goni, F.M. et al. (2005), Biophysics (and sociology) of ceramides, *Biochem. Soc. Symp.*, **72**, 177–188.

Goodwin, J.S. et al. (2005), Ras diffusion is sensitive to plasma membrane viscosity, *Biophys. J.*, **89**, 1398–1410.

Gorter, E. and Grendel, F. (1925), Bimolecular layers of lipoids on chromatocytes of blood, *J. Exp. Med.*, **41**, 439–443.

Green, D.E. and Brucker, R.F. (1973), Structure-function unitization model of biological membranes, *J. Bioenerg.*, **4**, 253–284.

Haque, M.E. and Lentz, B.R. (2004), Roles of curvature and hydrophobic interstice energy in fusion: studies of lipid perturbant effects, *Biochemistry*, **43**, 3507–3517.

Herbette, L. et al. (1985), The separate profile structures of the functional calcium pump protein and the phospholipid bilayer within isolated sarcoplasmic reticulum membranes determined by X-ray and neutron diffraction, *Biochim. Biophys. Acta*, **817**, 103–122.

Hildenbrand, M.F. and Bayerl, T.M. (2005), Differences in the modulation of collective membrane motions by ergosterol, lanosterol, and cholesterol: a dynamic light scattering study, *Biophys. J.*, **88**, 3360–3367.

Hommelgaard, A.M. et al. (2005), Caveolae: stable membrane domains with a potential for internalization, *Traffic*, **6**, 720–724.

Hong, W. (2005), SNAREs and traffic, *Biochim. Biophys. Acta*, **1744**, 493–517.

Jaffrezou, J.P. et al. (2002), Ceramide in regulation of apoptosis. Implication in multitoxicant resistance, *Subcell. Biochem.*, **36**, 269–284.

Killian, J.A. et al. (1992), Effects of temperature variation and phenethyl alcohol addition on acyl chain order and lipid organization in Escherichia coli derived membrane systems, *Biochim. Biophys. Acta*, 1105, **253**–262.

Koumanov, K.S. et al. (2005), Comparative lipid analysis and structure of detergent-resistant membrane raft fractions isolated from human and ruminant erythrocytes, *Arch. Biochem. Biophys.*, **434**, 150–158.

Lafont, F. et al. (2004), Bacterial subversion of lipid rafts, *Curr. Opin. Microbiol*. **7**, 4–10.

Langmuir, I. (1917), The constitution and fundamental properties of solids and liquids. II Liquids, *J. Am. Chem. Soc.*, **39**, 1848–1906.

Levine, Y.K. and Wilkins, M.H. (1971), Structure of oriented lipid bilayers, *Nat. New Biol.*, **230**, 69–72.

Mannock, D.A. et al. (1985), Phase behaviour of the membrane lipids of the thermophilic blue-green alga *Anacystis nidulans*, *Biochim. Biophys. Acta*, **821**, 153–164.

Martelli, A.M. et al. (2003), Nuclear lipids: new functions for old molecules?, *J. Cell. Biochem.*, **88**, 455–461.

Meer, G. van (2000), Cellular organelles: how lipids get there, and back, *Trends Cell. Biol.*, **10**, 550–552.

Miao, L. et al. (2002), From lanosterol to cholesterol: structural evolution and differential effects of lipid bilayers, *Biophys. J.* , **82**, 1429–1444.

Mitsuoka, K. et al. (1999), The structure of bacteriorhodopsin at 3.0 Å resolution based on electron crystallography: implication of the charge distribution, *J. Mol. Biol.*, **286**, 861–882.

Mombelli, E. et al. (2003), Hydrogen-bonding propensities of sphingomyelin in solution and in a bilayer assembly: a molecular dynamics study, *Biophys. J.*, **84**, 1507–1517.

Morris, R. et al. (2004), Rafts, little caves and large potholes: how lipid structure interacts with membrane proteins to create functionally diverse membrane environments, *Subcell. Biochem.*, **37**, 35–118.

O'Toole, P.J. et al. (2000), Investigations of spectrin-lipid interactions using fluoresceinphosphatidylethanolamine as a membrane probe, *Biochim. Biophys. Acta*, **1466**, 39–46.

Oka, T. et al. (2005), Structural transition of bacteriorhodopsin is preceded by deprotonation of Schiff base: microsecond time-resolved X-ray diffraction study of purple membrane, *Biophys. J.*, **88**, 436–442.

Oradd, G. et al. (2005), Lateral diffusion coefficients of separate lipid species in a ternary raft-forming bilayer: a Pfg-NMR multinuclear study, *Biophys. J.*, **89**, 315–320.

Overton, E. (1895), Über die osmotischen Eigenschaften der lebenden Pflanzen- und Tierzellen, *Zeitschr. Naturw. Ges. Zürich*, **40**, 159–201.

Park, S.H. et al. (2005), Rotational diffusion of membrane proteins in aligned phospholipid bilayers by solid-state NMR spectroscopy, *J. Magn. Reson.*, **178**, 165.

Parton, R.G. and Richards, A.A. (2003), Lipid rafts and caveolae as portals for endocytosis: new insights and common mechanisms, *Traffic*, **4**, 724–738.

Pike, L.J. et al. (2002), Lipid rafts are enriched in arachidonic acid and plasmenylethanolamine and their composition is independent of caveolin-1 expression: a quantitative electrospray ionization/mass spectrometric analysis, *Biochemistry*, **41**, 2075–2088.

Pohl, A. et al. (2005), Function of prokaryotic and eukaryotic ABC proteins in lipid transport, *Biochim. Biophys. Acta*, **1733**, 29–52.

Quinn, P.J. (1983), Models for adaptive changes in cell membranes, *Biochem. Soc. Trans.*, **11**, 329–330.

Quinn, P.J. (1985), A lipid-phase separation model of low-temperature damage to biological membranes, *Cryobiology*, **22**, 128–146.

Quinn, P.J. (2002), Plasma membrane phospholipid asymmetry, *Subcell. Biochem.*, **36**, 39–60.

Quinn, P.J. et al. (1995), Characterization of complexes formed in fully hydrated dispersions of dipalmitoyl derivatives of phosphatidylcholine and diacylglycerol, *Biophys. J.*, **68**, 1374–1382.

Radhakrishnan, A. et al. (2001), Stoichiometry of cholesterol-sphingomyelin condensed complexes in monolayers, *Biochim. Biophys. Acta*, **1511**, 1–6.

Rappolt, M. et al. (2003), Structural, dynamic and mechanical properties of POPC at low cholesterol concentration studied in pressure/temperature space, *Eur. Biophys. J.*, **31**, 575–585.

Rehm, M. et al. (2004), Endothelial glycocalyx as an additional barrier determining extravasation of 6% hydroxyethyl starch or 5% albumin solutions in the coronary vascular bed, *Anesthesiology*, **100**, 1211–1223.

Robertson, J.D. (1972), The structure of biological membranes. Current status, *Arch. Intern. Med.*, **129**, 202–228.

Rog, T. and Pasenkiewicz-Gierula, M. (2001), Cholesterol effects on the phospholipid condensation and packing in the bilayer: a molecular simulation study, *FEBS Lett.*, **502**, 68–71.

Rosconi, M.P. et al. (2004), Analyzing topography of membrane-inserted diphtheria toxin T domain using BODIPY-streptavidin: at low pH, helices 8 and 9 form a transmembrane hairpin but helices 5-7 form stable nonclassical inserted segments on the cis side of the bilayer, *Biochemistry*, **43**, 9127–9139.

Salaun, C. et al. (2004), Lipid rafts and the regulation of exocytosis, *Traffic*, **5**, 255–264.

Sanjuan, M.A. et al. (2001), Role of diacylglycerol kinase alpha in the attenuation of receptor signaling, *J. Cell. Biochem.*, **153**, 207–220.

Scheuring, S. et al. (2005), Structure of the dimeric PufX-containing core complex of *Rhodobacter blasticus* by in situ atomic force microscopy, *J. Biol. Chem.*, **280**, 1426–1431.

Siegel, D.P. et al. (2006), Transmembrane peptides stabilize inverted cubic phases in a biphasic length-dependent manner: implications for protein-induced membrane fusion, *Biophys. J.*, **90**, 200–211.

Simons, K. and Toomre, D. (2000), Lipid rafts and signal transduction, *Nat. Rev. Mol. Cell. Biol.*, **1**, 31–39.

Singer, S.J. and Nicolson, G.L. (1972), The fluid mosaic model of the structure of cell membranes, *Science*, **175**, 720–731.

Sot, J. et al. (2005), Different effects of long- and short-chain ceramides on the gel-fluid and lamellar-hexagonal transitions of phospholipids: a calorimetric, NMR, and X-ray diffraction study, *Biophys. J.*, **88**, 3368–3380.

Squier, T.C. et al. (1988), Rotational dynamics and protein-protein interactions in the Ca-ATPase mechanism, *J. Biol. Chem*, **263**, 9162–9170.

Staneva, G. et al. (2005), Detergents induce raft-like domains budding and fission from giant unilamellar heterogeneous vesicles: a direct microscopy observation, *Chem. Phys. Lipids*, **136**, 55–66.

Sugawara, S. et al. (2005), Catfish egg lectin causes rapid activation of multidrug resistance 1 P-glycoprotein as a lipid translocase, *Biol. Pharm. Bull.*, **28**, 434–441.

Tsvetkova, N.M. et al. (1994), Structural characteristics of thylakoid membranes of *Arabidopsis* mutants deficient in lipid fatty acid desaturation, *Biochim. Biophys. Acta*, **1192**, 263–271.

Unwin, N. (2005), Refined structure of the nicotinic acetylcholine receptor at 4A resolution, *J. Mol. Biol.*, **346**, 967–989.

Van Dort, H.M. et al. (1998), Effect of band 3 subunit equilibrium on the kinetics and affinity of ankyrin binding to erythrocyte membrane vesicles, *J. Biol. Chem*, **273**, 14819–14826.

Wang, T. (2003), Use of fats and oils in candle production, *Lipid Technol.*, **15**, 131–134.

Wiedmer, T. et al. (2000), Identification of three new members of the phospholipid scramblase gene family, *Biochim. Biophys. Acta*, **1467**, 244–253.

Williams, W.P. et al. (1982), Protein-lipid interactions in the photosynthetic membrane, *Biochem. Soc. Trans.*, **10**, 335–338.

Zwaal, R.F. et al. (2005), Surface exposure of phosphatidylserine in pathological cells, *Cell. Mol. Life Sci.*, **62**, 971–988.

8

CHEMICAL PROPERTIES

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8.1 Autoxidation and photo-oxidation*

The reactions discussed here differ from those in the following subchapters because autoxidation and photo-oxygenation proceed due to the presence of oxygen in air and are virtually inevitable while in other reactions the fatty substrate is deliberately exposed to selected reagents. Understanding lipid oxidation has been impeded by the fact that lipids are usually complex mixtures containing minor components that may catalyze or inhibit oxidation and because primary oxidation products are labile and easily convert to secondary products. The study of pure compounds, such as methyl oleate, linoleate, and linolenate, has provided insights into the oxidation process. The subject of lipid oxidation has been recently presented in an extensive book (Frankel, 2005) and summarized by Gunstone (2004), on both of which the present chapter draws heavily. Another review was provided by Porter et al. (1995). Analytical methods for studying the oxidation of lipids are discussed in the book edited by Kamal-Eldin and Pokorný (2005).

Lipid oxidation affects a variety of products, including foods and industrial products, such as biodiesel (Section 9.6). Not all studies in the literature can be readily applied to such products since the conditions may be too drastic or pure materials were studied while these products are mixtures containing a variety of trace pro- and antioxidants.

The primary oxidation products are allylic hydroperoxides [-CH=CHCH(OOH)-]. In these primary products, the original double bond(s) may have shifted or undergone isomerization from *cis* to *trans* configuration. These hydroperoxides are unstable and decompose to a variety of secondary oxidation products. Reactions of hydroperoxides include rearrangement to products of similar molecular weight, fission to give shorter-chain compounds (aldehydes and acids), and dimerization to give higher molecular weight materials. Mass spectrometry, often in combination with liquid chromatography, as well as ^{1}H- and ^{13}C-NMR, are common analytical methods for investigating the products of lipid oxidation.

Oxidation of lipids is promoted by factors such as elevated temperature, presence of light, or extraneous materials, such as metals or other oxidation initiators. The nature of the radicals also influences the products obtained. Double bond geometry can play a role (Tallman et al. 2004). Oxidation can be inhibited, but not prevented in the long term, by the presence of antioxidants.

The oxygen molecule, O_2, with which the olefinic lipids react, exists in two forms. These are the common ground state triplet form $^{3}O_2$, which is a diradical •O–O•, and the excited singlet form $^{1}O_2$, which is more reactive than the triplet form by 22.5 kcal/mol. Both forms are reactive in lipid oxidation, although some important differences exist and these are discussed in the "autoxidation" and "photo-oxygenation" sections. $^{3}O_2$ reacts at allylic positions of unsaturated fatty acid chains to give allylic hydroperoxides. In contrast $^{1}O_2$ is very electrophilic and reacts at an olefinic carbon atom, but also gives allylic hydroperoxides. The amounts and structure of the hydroperoxides obtained from the two forms of oxygen thus vary. The

* In the previous edition of *The Lipid Handbook* (1994), hydrogenation was discussed as the first chemical property. In this edition, it can be found in Section 4.2.)

TABLE 8.1 Relative rates of oxidation of oleate, linoleate, and linolenate

Reaction	Oleate	Linoleate	Linolenate
Autoxidation, $^3O_2$	1	27	77
Photo-oxidation, $^1O_2$	3×10^4	4×10^4	7×10^4
Ratio	30,000	1500	900

Source: Gunstone, F.D. (Ed.), in *The Chemistry of Fats and Oils,* Blackwell Publishing, Oxford, U.K., 2004, pp. 150–168.

relative rates of autoxidation and photo-oxidation are given in Table 8.1. A major factor is the number of double bonds in the molecule. In autoxidation, the number of double bonds separated from each other by one methylene group is particularly important. Other values given for relative rates of ester oxidation are oleate = 1, linoleate = 41, linolenate = 98, and arachidonate = 195 (Frankel, 2005). The corresponding relative oxidation rate of triacylglycerols is lower, that of trilinolenin being 50 relative to oleate.

8.1.1 Autoxidation

Autoxidation is a radical-based chain reaction with the typical stages of initiation, propagation and termination. The initiation step is:

$$RH + I\bullet \rightarrow R\bullet + IH$$

where I• = initiator. The direct oxidation of lipids is spin-forbidden because of opposite spins of $^3O_2$ and the lipid in its ground state. This obstacle can be overcome by the presence of initiators, such as trace metals, peroxides, and hydroperoxides present as impurities. The formation of the lipid alkyl radical and resulting delocalization of the odd electron is the cause of double bond migration observed in the primary hydroperoxide products.

The reaction of the alkyl radical R• of unsaturated lipids with oxygen to form peroxy radicals ROO• proceeds rapidly, followed by a hydrogen transfer reaction with other labile H in unsaturated lipids (or from an antioxidant, if present) as the rate-determining step, propagating the chain:

$$R\bullet + O_2 \rightarrow ROO\bullet$$

$$ROO\bullet + RH \rightarrow ROOH + R\bullet$$

Reversibility is a feature of the oxygen addition reactions, leading to β-scission or β-fragmentation of the C-O bond of the peroxy radicals. This can also influence the stereochemistry of the hydroperoxides. Figure 8.1 illustrates a hydroperoxide structure rising from methyl oleate autoxidation from which other structures discussed here can be visualized.

The peroxy radical can also rearrange with accompanying inversion of the double bond configuration from *cis* to *trans*:

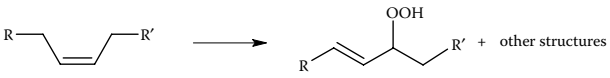


FIGURE 8.1

$$RCH{=}CHCH(OO\bullet)CH' \leftrightarrows RCH(OO\bullet)CH{=}CHR'$$

cis *trans*

If a β-olefinic system is present in the radical, cyclic peroxide and hydroperoxide structures may result. These are formed only in the autoxidation of species with three or more double bonds. In photo-oxidation, they can be formed from species such as linoleate with only two double bonds.

Various termination reactions are possible. Hydroperoxides are subject to homolysis, leading to peroxy or alkoxy radicals, which can also react. Thus, the following reactions are possible:

$$RO\bullet + RH \rightarrow ROH + R\bullet$$

$$RCH(O\bullet)R' \rightarrow R'CHO + R\bullet$$

While the formation of alcohols removes reactive species, unsaturated aldehydes may be unstable. Reaction of alkyl, alkoxy, and peroxy radicals leads to termination with dimer or dimer-like products:

$$ROO\bullet + ROO\bullet \rightarrow ROOR + O_2$$

$$RO\bullet + R\bullet \rightarrow R\text{-}O\text{-}R$$

$$ROO\bullet + R\bullet \rightarrow \text{stable products}$$

$$R\bullet + R\bullet \rightarrow R\text{-}R \text{ (dimers)}$$

Termination involving an antioxidant occurs as follows:

$ROO\bullet + AH \rightarrow ROOH + A\bullet \rightarrow$ products where AH is a phenolic antioxidant

$ROO\bullet + B \rightarrow ROOB\bullet \rightarrow$ products where B is an unsaturated compound such as β-carotene

The latter reaction affects autoxidation at its initiation stage (reaction with R•) and this is the most desirable point of attack.

The autoxidation process usually has an induction time during which the overall reaction is slow. A more rapid stage of oxidation then ensues. It is the purpose of antioxidants to either prolong the onset of the initiation reaction or to enhance termination thereby reducing the length of the propagation sequence.

When unsaturated compounds are contained in emulsions, as is the case with most biological systems and food, oxidation differs in detail from the above process. Also, the rates of oxidation in natural mixtures, such as

vegetable oils, can differ from those in studies on pure compounds due to the effects of present pro- and antioxidants.

8.1.2 Photo-oxygenation

As indicated above, because of the greater reactivity of $^1O_2$, photo-oxidation is a much faster process than autoxidation with less reactivity differences depending on the degree of unsaturation (see Table 8.1). Photo-oxidation is promoted by the presence of sensitizers, such as chlorophyll, methylene blue, rose Bengal, or erythrosine, which cause the formation of $^1O_2$ from $^3O_2$. Some early studies on photo-oxidation are reported by Chan (1977), Clements et al. (1973), Frankel et al. (1979), Neff et al. (1983), Neff and Frankel (1984), Rawls and van Santen (1970), and Terao and Matsushita (1977, 1980).

The reaction mechanism of photo-oxidation differs from that of autoxidation. Due to the high electrophilicity of $^1O_2$, the reaction is fast and without an induction period. However, the hydroperoxides formed by photo-oxidation can serve as initiators of the free radical oxidation (autoxidation). Thus, there is some connection between the two oxidation mechanisms.

Other differences between autoxidation and photo-oxidation involve the mechanism. Photo-oxidation is not a chain reaction but an ene reaction between $^1O_2$ and the double bond and occurs by insertion of oxygen at either end of the carbon double bond with concomitant migration of the double bond to an allylic position and isomerization to *trans* configuration:

$$RCH{=}CHCH_2CHR' + {}^1O_2 \leftrightarrows RCH(OOH)CH{=}CHR'$$

cis *trans*

Photo-oxidation is not affected by the antioxidants used to inhibit autoxidation, but is affected by $^1O_2$ quenchers, such as carotene. In accordance with the mechanism, the reactivity ratio between oleate, linoleate, and linolenate is approximately proportional to the number of double bonds. The distribution of hydroperoxides differs from that of autoxidation (Table 8.2 to Table 8.5).

FIGURE 8.2

$^1O_2$ can also react with conjugated dienes to form endoperoxides (Figure 8.2). This is of significance because in the course of oxidation some conjugation of double bonds may arise.

8.1.3 Hydroperoxides formed

Table 8.2 to Table 8.5 provide information on the various hydroperoxides formed from methyl oleate, methyl linoleate, and methyl linolenate under conditions of autoxidation and photo-oxidation. Temperature dependence of autoxidation products can be obtained from the data in Table 8.2 to Table 8.4. Differences in the nature and amounts of hydroperoxides are visible from these data. The results, summarized in Table 8.5, show the formation of hydroperoxides with the OOH moiety at the positions of the olefinic carbons, as discussed above. The "inner" hydroperoxides (OOH at C-8 and C-12) formed through photo-oxidation of methyl linoleate possess a β-hydroxy alkene system and can form hydroperoxy peroxides (Figure 8.3), which can be the cause of lower levels of "inner" hydroperoxides. Besides the review literature mentioned above, primary literature on the formation of hydroperoxides, mainly formed by autoxidation, and their analysis is due to Brash (2000), Coxon et al. (1981), Frankel et al. (1961a, 1977a-c, 1984a, 1990a), Neff et al. (1988, 1990), Porter and Wujek (1984), and Yin and Porter (2005).

8.1.4 Secondary oxidation products (decomposition of hydroperoxides)

Besides species with relatively unchanged molecular weight, secondary oxidation products are a variety of polymeric and lower-molecular weight compounds. The complicated decomposition process of hydroperoxides is reflected in this complex variety of products.

Information on the secondary oxidation products that can result from the initially formed hydroperoxides is summarized in Table 8.6. The secondary products are categorized as monomeric (products with the same chain length

TABLE 8.2 Distribution of hydroperoxides among total hydroperoxides from autoxidation of methyl oleate at different temperatures

Temp. (°C)	8-OOH, Δ-9			9-OOH, Δ-10			10-OOH, Δ-8			11-OOH, Δ-9		
	cis	*trans*	total	*cis*	*trans*	total	*cis*	*trans*	total	*cis*	*trans*	total
25	14.1	12.3	26.4	1.1	23.1	24.2	1.1	21.7	22.8	13.7	12.9	26.6
50	8.3	17.8	26.1	2.2	22.5	24.7	2.2	21.3	23.5	8.3	17.4	25.7
75	6.1	19.0	25.1	2.7	22.5	25.1	2.9	22.0	24.9	5.4	19.5	24.9

Source: Adapted from Gunstone, F.D. (Ed.), in *The Chemistry of Fats and Oils,* Blackwell Publishing, Oxford, U.K., 2004, pp. 150–168; and Frankel, E.N., *Lipid Oxidation*, 2nd ed., The Oily Press, Bridgwater, U.K., 2005.

TABLE 8.3 Distribution of hydroperoxides among total hydroperoxides from autoxidation of methyl linoleate at different temperatures

Temp. (°C)	9-OOH			13-OOH		
	cis, trans	*trans, trans*	total	*cis, trans*	*trans, trans*	total
25	29.7	19.2	48.9	31.0	20.1	51.2
50	22.9	29.6	52.5	19.5	28.0	47.5
65	17.5	30.4	47.9	18.6	33.5	52.1

Source: Frankel, E.N., *Lipid Oxidation*, 2nd ed., The Oily Press, Bridgwater, U.K., 2005.

TABLE 8.4 Distribution of hydroperoxides among total hydroperoxides from autoxidation of methyl linolenate at different temperatures

Temp. (°C)	9-OOH; Δ10,12,15	12-OOH; Δ9,13,15	13-OOH; Δ9,11,15	16-OOH; Δ9,12,14
25	30.6	10.1	10.8	48.6
60	29.4	11.4	11.8	47.5
80	34.2	11.1	11.5	43.3

Source: Adapted from Gunstone, F.D. (Ed.), in *The Chemistry of Fats and Oils,* Blackwell Publishing, Oxford, U.K., 2004, pp. 150–168; and Frankel, E.N., *Lipid Oxidation*, 2nd ed., The Oily Press, Bridgwater, U.K., 2005.

TABLE 8.5 Distribution of hydroperoxides (%) among total hydroperoxides from photo-oxygenation of methyl oleate, linoleate, and linolenate

Hydroperoxide	Me Oleate	Me Linoleate	Me Linolenate
t 9-OOH	48–51		
t 10-OOH	49–52		
t,c 9-OOH		28	
t,c 10-OOH		18	
c,t 12-OOH		21	
c,t 13-OOH		34	
t,c,c 9-OOH			20–23
t,c,c 10-OOH			13
c,t,c 12-OOH			12–14
c,t,c 13-OOH			14–15
c,c,t 15-OOH			12–13
c,c,t 16-OOH			25–26

Source: Frankel, E.N., *Lipid Oxidation*, 2nd ed., The Oily Press, Bridgwater, U.K., 2005.

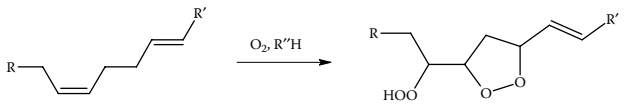


FIGURE 8.3

as the original material, but with different functional groups), oligomeric (products of higher molecular weight), and volatile (short-chain) compounds. Various types of reactions can occur during the decomposition process including dehydration, cyclization, rearrangement, radical substitution, chain cleavage, dimerization, etc. In many cases, a combination of these reactions occurs to form the final "secondary" oxidation product. The formation of some of the observed volatiles cannot be readily explained by classical cleavage mechanisms, but may be attributed to oxidation of the unsaturated aldehydes and, thus, may be "tertiary" products. Thermal and metal catalyzed reactions of hydroperoxides give homolytic cleavage while acidic decomposition proceeds by heterolytic cleavage, leading to less complex products consisting mainly of aldehydes.

The reactions that occur during the formation of the secondary products are influenced by parameters, such as temperature and the presence of light or air or extraneous materials, which also affect the initial oxidation step of hydroperoxide formation. It may be noted that the various secondary oxidation products listed in Table 8.6 are formed in different proportions.

The location of the functional groups in the chain is largely determined by the location of the original double bonds and, thus, the resulting hydroperoxides as given in Table 8.2 to Table 8.5. The unsaturated aldehydes formed as volatile secondary products are important because these are responsible for the off-flavor of foods containing oxidized oils. These flavor-affecting compounds are often formed in very small amounts (ppb levels). The differences noted in Table 8.6. for triacylglycerols and methyl esters show that using the latter as models for oxidation of triacylglycerols or fats and oils, in general, may not always be suitable. Discussing the details of the formation of all possible products is beyond the scope of this section. The reader is referred to more detailed references, such as the book by Frankel (2005) and some original literature (Evans et al., 1965; Frankel et al., 1960, 1961b, 1983, 1984b, 1988; Frankel and Neff, 1983, Lee and Blair, 2000, Schneider et al., 2001, 2004, 2005; Seppanen and Csallany, 2001, 2002; Spitteller et al., 2001; and Ullrich and Grosch, 1987).

8.1.5 Oxidation of cholesterol

Cholesterol (see Dictionary section for the structure) possesses a cyclic double bond as well as side chains containing tertiary carbons also susceptible to oxidation. Cholesterol oxides, although normally produced in normal cholesterol metabolism, negatively influence human health at higher levels through participation in developing atherosclerosis. Cholesterol oxides influence properties, such as stability, permeability, and fluidity, of the cell membrane. Fresh foods do not contain oxidized cholesterol; however, oxidized animal-based products are a major source of cholesterol oxides. Primary oxidation products include 7-α-OH-, 7-β-OH, and 7-keto-cholesterol, α- and β-epoxides, 3,5,6-trihydroxycholestero 1, and 20- and 25-hydroxycholesterol (Cuppett et al., 2003).

TABLE 8.6 Secondary oxidation products derived from the decomposition of hydroperoxides

Secondary products	Fatty compound			
	Oleate	**Linoleate**	**Linolenate**	**Triacylglycerols**
Monomeric	Allylic keto-oleates (keto at C8-11) Epoxy stearate Epoxy oleates Dihydroxystearates	Keto-linoleate (C9-C13) Epoxyhydroxy oleate Hydroxy dienes Dihydroxyoleates Trihydroxyoleates	Hydroperoxy epidioxides, dihydroperoxides	
Oligomeric		Dimers linked via peroxy or ether groups with OOH, OH or C=O groups C-C linked dimers (under N_2) Dimers and oligomers containing conjugated diene-triene, dihydroperoxides, or hydroperoxy epidioxide units		Di- and trihydroperoxides; less tendency to dimerize than with methyl esters; more significant at higher temperatures (frying), giving "thermal" and "oxidative" dimers
Volatiles	Saturated and unsaturated short-chain carbonyl compounds, alcohols, and hydrocarbons; examples given below			
	Heptanal, nonanal, decanal 2-nonenal, 2-undecenal heptane, octane oxo esters methyl nonanoate	From peroxide-linked dimers pentane, 1-pentanol, pentanal, hexanal, 3-nonenal, 2,4-decadienal, methyl octanoate, methyl 9-oxononanoate Others: acetaldehyde, 2-pentylfuran, methyl heptanoate, 2-octenal, 2,4-nonadienal, 2-4-decadienal, methyl 8-oxooctanoate, methyl 10-oxodecanoate; 2*c*-octenal, 2*t*-nonenal, 1-octene-3-one, 3-octene-2-one, 2*t*-octenal, 4-hydroxy-2-nonenal, 4-oxo-2-nonenal; volatile epoxy acids (*t*-2,3-epoxyoctanoic acid)	Decatrienal, methyl octanoate, propanal, 2-pentenal, 3-hexenal, 2,4-heptadienal, ethane, acetaldehyde, butanal, methyl 9-oxononanoate, 2-butenal, 2-butylfuran, 2*t*,6*c*-nonadienal, 1,5*c*-octadien-3-one, 3*c*-hexenal, 3-nonenal, hexanal; *t*-4,5-epoxy-2-heptenal	Oxoglycerides or aldehydo-glycerides From trilinolein: pentane, hexanal, 2-heptenal, 2,4-decadienal From trilinolenin: propanal, 2,4-heptadienal, 2,4,7-decetrienal In vegetable oils: ethane, propane, pentane, hexane. dialdehydes, ketones, ethyl esters, nonane, decane, undecane, 2-pentylfuran, lactones, benzene, benzaldehyde, acetophenone
Volatiles from secondary products		Alkanals, glyoxal, α-keto aldehydes, dialdehydes	Malonaldehyde (typical for lipids with three or more double bonds)	

Source: Adapted from Gunstone, F.D. (Ed.), in *The Chemistry of Fats and Oils,* Blackwell Publishing, Oxford, U.K., 2004, pp. 150–168; and Frankel, E.N., *Lipid Oxidation*, 2nd ed., The Oily Press, Bridgwater, U.K., 2005.

8.1.6 Antioxidants

Several approaches can be utilized to either prevent oxidation or decelerate its rate. One is obviously to prevent contact of the lipid material with air. Another is to avoid contact with pro-oxidants, elevated temperatures, or presence of light. Since these solutions are not always possible or practical, antioxidants are of significant interest. Their function has been described briefly in the preceding text.

Using antioxidants is not a method for preventing oxidation. Their use only retards the onset of oxidation, i.e., extends the induction period until the antioxidant is exhausted and oxidation commences. Preventing exposure of the lipid material to oxidation-promoting factors as far as possible, therefore, is advisable even when using antioxidants. The antioxidants discussed here affect autoxidation. Photo-oxidation is not affected by these materials, except for the connection between the two mechanisms discussed above. Quenchers of singlet oxygen, e.g., β-carotene, inhibit photo-oxidation.

Antioxidants occur either naturally in the lipid mixture, such as vitamin E (tocopherols and tocotrienols, four species of each, α, β, γ, δ, exist) or are deliberately added synthetic materials, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), *tert*-butythydroquinone (TBHQ), or propyl gallate (PG) (Figure 8.4). The level of natural antioxidants in vegetable oils is affected by the refining process. Phenols active as antioxidants are found in rosemary, sesame, tea, etc. Herbs and spices also represent a source of natural antioxidants. Vitamin C is an oxygen scavenger. Recently reported pyrimidinols (Figure 8.5) contain a strongly electron-donating

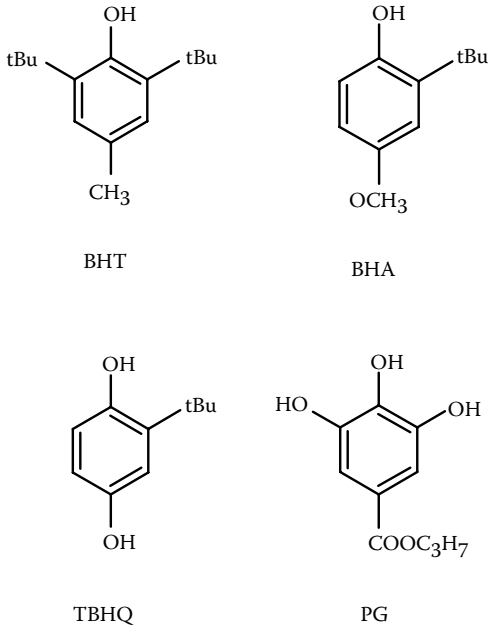


FIGURE 8.4

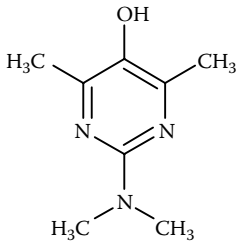


FIGURE 8.5

dialkylamino group and are among the strongest synthetic antioxidants known. Pyridinols, which have only one nitrogen in the ring may be even more effective (Wijtmans et al., 2004). Antioxidants, such as phenols and amines, have a hydrogen atom that can be "donated" to interrupt the chain reaction, whereby, for example, phenols become quinones. Alternatively, antioxidants can react with a radical in an additive fashion.

Not all synthetic antioxidants may be used everywhere. For example, TBHQ is not permitted for food use in the European Union (EU), while it is acceptable in the U.S. and other countries. The synthetic antioxidants also possess somewhat differing solubilities or effectiveness in oils and fats. For example, propyl gallate is probably the least soluble of the antioxidants in Figure 8.4. Some are synergistic with other antioxidants. They are generally used at levels up to 100 to 200 ppm.

The effectiveness of particular antioxidants can depend on a variety of factors, including the fatty acid profile of the oil or fat, the amount of naturally occurring antioxidants, as well as storage or other conditions. It is also possible to use more than one antioxidant to influence different stages of the reaction process. Synergistic effects between antioxidants can then influence their effectiveness.

Other materials with antioxidant effect include chelating agents, such as ethylenediamine tetraacetic acid (EDTA), citric acid, phosphoric acid, or amino acids, which help to remove metal ions. These materials are often described as secondary antioxidants. Oxygen scavengers or reducing agents, such as ascorbic acid, which can regenerate spent antioxidant, and singlet oxygen quenchers, such as β-carotene, are also used as oxidation inhibitors.

References

Brash, A.R., Autoxidation of methyl linoleate: identification of the *bis*-allylic 11-hydroperoxide, *Lipids,* **35**, 947–952, 2000.

Chan, H.W.-S., Photo-sensitized oxidation of unsaturated fatty acid methyl esters. The identification of different pathways, *J. Am. Oil Chem. Soc.,* **54**, 100–104, 1977.

Clements, A.H. et al. Participation of singlet oxygen in photosensitized oxidation of 1,4-dienoic systems and photooxidation of soybean oil, *J. Am. Oil Chem. Soc.,* **50**, 325–330, 1973.

Coxon, D.T. et al. Formation, isolation and structure determination of methyl linolenate diperoxides, *Chem. Phys. Lipids,* **28**, 365–378, 1981.

Cuppett, S.L., Cholesterol oxides: sources and health implications, *Lipid Technol. Newsl.,* **9**, 9–13, 2003.

Evans, C.D. et al. Chromatographic studies on oxidative and thermal fatty acid dimers, *J. Am. Oil Chem. Soc.,* **42**, 764–770, 1965.

Frankel, E.N. et al. Thermal dimerization of fatty ester hydroperoxides, *J. Am. Oil Chem. Soc.,* **37**, 418–424, 1960.

Frankel, E.N. et al. Autoxidation of methyl linolenate. isolation and characterization of hydroperoxides, *J. Org. Chem.*, **26**, 4663–4669, 1961a.

Frankel, E.N. et al. Analysis of lipids and oxidation products by partition chromatography. Dimeric and polymeric products, *J. Am. Oil Chem. Soc.,* **38**, 130–134, 1961b.

Frankel, E.N. et al. Analysis of autoxidized fats by gas chromatography-mass spectrometry. I. Methyl oleate, *Lipids,* **12**, 901–907, 1977a.

Frankel, E.N. et al. Analysis of autoxidized fats by gas chromatography-mass spectrometry. II. Methyl linoleate, *Lipids,* **12**, 908–913, 1977b.

Frankel, E.N. et al. Analysis of autoxidized fats by gas chromatography-mass spectrometry. III. Methyl linolenate, *Lipids,* **12**, 1055–1061, 1977c.

Frankel, E.N. et al. Analysis of autoxidized fats by gas chromatography-mass spectrometry. V. Photosensitized oxidation, *Lipids,* **14**, 961–967, 1979.

Frankel, E.N. and Neff, W.E., Formation of malonaldehyde from lipid oxidation products, *Biochim. Biophys. Acta*, **754**, 264–270, 1983.

Frankel, E.N. et al. Analysis of autoxidized fats by gas chromatography-mass spectrometry. VIII. Volatile thermal decomposition products of hydroperoxy cyclic peroxides, *Lipids,* **18**, 353–357, 1983.

Frankel, E.N. et al. Stereochemistry of olefin and fatty acid oxidation. III. The allylic hydroperoxides fom the autoxidation of methyl oleate. Methyl linolenate, *J. Chem. Soc., Perkin Trans.,* I, 2233–2240, 1984a.

Frankel, E.N. et al. Analysis of autoxidized fats by gas chromatography-mass spectrometry. IX. Homolytic vs. heterolytic cleavage of primary and secondary oxidation products, *Lipids,* **19**, 790–800, 1984b.

Frankel, E.N. et al. Analysis of autoxidized fats by gas chromatography-mass spectrometry. X. Volatile thermal decomposition products of methyl linolenate dimers, *Lipids,* **23**, 295–298, 1988.

Frankel, E.N. et al. Autoxidation of polyunsaturated triacylglycerols. II. Trilinolenoylglycerol, *Lipids,* **25**, 40–47, 1990.

Frankel, E.N., *Lipid Oxidation*, 2nd ed., The Oily Press, Bridgwater, U.K., 2005.

Gunstone, F.D. Ed., Oxidation through reaction with oxygen, in *The Chemistry of Fats and Oils*, Blackwell Publishing, Oxford, U.K., 2004, pp. 150–168.

Kamal-Eldin, A., and Pokorný, J. Eds., *Analysis of Lipid Oxidation*, AOCS Press, Champaign, IL, 2005.

Lee, S.H. and Blair, I.A., Characterization of 4-oxo-2-nonenal as a novel product of lipid peroxidation, *Chem. Res. Toxicol.,* **13**, 698–702, 2000.

Neff, W.E. et al. Photosensitized oxidation of methyl linoleate monohydroperoxides: hydroperoxy cyclic peroxides, dihydroperoxides, keto esters, and volatile thermal decomposition products, *Lipids,* **18**, 868–876, 1983.

Neff, W.E. and Frankel, E.N., Photosensitized oxidation of methyl linolenate and monohydroperoxides: hydroperoxy cyclic peroxides, dihydroperoxides, and hydroperoxy biscyclic peroxides, *Lipids,* **19**, 952–957, 1984.

Neff, W.E. et al. Autoxidative dimerization of methyl linolenate and its monohydroperoxides, hydroxy epidioxides and dihydroperoxides, *J. Am. Oil Chem. Soc.,* **65**, 616–623, 1988.

Neff, W.E. et al. Autoxidation of polyunsaturated triacylglycerols. I. Trilinoleoylglycerol, *Lipids,* **25**, 33–39, 1990.

Porter, N.A. and Wujek, D.G., Autoxidation of polyunsaturated fatty acids, an expanded mechanistic study, *J. Am. Chem. Soc.,* **106**, 2626–2629, 1984.

Porter, N.A. et al. Mechanisms of free radical oxidation of unsaturated lipids, *Lipids,* **30**, 277–290, 1995.

Rawls, H.R. and van Santen, P.J., A possible role of singlet oxygen in the initiation of fatty acid autoxidation, *J. Am. Oil Chem. Soc.,* **47**, 121–125, 1970.

Schneider, C. et al. Two distinct pathways of formation 4-hydroxynonenal. Mechanisms of nonenzymatic transformation of the 9- and 13-hydroperoxides of linoleic acid to 4-hydroxyalkenals, *J. Biol. Chem.,* **276**, 20831–20838, 2001.

Schneider, C. et al. Autoxidative transformation of chiral hydroxy linoleic and arachidonic acids to chiral 4-hydroxy-2(*E*)-nonenal, *Chem. Res. Toxicol*., **17**, 937–941, 2004.

Schneider, C. et al. Synthesis of dihydroperoxides of linoleic and linolenic acids and studies on their transformation to 4-hydroperoxynonenal, *Lipids,* **40**, 1155–1162, 2005.

Seppanen, C.M. and Csallany, A.S., Simultaneous determination of lipophilic aldehydes by high-performance liquid chromatography, *J. Am. Oil Chem. Soc.,* **78**, 1253–1260, 2001.

Seppanen, C.M. and Csallany, A.S., Formation of 4-hydroxynonenal, a toxic aldehyde, in soybean oil at frying temperature, *J. Am. Oil Chem. Soc.,* 79, 1033–1038, 2002.

Spitteller, P. et al. Aldehydic lipid peroxidation products from linoleic acid, *Biochim. Biophys. Acta,* **1531**, 188–208, 2001.

Tallman, K.A. et al. Factors influencing the autoxidation of fatty acids: effect of olefin geometry of the nonconjugated diene, *J. Amer. Chem. Soc.,* **126**, 9240–9247, 2004.

Terao, J. and Matsushita, S., Products formed by photosensitized oxidation of unsaturated fatty acid esters, *J. Am. Oil Chem. Soc.,* **54**, 234–238, 1977.

Terao, J. and Matsushita, S., The isomeric compositions of monohydroperoxides produced by oxidation of unsaturated fatty acid esters with singlet oxygen, *Food Process. Preserv.,* **3**, 329–337, 1980.

Ullrich, F. and Grosch, W., Identification of the most intense volatile flavour compounds formed during autoxidation of linoleic acid, *Z. Lebensm. Unters. Forsch.,* **184**, 277–282, 1987.

Wijtmans, M. et al. Synthesis and reactivity of some 6-substituted-2,4-dimethyl-3-pyridinols, a novel class of chainbreaking antioxidants, *J. Org. Chem.*, **69**, 9215–9223, 2004.

Yin, H., and Porter, N.A., New insights regarding the autoxidation of polyunsaturated fatty acids. *Antiox. Redox Sign.,* **7**, 170–184, 2005.

8.2 Enzymatic oxidation

Enzymatic oxidation often leads to products that are not available, or only with difficulty, by chemical synthesis. These reactions generally proceed with great stereospecificity. The products have interesting properties and can serve as intermediates in reaction sequences, with the other synthetic steps being of chemical nature. The *in vivo* oxidation of fatty compounds is discussed in Section 8.1. (Bornscheuer (2000). edited a book on this subject.)

8.2.1 Formation of hydroxy and keto fatty acids

Probably the most investigated reaction is the hydration of oleic acid to 10-hydroxystearic acid, which was first reported by Wallen et al. (1962). This reaction was then shown to proceed with great stereospecificity (Schroepfer and Bloch, 1963; Schroepfer, 1965), the product having *R* configuration. Subsequent mechanistic studies (Schroepfer, 1966; Niehaus et al., 1970b) were carried out. 10-ketooctadecanoic acid is also a common product of enzymatic oxidations and likely results from further oxidation of 10-hydroxyoctadecanoic acid. Another system that has found interest is the conversion of the double bond in oleic acid to a 2(*E*)-ene-1,4-diol with double bond shift to give 7,10-dihydroxy-8(*E*)-octadecenoic acid (Parra et al., 1990; Hou et al., 1991; Kuo et al., 1998) with this compound possessing *S*,*S* configuration (Gardner and Hou, 1999). Hydroxy fatty acids, including species obtained by enzymatic hydroxylation, possess good surfactant properties (Parra et al., 1990; Knothe et al., 1995), with a ricinoleic acid-derived trihydroxylated species (7,10,12-trihydroxy-8(*E*)-octadecenoic acid) displaying anomalous surface tension behavior (Kuo and Knothe, 2004).

These processes are very sensitive to substrate structure. For example, while a free fatty acid may undergo oxidation, its alkyl esters may not. Products and/or yields can also differ depending on chain length as well as double bond position, configuration, and number. Different species can give the same product when using the same fatty substrate, but yields may differ. Some of these processes may proceed via isolable intermediates that are themselves of interest. Glycolipidic compounds, formed from fatty compounds and carbohydrates present in the culture broth, may also arise during such reactions.

A selection of various oxygenated fatty acid derivatives available by such reactions is listed in Table 8.7. Details of the procedures are available from these references. Some of the reactions also lead to minor products, which are not listed, but are discussed in the references. Some review articles are by Kuo and Hou (1999), Hou (2000), and Kuo et al. (2002a).

While the reactions discussed above usually occur with unsaturated fatty acids, a double bond need not always be present for enzymatic hydroxylation. Thus, the α–oxidation enzyme system of peas in the presence of molecular oxygen was used in the formation of enantiomerically pure 2(*R*)-hydroxy acids, with saturated C_7-C_{16} fatty acids and oleic acid serving as substrates (Adam et al., 1996, 1998).

8.2.2 Formation of hydroperoxides

Generally, lipoxygenases (LOX) are iron-containing enzymes that are widely distributed through the plant kingdom and catalyze the formation of hydroperoxides (-OOH moiety) from polyunsaturated fatty acids. The natural substrate is linoleic acid, which is converted to 13(*S*)-hydroperoxyoctadeca-9(*Z*),11(*E*)-dienoic acid and/or 9(*R*)-hydroperoxyoctadeca-10(*E*),12(*Z*)-dienoic acid. Attack is initiated by removal of hydrogen from C-(*n*-8) between the two double bonds. Removal of the pro-*S* hydrogen atoms leads to the 13(*S*)-hydroperoxide, while removal of the pro-*R* hydrogen atom leads to the 9(*R*)-hydroperoxide.

However, LOX containing other metals may also be effective. A manganese-containing linoleic 13(*R*) LOX from the fungus *Gäumannomyces graminis* was purified and characterized (Su and Oliw, 1998). It oxidizes linolenic acid about twice as quickly as linoleic acid. Incubation with linoleic acid yields 13(*R*)-hydroperoxy-9(*Z*),11(*E*)-octadecadienoic acid and a new hydroperoxide, 11(*S*)-hydroperoxy-9(*Z*),12(*Z*)-octadecadienoic acid (Hamberg et al., 1998). The kinetics of manganese LOX were investigated later and it was suggested that its kinetic and redox properties are similar to those of iron LOX (Su et al., 2000). It was then shown that the aforementioned 11-hydroperoxide compound can also be formed by an iron-dependent LOX (Oliw et al., 2004).

The detailed composition of the hydroperoxide mixture depends on the source of the enzymes and also, to some extent, on the pH and the oxygen pressure. Enzymes from flaxseed, soybean, peanut, or eggplant (aubergine) produce the 13-hydroperoxide mainly; the 9-hydroperoxide is the main product with enzyme preparations from rice, corn, potato, and tomato. The oxidation process is inhibited by eicosa-5,8,11,14-tetraynoic acid (the acetylenic analogue of arachidonic acid). A stereochemical investigation of soybean LOX catalysis was reported by Coffa et al. (2005). γ-Linolenic acid gives its 9-hydroperoxide (6*Z*,8*E*,12*Z*) with soybean lipoxygenase in a maximum yield of 35% using a hexane-aqueous, two-phase system at pH 6.5 and 10°C (Hiruta et al., 1988). A facile method for preparing 9-hydroperoxides is LOX from maize (corn) *Zea mays,* which oxidizes linoleic and linolenic acid to give 9(*S*)-hydroperoxy-10(*E*),12(*Z*)-octadecadienoic and 9(*S*)-hydroperoxy-10(*E*),12(Z),15(*Z*)-octadecatrienoic acid, respectively (Gardner and Grove, 2001). The LOX of sperm whale myoglobin oxidizes linoleic acid to an 84:16 mixture of the 9(*S*):9(*R*) enantiomers of 9-hydroperoxy-10(*E*),12(*Z*)-octadecadienoic acid (Rao et al., 1994).

TABLE 8.7 Starting materials used and products obtained from enzymatic oxidations

Starting material	Product	Species	References
Oleic acid	10-Hydroxyoctadecanoic acid	*Pseudomonas* species	Wallen et al. (1962)
Oleic acid	10-Hydroxyoctadecanoic acid	*Saccharomyces cerevisiae*	El-Sharkawy et al. (1992)
Oleic acid	10-Hydroxyoctadecanoic acid	*Nocardia cholesterolicum*	Koritala et al. (1989)
Oleic acid	10-Hydroxyoctadecanoic acid	*Nocardia paraffinae*	Latrasse et al. (1997)
Oleic acid	10-Hydroxyoctadecanoic acid, 10-ketooctadecanoic acid	*Corynebacterium* sp. S-401	Seo et al. (1981)
Oleic acid	10-Ketooctadecanoic acid	*Nocardia aurantia*, *Nocardia* sp. (NRRL 5646), *Mycobacterium fortuitum*	El-Sharkawy et al. (1992)
		Flavobacterium (NRRL B-14859)	Hou (1994a)
Oleic acid	10-Ketooctadecanoic acid	*Staphylococcus warneri*	Lanser and Nakamura (1996)
Oleic acid	10-Ketooctadecanoic acid	*Sphingobacterium* sp. O22	Kuo et al. (1999)
Oleic acid	10-Ketooctadecanoic acid	*Bacillus sphaericus*	Kuo et al. (2002b)
Oleic acid	10-Ketooctadecanoic acid, 4-ketostearic acid, 1,7-heptanedicarboyxlic acid	*Sarcina lutea*	Blank et al. (1991)
Oleic acid	10-Hydroxyoctadecanoic acid, 10-ketooctadecanoic acid, 4-ketododecanoic acid	*Micrococcus luteus*	Esaki et al. (1994)
Oleic acid	15-, 16-, 17-Hydroxy-9-octadecenoic acids	*Bacillus pumilus*	Lanser et al. (1992)
Oleic acid	7,10-Dihydroxy-8(*E*)-octadecenoic acid	*Pseudomonas* 44T1	Parra et al. (1990)
		Pseudomonas 42A2	Hou et al. (1991)
		Pseudomonas aeruginosa PR3.	Kuo et al. (1998)
Oleic acid	10-Hydroxy-8(*E*)-octadecenoic acid	*Pseudomonas* 42A2	Guerrero et al. (1997)
Oleic acid	10-Hydroperoxy-8(*E*)-octadecenoic acid	*Pseudomonas* 42A2	Guerrero et al. (1997)
Oleic acid	7-Hydroxy-16-oxo-9-*cis*-octadecenoic acid 7-Hydroxy-17-oxo-9-*cis*-octadecenoic acid	*Bacillus* NRRL BD-447	Lanser et al. (1998) Lanser & Manthey (1999)
Ricinoleic acid	7,10,12-Trihydroxy-8(*E*)-octadecenoic acid	*Pseudomonas aeruginosa* PR3.	Kuo et al. (1998)
Ricinoleic acid	10,12-Trihydroxy-8(*E*)-octadecenoic acid	*Pseudomonas aeruginosa* PR3.	Kim et al. (2000)
Ricinoleic acid	γ-Decalactone, 4-hydroxydecanoic acid	*Sporidiobolus salmonicolor*, *S. ruineii*	Feron et al. (1996)
12-Hydroxyoctadecanoic acid	5-*n*-Hexyl-Tetrahydrofuran-2-Acetic Acid	*Corynebacterium* (FUI-2) / *bacillus* (NRRL B-14864)	Huang et al. (1995)
9,10-Epoxyoctadecanoic acid[a]	9,10-Dihydroxyoctadecanoic acid	*Pseudomonas* species	Niehaus et al. (1970b)
Linoleic acid	10-Hydroxy-12(*Z*)-octadecenoic acid	*Pseudomonas* species	Schroepfer et al. (1970)
		Flavobacterium sp. NRRL B-14859	Hou (1994b)
		Nocardia cholesterolicum	Koritala and Bagby (1992)
Linoleic acid	9,10,13-Trihydroxy-11(*E*)-octadecenoic acid; 9,12,13-Trihydroxy-(10*E*)-octadecenoic acid	*Pseudomonas aeruginosa* PR3	Kim et al. (2000)
Linoleic acid	12,13.17-Trihydroxy-9(*Z*)-octadecenoic acid; 12-Hydroxy-13,16-epoxy-9(*Z*)-octadecenoic acid; 7,12-Dihydroxy-13,16-epoxy-9(*Z*)-octadecenoic acid; bicyclic fatty acids	*Clavibacter* sp. ALA2	Hou (1996) Gardner et al. (2000)
Linoleic acid	12,13,16-Trihydroxy-9(*Z*)-octadecenoic acid	*Clavibacter* sp. ALA2	Hou et al. (2001).
12,13,16-Trihydroxy-9(*Z*)-octadecenoic acid	12-Hydroxy-13,16-epoxy-9(*Z*)-octadecenoic acid; 7,12-Dihydroxy-13,16-epoxy-9(*Z*)-octadecenoic acid	*Clavibacter* sp. ALA2	Hosokawa et al. (2003b)

(Continued)

TABLE 8.7 continued

Starting material	Product	Species	References
Linolenic acid	10-Hydroxy-12(*Z*),15(*Z*)-octadecadienoic acid	*Nocardia cholesterolicum*	Koritala and Bagby (1992)
α- and γ-Linolenic acids	10-Hydroxy-12(*Z*),15(*Z*)-octadecadienoic acid; 10-Hydroxy-6(*Z*),12(*Z*)-octadecadienoic acid	*Flavobacterium* sp. DS5	Hou et al. (1995)
n-3 and n-6 polyunsaturated fatty acids	Various hydroxy furanoid fatty acids depending if n-3 or n-6 substrates used	*Clavibacter* sp. ALA2	Hosokawa et al. (2003a)
Various saturated and unsaturated fatty acids	(T-1)-, (T-2), and (T-3) hydroxy fatty acids	*Bacillus megaterium*	Miura and Fulco (1974)

[a] The *cis*- and *trans*-epoxy moieties give *threo*- and *erythro*-dihydroxy, respectively.

Similar hydroperoxides are produced from *n*–3 (18:3, 18:4, 19:3, 20:3, 20:5 and 22:6) and *n*–6 (17:3, 18:3, 19:3, 20:2, 20:3, 20:4, 21:3, 21:4 and 22:3) acids, all of which contain the n-6, n-9 diene unit, as well as from the (*n*–4)-16:3, (*n*–5)-17:3, and (*n*–7)-18:3 acids. LOX from potato tuber catalyzed the aerobic formation of four major derivatives of DHA (docosahexaenoic acid), two of which (7,17(*S*)-and 10,17(*S*)-dihydroperoxy-DHA) were also formed from soybean LOX (Butovich et al., 2005). The new materials were 10(*S*)-hydroperoxy-DHA and 10,20-dihydroperoxy-DHA.

Among the complete series of methylene-interrupted 18:2 acids, the 9,12 isomer is the best substrate, but there is significant oxidation of one other isomer (13*Z*,16*Z*), which gives the 17(*S*)- and 13-hydroperoxides in an 85:15 ratio. Acids with an appropriately placed 1,4,7-triene unit yield a monohydroperoxide fairly quickly and then a bis-hydroperoxide more slowly if the monohydroperoxide still contains a pentadiene unit. Thus α-linolenate gives only the 13-hydroperoxide whereas γ-linolenate gives the 6,13-*bis*-hydroperoxide.

Conjugated dienes result from enzymatic oxidation of three triene acids (9*Z*,12*Z*,15*Z*; 5*Z*,9*Z*,12*Z*; 5*E*,9*Z*,12*Z*) almost as efficiently as from linoleic acid. Other polyene acids, such as γ-linolenic acid, arachidonic acid, EPA, and DHA, give lower yields of conjugated diene because the hydroperoxide readily oxidizes to a *bis*-hydroperoxide with conjugated triene unsaturation. This further oxidation is apparent at pH 9, but is inhibited at pH 11 (Takagi et al., 1987).

Secondary products can arise from the decomposition, rearrangement or other reactions of hydroperoxy compounds. Those produced from the 9- and 13-hydroperoxides of linoleic acid include oxo-2,4-dienes, hydroxy-2,4-dienes, hydroxyepoxides, ketols (2-ene-1-ol-4-ones; 4-ene-1-ol-2-ones), ketodiols, 1,2- and 1,4-diols, triols as well as unsaturated fatty ethers. The detailed stereochemistry of the 9,10,12- and 9,12,13-trihydroxy acids obtained from the 9- and 13-linoleate hydroperoxides has been reported (Hamberg, 1991). Colneleic and colnelenic acids are formed by rearrangement of the appropriate 9-hydroperoxy derivatives of linoleic and α-linolenic acids produced by enzymes in potato tuber. They are 18:3 and 18:4 fatty acids, respectively, with an oxygen atom inserted between C9 and C10, a double bond at C8/9 and two conjugated double bonds extending from C10 as well as a double bond at C15 in case of colnelenic acid (Crombie et al., 1991, see also Section 8.1 for the compound structure). A hydroperoxy lyase from the alga *Chlorella pyrenoidosa*) cleaves the 13-hydroperoxy derivatives of linoleic and linolenic acids into a volatile component and 13-oxo–9(*Z*),11(*E*)-tridecadienoic acid (Nuñez et al., 2000). The volatile component was pentane in case of the 13-hydroperoxide derivative of linoleic acid, while it was a more complex mixture in case of the 13-hydroperoxide derivative of linolenic acid.

Other products are only produced in compounds with more than two double bonds, such as a fatty acid with a cyclopentenone moiety produced from linolenic acid by the flaxseed enzyme and the compounds produced from linolenic acid and the soybean flour enzyme(s).

Other plant systems cause hydroperoxides to degrade to short-chain aldehydes, which have important flavor properties. For example, when cucumber cells are broken and the enzymes liberated, lipolysis, enzymatic oxidation, and subsequent chain fission give hexanal (from 18:2), non-2-enal (from 18:2), nona-2,6-dienal (from 18:3), $OHC(CH_2)_7COOH$ (from 18:2 and 18:3), and $OHCCH{=}CH(CH_2)_8COOH$ (from 18:2 and 18:3). Similar products have been identified in tomatoes and in tea leaves.

Fatty acid hydroperoxides can be rapidly identified without derivatization by liquid chromatography with electrospray ionization — tandem mass spectrometry (Schneider et al., 1997). The position of the hydroperoxy units was also determined by liquid chromatography in conjunction with electron impact mass spectrometry, also not requiring extensive derivatization of the thermally unstable hydroperoxides for GC-MS (Nuñez et al., 2001). The results in this work indicated that two lipoxygenases interacting with linoleic acid produce the 13- and 9-hydroperoxy derivatives and that the 9-lipoxygenase enzyme cleaves the 13-hydroperoxy derivative to 13-oxo-tridecedieonic acid and pentane, but does not affect the

9-hydroperoxy derivative. Analytical methods for lipid hydroperoxides have been reviewed (Dobarganes and Velasco, 2002).

Epoxidation of fatty acid chains can be also carried out enzymatically. Enzymatic reactions giving epoxidized products are discussed in Section 8.3.

References

Adam, W. et al. Enantioselective α-hydroxylation of carboxylic acids with molecular oxygen catalyzed by the α-oxidation enzyme system of young pea leaves (*Pisum sativum*): A substrate selectivity study, *Tetrahed.: Asymm.*, **7**, 2287–2292, 1996.

Adam, W. et al. α-Hydroxylation of carboxylic acids with molecular oxygen catalyzed by the α-oxidase of peas (Pisum sativum): a novel biocatalytic synthesis of enantiomerically pure (R)-2-hydroxy acids, *J. Am. Chem. Soc.*, **120**, 11044–11048, 1998.

Blank, W. et al. Transformation of oleic acid and its esters by *Sarcina lutea*, *Agric. Biol. Chem.,* **55**, 2651–2652, 1991.

Bornscheuer, U.T. Ed. *Enzymes in Lipid Modification*. Wiley-VCH, Weinheim Germany, 2000.

Butovich, I.A. et al. Novel oxylipins formed from docosahexaenoic acid by potato lipoxygenase — 10(*S*)-hydroxydocosahexenoic acid and 10,20-dihydroxydocosahexaenoic acid, *Lipids*, **40**, 249–257, 2005.

Coffa, G. et al. On the relationships of substrate orientation, hydrogen abstraction, and product stereochemistry in single and double dioxygenations by soybean lipoxygenase-1 and its Ala542Gly mutant, *J. Biol. Chem.,* **280**, 38756–38766, 2005.

Crombie, L. et al. An isotopic study (^{2}H and ^{18}O) of the enzymic conversion of linoleic acid into colneleic acid with carbon chain fracture: the origin of shorter chain aldehydes, *J. Chem. Soc., Perkin Trans.* I, 567–575, 1991.

Dobarganes, M.C. and Velasco, J., Analysis of lipid hydroperoxides, *Eur. J. Lipid Sci. Technol.,* **104**, 420–408 (2002).

El-Sharkawy, S.H. et al. Microbial oxidation of oleic acid, *Appl. Environm. Microbiol.,* **58**, 2116–2122, 1992.

Esaki, N. et al. Biotransformation of oleic acid by *Micrococcus luteus* cells, *Biosci. Biotech. Biochem.,* **58**, 319–321, 1994.

Feron, G. et al. Production, identification, and toxicity of γ-decalactone and 4-hydroxydecanoic acid from *Sporidiobolus* spp., *Appl. Environm. Microbiol.,* **62**, 2826–2831, 1996.

Gardner, H.W. and Grove, M.J., Method to produce 9(*S*)-hydroperoxides on linoleic and linolenic acids by maize lipoxygenase, *Lipids,* **36**, 529–533, 2001.

Gardner, H.W. and Hou, C.T., All (*S*) stereoconfiguration of 7,10-8(*E*)-octadecenoic acid from bioconversion of oleic acid by *Pseudomonas aeruginosa*, *J. Am. Oil Chem. Soc.,* **76**, 1151–1156, 1999.

Gardner, H.W. et al. Biotransformation of linoleic acid by *Clavibacter* sp. ALA2: Heterocyclic and heterobicyclic fatty acids, *Lipids,* **35**, 1055–1060, 2000.

Guerrero, A. et al. Oxidation of oleic acid to (*E*)-10-hydroperoxy-8-octadecenoic and (*E*)-10-hydroxy-8-octadecenoic acids by *Pseudomonas* sp. 42A2, *Biochim. Biophys. Acta,* **1347**, 75–81, 1997.

Hamberg, M., Regio- and stereochemical analysis of trihydroxyoctadecenoic acids derived from linoleic acid 9- and 13-hydroperoxides, *Lipids*, **26**, 407–415, 1991.

Hamberg, M. et al. Manganese lipoxygenase. Discovery of a *bis*-allylic hydroperoxide as product and intermediate in a lipoxygenase reaction, *J. Biol. Chem.,* **273**, 13080–13088, 1998.

Hiruta, O. et al. Production of 9-hydroperoxy-γ-linolenic acid by soybean lipoxygenase in a two-phase system, *J. Am. Oil Chem. Soc*., **65**, 1911-1914, 1988.

Hosokawa, M. et al. Bioconversion of n-3 and n-6 PUFA by *Clavibacter* sp. ALA2, *J. Am. Oil Chem. Soc.,* **80**, 1085–1091, 2003a.

Hosokawa, M. et al. Biosynthesis of tetrahydrofuranyl fatty acids from linoleic acid by *Clavibacter* sp. ALA2, *J. Am. Oil Chem. Soc.,* **80**, 145–149, 2003b.

Hou, C.T., Production of 10-ketostearic acid from oleic acid by *Flavobacterium* sp. Strain DS5 (NRRL B-14859), *Appl. Environm. Microbiol.,* **60**, 3760–3763, 1994a.

Hou, C.T., Conversion of linoleic acid to 10-hydroxy-12(*Z*)-octadecenoic acid by *Flavobacterium* sp. (NRRL B-14859), *J. Am. Oil Chem. Soc.,* **71**, 975–978, 1994b.

Hou, C.T., Production of hydroxy fatty acids from unsaturated fatty acids by *Flavobacterium* sp. DS5 hydratase, a C-10 positional- and *cis* unsaturation-specific enzyme, *J. Am. Oil Chem. Soc.,* **72**, 1265–1270, 1995.

Hou, C.T., A novel compound, 12,13,17-trihydroxy-9(*Z*)-octadecenoic acid, from linoleic acid by a new microbial isolate *Clavibacter* sp. ALA2, *J. Am. Oil Chem. Soc.,* **73**, 1359–1362, 1996.

Hou, C.T., Biotransformation of unsaturated fatty acids to industrial products, *Adv. Appl. Microbiol*., **47**, 201–220, 2000.

Hou, C.T. et al. A novel compound, 7,10-dihydroxy-8(*E*)-octadecenoic acid, from oleic acid by bioconversion, *J. Am. Oil Chem. Soc.,* **68**, 99–101, 1991.

Hou, C.T. et al. 12,13,16-Trihydroxy-9(*Z*)-octadecenoic acid, a possible intermediate in the bioconversion of linoleic acid to tetrahydrofuranyl fatty acids by *Clavibacter* sp. ALA2, *J. Am. Oil Chem. Soc.,* **78**, 1167–1169, 2001.

Huang, J.-K. et al. Microbial transformation of 12-hydroxyoctadecanoic acid to 5-*n*-hexyl-tetrahydrofuran-2-acetic acid, *J. Am. Oil Chem. Soc.,* **72**, 323–326, 1995.

Kim, H. et al. Production of isomeric 9,10,13 (9,12,13)-trihydroxy-11(*E*) (10*E*)-octadecenoic acid from linoleic acid by *Pseudomonas aeruginosa* PR3, *J. Industr. Microbiol. Biotech,*. **25**, 109–115, 2000.

Kim, H. et al. Production of 10,12-dihydroxy-8(*E*)-octadecenoic acid, an intermediate in the conversion of ricinoleic acid to 7,10,12-trihydroxy-8(*E*)-octadecenoic acid by *Pseudomonas aeruginosa* PR3, *J. Industr. Microbiol. Biotechnol.,* **24**, 167–712, 2000.

Knothe, G. et al. Surface tension studies on novel allylic mono- and dihydroxy Fatty Compounds. A method to distinguish *erythro* / *threo* diastereomers, *J. Am. Oil Chem. Soc.,* **72**, 43–47, 1995.

Koritala, S. and Bagby, M.O., Microbial conversion of linoleic and linolenic acids to unsaturated hydroxy fatty acids, *J. Am. Oil Chem. Soc.,* **69**, 575578, 1992.

Koritala, S. et al. Microbial conversion of oleic acid to 10-hydroxystearic acid, *Appl. Microbiol. Biotechnol.,* **32**, 299–304, 1989.

Kuo, T.M. and Hou, C.T., Bioconversion of unsaturated fatty acids by *Pseudomonas aeruginosa* PR3, *Recent Res. Devel. Oil Chem.,* **3**, 1–10, 1999.

Kuo, T.M. and Knothe, G., Production and properties of 7,10,12-trihydroxy-8(*E*)-octadecenoic acid from ricinoleic acid conversion by *Pseudomonas aeruginosa*, *Eur. J. Lipid Sci. Technol.,* **106**, 405–411, 2004.

Kuo, T.M. et al. Fatty acid bioconversions by *Pseudomonas aeruginosa* PR3, *J. Am. Oil Chem. Soc.,* **76**, 875–879, 1998.

Kuo, T.M. et al. Microbiological conversions of fatty acids to value-added products. In *Lipid Biotechnology*, Marcel Dekker, New York, Basel, T.M. Kuo and H.W. Gardner, Eds., pp. 605–628, 2002.

Kuo, T.M. et al. Conversion of oleic acid to 10-ketostearic acid by *Sphingobacterium* sp. 22, *J. Am. Oil Chem. Soc.,* **76**, 709–712, 1999.

Kuo, T.M. et al. Production of 10-ketostearic acid and 10-hydroxystearic acid by strains of *Shingobacterium thalpophilum* isolated from composted manure, *Curr. Microbiol.,* **40**, 105–109, 2000.

Kuo, T.M. et al. Conversion of fatty acids by *Bacillus sphaericus*-like organisms, *Curr. Microbiol.,* **45**, 265–271, 2002b.

Lanser, A.C., Bioconversion of oleic acid to a series of keto-hydroxy and dihydroxy acids by *Bacillus* species NRRL BD-447: Identification of 7-hydroxy-16-oxo-9-*cis*-octadecenoic acid, *J. Am. Oil Chem. Soc.,* **75**, 1809–1813, 1998.

Lanser, A.C. and Manthey, L.K., Bioconversion of Oleic acid to a series of keto-hydroxy and dihydroxy acids by *Bacillus* Species NRRL BD-447: Identification of 7-hydroxy-16-oxo-9-*cis*-octadecenoic acid. *J. Am. Oil Chem. Soc.,* **76**, 1023–1026, 1999.

Lanser, A.C. and Nakamura, L.K.,, Identification of a *Staphylococcus warneri* species that converts oleic acid to 10-ketostearic acid, *Curr. Microbiol.,* **32**, 260–263, 1996.

Lanser, A.C. et al. Production of 15-, 16-, 17-hydroxy-9-octadecenoic acids by bioconversion of oleic acid with *Bacillus pumilus*, *J. Am. Oil Chem. Soc.,* **69**, 363–366, 1992.

Latrasse, A. et al. Conversion of oleic acid to 10-hydroxystearic acid by *Nocardia paraffinae*, *Biotechnol. Lett.,* **198**, 715–718, 1997.

Miura, Y. and Fulco, A.J., (T–2) Hydroxylation of fatty acids by a soluble system from *Bacillus megaterium*. *J. Biol. Chem.,* **249**, 1880–1888, 1974.

Niehaus, Jr. et al. Stereospecific hydration of the Δ^9 double bond of oleic acid, *J. Biol. Chem.,* **245**, 3790–3797, 1970a.

Niehaus, Jr., W.G. et al. Stereospecific hydration of *cis*- and *trans*-9,10-epoxyoctadecanoic acids,. *J. Biol. Chem.,* **245**, 3802–3809, 1970b.

Nuñez, A. et al. Anaerobic lipoxygenase activity from *Chlorella pyrenoidosa* responsible for the cleavage of the 13-hydroperoxides of linoleic and linolenic acids, *Eur. J. Lipid Sci. Technol.,* **102**, 181–188, 2000.

Nuñez, A. et al. Characterization of lipoxygenase oxidation products by high-performance liquid chromatography with electron impact-mass spectrometric detection, *Lipids*, **36**, 851–856, 2001.

Oliw, E.H. et al. Biosynthesis and isomerization of 11-hydroperoxylinoleates by manganese- and iron-dependent lipoxygenases, *Lipids,* **39**, 319–323, 2004.

Parra, J.L. et al. Studies of biosurfactants obtained from olive oil. *Tenside, Surfactants, Deterg.,* **27**, 302–306, 1990.

Rao, S.I. et al. The lipoxygenase activity of myoglobin. Oxidation of linoleic acid by the ferryl oxygen rather than protein radical, *J. Biol. Chem.,* **269**, 7210–7216, 1994.

Schneider, C. et al. Analysis of lipoxygenase-derived fatty acid hydroperoxides by electrospray ionization tandem mass spectrometry, *Lipids*, **32**, 331–336, 1997.

Schroepfer, G.J., Jr., Enzymatic stereospecificity in the conversion of oleic acid to 10-hydroxystearic acid, *J. Am. Chem. Soc.,* **87**, 1411–1412, 1965.

Schroepfer, G.J., Jr., Stereospecific conversion of oleic acid to 10-hydroxystearic acid, *J. Biol. Chem.,* **241** (22), 5441–5447, 1966.

Schroepfer, G.J., Jr. and Bloch, K., Enzymatic stereospecificity in the dehydrogenation of stearic acid to oleic acid, *J. Am. Chem. Soc.,* **85**, 3310–3311, 1963.

Schroepfer, G.J., Jr. et al. Enzymatic conversion of linoleic acid to 10D-hydroxy-)^{12}-*cis*-octadecenoic acid, *J. Biol. Chem.,* **245**, 3798–3801, 1970.

Seo, C.W. et al. Hydration of squalene and oleic acid by *Corynebacterium* sp. S-401. *Agric. Biol. Chem.,* **45**, 2025–2030, 1981.

Su, C. and Oliw, E., Manganese lipoxygenase. Purifications and characterization, *J. Biol. Chem.,* **273**, 13072–13079, 1998.

Su, C. et al. Kinetics of manganese lipoxygenase with a catalytic mononuclear redox center, *J. Biol. Chem.,*. **275**, 18830–18835, 2000.

Takagi, T. et al. Formation of conjugated diene and triene products in lipoxygenase oxidation of C18, C20, C22 PUFAs, *J. Am. Oil Chem. Soc*., **64**, 1320–1323, 1987.

Wallen, L.L. et al. The microbiological production of 10-hydroxystearic acid from oleic acid, *Arch. Biochem. Biophys.,* **99**, 249–253, 1962.

8.3 Epoxidation, hydroxylation and oxidative fission

8.3.1 Epoxidation

Epoxidation, the reaction by which a double bond is converted to an epoxide, is probably one of the most commonly applied procedures in fatty acid chemistry and is carried out both on laboratory and commercial scales. Gunstone (1997) has provided an overview of epoxidized oils. Numerous naturally occurring epoxy fatty acids are known (Section 1.1.2.10). Some reactions in which epoxides serve as intermediates in multistep sequences are mentioned in other sections of this chapter. Epoxidation reactions are exothermic; high concentrations of peroxy acids should be avoided and the epoxy products themselves are unstable towards acids. Fatty acid composition of an oil or fat influences the epoxidation kinetics (La Scala and Wool, 2002).

Epoxidized vegetable oils are of significant commercial interest. A common application is the use of epoxidized vegetable oil as plasticizer-stabilizer additive for poly(vinyl chloride) (PVC). The application in powder coatings has

been discussed (Buisman et al., 1999). Other uses include lubricating oils, composites, paint diluents, etc. *Vernonia galamensis* seed oil, a rich source of vernolic acid, was studied as a source of C_8, C_9, and C_{12} dibasic acids by oxidation with nitric or chromic acids (Ayorinde et al., 1988, 1989). Reaction of epoxide with a polyol gives a polyhydroxy product of high molecular weight, which can be used to make polyurethanes. Epoxides of tall oil methyl esters were converted to polyethylene glycol ethers with potential application as surfactants (Hedman et al., 2003). The triethylamine salt of 10,11-epoxyundecanoic acid shows rust-inhibiting properties in a water-based cutting fluid (Watanabe et al., 1988). Fatty epoxides are also reported to show biological effects, such as antifungal action against rice blast disease, serving in controlling secondary metabolism, and being active against microbial and pathogenic attacks in the cutin of green plants (Omar et al., 2003).

The epoxidation reaction proceeds by *cis* addition. Thus, oleic acid yields *cis*-9,10-epoxystearic acid and elaidic acid affords *trans*-9,10-epoxystearic acid. Linoleic acid furnishes *cis*-9,10, *cis*-12,13-diepoxystearic acid. This exists in two (racemic) stereochemical forms, which have been separated by crystallization. Partial epoxidation of methyl linoleate gives diepoxide, unreacted linoleate and a mixture of two monoepoxides (*cis*-9,10-epoxy 12c-18:1 and *cis*-12,13-epoxy 9c-18:1).

Most epoxidations are carried out with peroxy acids, which are often preformed and used *in situ* for epoxidation, though some can be stored and used as required. The peroxy acids are made by interaction of carboxylic acids, anhydrides or acid chlorides with hydrogen peroxide, with the first of these requiring an acidic catalyst, such as sulphuric acid or sulphonic acid (Gunstone, 1979). Additionally, peroxyacetic acid is made by oxidation of ethanal. The most commonly used peroxy acids include aliphatic (peroxyformic, peroxyacetic, peroxytrifluoroacetic, peroxylauric), aromatic (peroxybenzoic, m-chloroperoxybenzoic) species as well as monoperoxy acids based on dibasic acids (succinic, maleic, phthalic). These peroxy acids are unlikely to be pure and should be analyzed before use. Epoxidation with 3-chloroperbenzoic acid occurs in 3 to 5 min in high yield in a microwave oven (Lie Ken Jie and Cheung, 1988), and a smooth epoxidation in aqueous suspension at 0°C in 30 to 60 min was also reported (Fringuelli et al., 1989). Routes leading to the selective epoxidation of arachidonic acid have been described (Corey et al., 1979). It was shown that peroxyarachidonic acid undergoes intramolecular reaction to give only *cis*-14,15-epoxy 20:3, possibly via a cyclic intermediate. Selective epoxidation of the 5,6 double bond can be achieved by the iodolactonization reaction as discussed later in this chapter.

Other epoxidizing reagents that have been studied, but not necessarily extensively used, with olefinic acids include TBHP and transition-metal salts, hydrogen peroxide and isocyanate, sodium chlorate and osmium tetroxide, iodine and silver oxide, and hydrogen peroxide and ortho esters. The epoxidation of a conjugated linoleic acid isomer, methyl (9*Z*,11*E*)-octadecadienoate, with various epoxidizing reagents, such as m-chloroperoxybenzoic acid, dimethyl dioxirane, methyltrioxorhenium/H_2O_2, potassium peroxomonosulfate (Oxone, $2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$)/tetrahydrothiopyran-4-one, and Novozyme 435/H_2O_2 yielded the corresponding mono-epoxy (methyl (11,12*E*)-epoxy-(9*Z*)-octadecenoate and methyl (9,10*Z*)-epoxy-(11*E*)-octadecenoate) and a mixture of diastereomers of *syn*- and *anti*-diepoxy-stearate (methyl (9,10*Z*;11,12*E*)-diepoxystearate) (Lie Ken Jie et al., 2003). A previous use of potassium peroxomonosulfate is reported by Lie Ken Jie and Pasha (1998). An overview of various catalysts that have been investigated for the epoxidation reaction, including methyltrioxorhenium (MTORe; CH_3ReO_3), tungstates (for example, Na_2WO_4-H_3PO_4), and titanosilicates was provided by Piazza (1999). The use of titanosilicates (Campanella et al., 2004, Guidotti et al., 2003) and MTORe (Gerbase et al., 2002; Du et al., 2004) was investigated in some additional publications.

Asymmetric Sharpless epoxidation can be used to enrich specific isomers in the epoxidized product mixture. Thus, the reaction of methyl 13(*S*)-hydroxy-9*Z*,11*E*-octadecadienoate with *tert.*-butyl hydroperoxide (TBHP) catalysed by titanium tetraisopropoxide [$Ti(iOPr)_4$] in the presence of L(+)-di-isopropyl tartrate gave methyl 13*S*-hydroxy-11*S*,12*S*-epoxy-9*Z*-octadecenoate (*erythro* diastereomer) in 84% diastereomeric excess (de). Epoxidation with TBHP catalyzed by $Ti(iOPr)_4$ in the presence of D(-)-diisopropyl tartrate yielded methyl 13(*S*)-hydroxy-11(*R*),12(*R*)-epoxy-9(*Z*)-octadecenoate (*threo* diastereomer) in 76% de. The use of m-CPBA did not give chiral products (Omar et al., 2003).

Besides chemical systems, epoxidation can be carried out enzymatically with H_2O_2. One of the most important factors affecting this procedure is the concentration of H_2O_2 (Orellana-Coca et al., 2005). Immobilized oat peroxygenase enzyme was used to epoxide linoleic and linolenic acids (Piazza et al., 2003). The epoxidation of linoleic acid gave mono- and diepoxides in nearly equal amounts while the epoxidation of linolenic acid afforded 9,10-15,16-diepoxyoctadecanoic acid with only small amounts of triepoxide. An advantage of this procedure is that no opening of the epoxy ring occurs.

Unsaturated fatty acids can be "self-epoxidized" using immobilized lipase (Novozym 435) from *Candida antarctica* and H_2O_2 without using a mineral acid (Warwel and Rüsch gen. Klaas, 1995). The unsaturated acid is converted to the corresponding peroxy acid, which then acts as epoxidizing agent with back-formation of the acid. This procedure was applied with high epoxidation yields to a variety of vegetable oils (Rüsch gen. Klaas and Warwel, 1996, 1999). The reaction may be limited to unbranched carboxylic acids (Rüsch gen. Klaas and

Warwel, 1997). The formation of peroxy carboxylic acids from saturated fatty acids in presence of lipase was originally applied to the epoxidation of alkenes (Björkling et al., 1990; Björkling et al., 1992). A kinetic model for self-epoxidation of oleic acid has been developed (Yadav and Manjula, 2001).

The high reactivity of the epoxy ring and, therefore, the possibility of easily obtaining other fatty acid derivatives via an epoxy intermediate have led to the development of numerous procedures for epoxidation. Epoxides can be converted via the overall reaction

in which X can be H_2 (giving the alcohol), H_2O (diol), ROH (alkoxy alcohol), RCOOH (acyloxy alcohol), $RCONH_2$ (acylamido alcohol), H_2S (mercapto alcohol), R_2NH (amino alcohol) HCN (cyano alcohol), HCl (chlorohydrin), and $NaHSO_3$ (sodium hydroxy sulfonate) (Baumann et al., 1988). Another report on the opening of the epoxy (oxirane) ring with various carboxylic acids, alcohols, and butylamine is provided by Pagès-Xatart-Parès et al. (1999). The regeneration of olefinic compounds from epoxides by a number of stereospecific deoxygenation reactions is also possible.

8.3.2 Hydrolysis of epoxides

Epoxides are easily hydrolyzed to a diol (or its monoester), especially under acidic conditions. These reactions occur stereospecifically with inversion, so the *cis*- and *trans*-epoxides give the *threo*-and *erythro*-diols, respectively. For example, this change is smoothly effected by heating with acetic acid (acetolysis) followed by hydrolysis of the monoacetate.

Linoleic acid readily forms a diepoxide, but its conversion to 9,10,12,13-tetrahydroxystearic acid is somewhat less satisfactory. The reaction is reported to take place smoothly with anhydrous DMSO or with 97% formic acid followed by alkaline hydrolysis. With more conventional reagents, a small amount of tetrahydroxy acid is accompanied by cyclic ethers (1,4- and/or 1,5-epoxides) and by the cyclopropanediol shown in Figure 8.3.

8.3.3 Hydroxylation

Besides obtaining dihydroxy compounds via epoxides, hydroxylation of double bonds can be conducted with a variety of other reagents that cause *cis*- or *trans*-hydroxylation to give the *threo*- and *erythro*-diols. Hydroxylations can also be conducted enzymatically (Section 10.3).

cis-Hydroxylation (*cis* double bonds give *erythro* diols; *trans* double bonds lead to *threo* diols) is effected by dilute

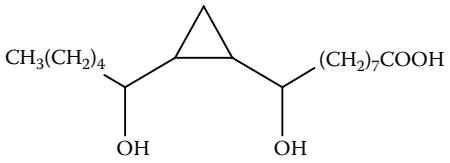


FIGURE 8.6

alkaline potassium permanganate, by osmium tetroxide (OsO_4) alone, by OsO_4 as a catalyst in conjunction with an oxidizing agent, such as metal chlorate, hydrogen peroxide/t-butanol, t-butylhydroperoxide, or N-methylmorpholine N-oxide, or by the Woodward reaction, requiring iodine, silver acetate and wet acetic acid.

trans-Hydroxylation (*cis* double bonds give *threo* diols, *trans* double bonds lead to *erythro* diols) results from the Prévost reaction (iodine and silver benzoate under anhydrous conditions) or, more easily, from epoxidation followed by acid-catalyzed hydrolysis. Cyclic selenites are intermediates in the formation of *threo* vicinal diols from *cis* unsaturated fatty compounds when applying SeO_2 in combination with H_2O_2 (Knothe et al., 1997).

Oleic acid forms two diastereomeric (four enantiomers) vicinal diols (*erythro*/*threo* diastereomers). The stereoisomers increase with the number of double bonds, enantiomers increasing by 2^n and diastereomers by 2^{n-1} where n is the number of chiral centres (originally olefinic carbons). The diastereomers can easily be distinguished by differing physical properties, such as melting point. Eight diastereomeric 9,10,12,13-tetrahydroxystearic acids and 32 diastereomeric 9,10,12,13,15,16-hexahydroxystearic acids exist, not all of which have been identified. Certain difficulties in converting diepoxides to tetrols exist. Ricinoleic acid, which itself occurs in enantiomeric form (*R* configuration), gives four enantiomeric trihydroxystearic acids upon hydroxylation.

The vicinal diols can be cleaved by periodic acid (to aldehydes) and by permanganate (to acids). After suitable derivatization, the hydroxy compounds can be separated by gas chromatography and identified by mass spectrometry (Section 6.4).

Other hydroxy fatty compounds can be obtained through a variety of reactions, with the following examples illustrating this. Chiral oxazolidinone carboximides were used in the asymmetric synthesis of 2-hydroxy fatty acid methyl esters (Hwang and Erhan, 2001). The hydroxylation of isolated double bonds with SeO_2/TBHP affords a mixture of positional isomers of allylic monohydroxy compounds and *erythro*/*threo* diastereomers of the dihydroxy products (2(*E*)-ene-1,4-diols) with some of the corresponding enones as side products. (Knothe et al., 1993; Knothe et al., 1994**).** When applying this oxidation system to fatty compounds with triple bonds, a variety of keto, hydroxy and hydroxy-keto compounds were obtained (Lie Ken Jie et al., 1997).

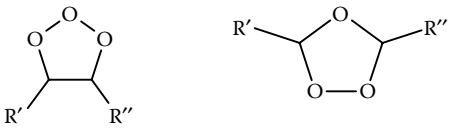
8.3.4 Oxidative fission

Unsaturated acids can be cleaved at their unsaturated centers. These processes are important for two reasons. On an analytical scale, identification of the cleavage products provides evidence of double-bond position. This may be necessary in the identification of acids of unknown structure and in the analysis of mixtures of acids such as oleate and petroselinate or mixtures of monoene isomers resulting from partial hydrogenation. Mixtures of diene esters present even more difficulties. When used for analytical purposes simple procedures giving unequivocal results with small quantities of materials are required. However, methods, such as mass spectrometry and nuclear magnetic resonance (Section 6.5), can provide such information without the necessity for cleavage reactions. On a preparative and industrial scale, oxidative fission may be used to produce new and interesting compounds. For example, petroselinic acid gives a series of C_6 difunctional and C_{12} monofunctional compounds. Only two procedures, von Rudloff oxidation and ozonolysis, have been widely employed.

Reaction with permanganate, the classical method of oxidative cleavage, leads to over-oxidation, i.e., the initially formed oxidation products are subject to chain shortening by further oxidation. However, olefinic and, especially, acetylenic bonds in unsaturated fatty esters can be cleaved using $KMnO_4$ in aqueous acetic acid under ultrasound, quantitatively yielding the corresponding carboxylic acids (Lie Ken Jie and Kalluri, 1996). This difficulty is also overcome in the von Rudloff procedure, which employs a 1:39 mixture of potassium permanganate and sodium metaperiodate, but is not suitable for the determination of triple bonds. Under von Rudloff conditions, permanganate is never present in high concentration, but is continuously regenerated by oxidation with metaperiodate. Acids are oxidized in aqueous alkaline solution and esters in aqueous t-butanol solution for 6 to 12 hours at room temperature. The overall reaction is:

$$R^1CH{=}CHR^2 \rightarrow R^1COOH + HOOCR^2$$

Ozonolysis is now more extensively employed for both analytical and preparative purposes. Reaction occurs via the cyclic molozonide (Figure 8.7), which breaks down to aldehyde and the Criegee zwitterion RC^+OO^-. The latter then reacts with aldehyde to give cyclic ozonide (Figure 8.7) or with solvent to give alkoxyhydroperoxide (alcohol) or acylhydroperoxide (carboxylic acid). All of these subsequently break down to the same ozonolysis products (Privett and Nickell, 1966). The ozonide exists in *cis* and *trans* forms. Thus, unsymmetrical alkenes produce six ozonides, namely *cis*/*trans* isomers of symmetrical and unsymmetrical compounds (substitution of the ozonide ring by R^1R^2, $(R^1)_2$, and $(R^2)_2$) as *cis*/*trans* isomers. The six ozonides from methyl oleate were separated by HPLC and characterized spectroscopically (Wu et al., 1992).



Molozonide Ozonide

FIGURE 8.7

Of greater importance are the fission products resulting from further reaction of the ozonide or the alkoxy- or acyloxyhydroperoxide. These may be alcohols, aldehydes, acids or amines depending on the method of cleavage. Alcohols are formed by reduction of the ozonide with metal hydrides (lithium aluminum hydride or sodium borohydride) or by catalytic hydrogenation with nickel or platinum catalysts. Aldehydes result under milder reducing conditions, and zinc/acid, triphenylphosphine, dimethylsulfide or Lindlar's catalyst are commonly employed for the purpose. Acids are formed with a wide range of oxidizing agents, including peroxy acids or silver oxide. Amines result from reduction of ozonides over Raney nickel in the presence of ammonia or may be prepared from the aldehydes by reduction of their oximes. When HCl/MeOH was used to decompose ozonides, methyl esters or methyl acetals were produced (Mittelbach and Poklular, 1990). Both EPA and DHA give the acetal ester $(MeO)_2CHCH_2CO_2Me$ as the major C_3 product (about 45% of the theoretical yield). The same authors also studied the partial ozonolysis of C_{18} diene and triene esters.

The ozonolysis of readily available monoene acids is carried out on an industrial scale as a source, in particular, of dibasic acids. The C_9, C_{10}, C_{12}, C_{13} and C_{15} acids of this type are readily available from appropriate monoenes. Modification of the reaction process leads to other difunctional compounds (Klein, 1984).

Two alternatives to ozonolysis have been described. One employs oxygen with aldehydes as the oxidizing agent (Hinze, 1987) and the other uses sodium hypochlorite (Zaidman et al., 1988). The latter occurs in three stages: (1) emulsification of oleic acid in water, (2) noncatalytic oxidation to diol with sodium hypochlorite and sodium hydroxide, and (3) cleavage of the diol with sodium hypochlorite and ruthenium chloride ($RuCl_3$) as catalyst.

References

Ayorinde, F.O. et al. Synthesis of azelaic acid and suberic acid from *Vernonia galamensis* oil, *J. Am. Oil Chem. Soc*., **65**, 1774, 1988.

Ayorinde, F.O. et al. Synthesis of dodecanedioic acid from *Vernonia galamensis* oil, *J. Am. Oil Chem. Soc*., **66**, 690, 1989.

Baumann, H. et al. Natural fats and oils — renewable raw materials for the chemical industry, *Angew. Chem., Int. Edn*, **27,** 41–62, 1988.

Björkling, F. et al. Lipase-mediated formation of percarboxylic acids used in catalytic epoxidation of alkenes, *J. Chem. Soc., Chem. Commun.*, 1301–1303, 1990.

Björkling, F. et al. Lipase catalyzed synthesis of peroxycarboxylic acids and lipase mediated oxidations, *Tetrahedron* **48**, 4587–4592, 1992.

Buisman, G.J.H. et al. Synthesis of epoxidized novel fatty acids for use in paint applications, in *Recent Developments in the Synthesis of Fatty Acid Derivatives*, Knothe, G. and Derksen, J.T.P., Eds., AOCS Press, Champaign, IL, 1999, pp. 128–140.

Campanella, A. et al. Campos-Martin, J.M., and Fierro, J.L., Soybean oil epoxidation with hydrogen peroxide using an amorphous Ti/SiO_2 catalyst, *Green Chem.,* **6**, 330–334, 2004.

Corey, E.J. et al. Selective epoxidation of eicosa-*cis*- 5,8,11,14-tetraenoic (arachidonic) acid and eicosa-*cis*-8,11,14-trienoic acid, *J. Am. Chem. Soc*., **101**, 1586–1587, 1979.

Du, G. et al. Catalytic epoxidation of methyl linoleate, *J. Am. Oil Chem. Soc.* 81, 477–480, 2004.

Fringuelli, F. et al. Epoxidation reaction with *m*-chloroperoxybenzoic acid in water, *Tetrahedron Lett.*, **30**, 1427–1428, 1989.

Gerbase, A.E. et al. Epoxidation of soybean oil by the methyltrioxorhenium-CH_2Cl_2 / H_2O_2 catalytic biphasic system, *J. Am. Oil Chem. Soc.,* **79**, 179–181, 2002.

Guidotti, M. et al. Heterogeneous catalytic epoxidation of fatty acid methyl esters on titanium-grafted silicas, *Green Chem.,* **5**, 421–424, 2003.

Gunstone, F.D., in *Fatty Acids*, Pryde, E.H., Ed., American Oil Chemists' Society, Champaign, IL, p. 379, 1979.

Gunstone, F.D., Epoxidized oils, in *Lipid Technologies and Applications*, Gunstone, F.D., and Padley, F.B., Eds., pp. 759–769, 1997.

Hedman, B. et al. Synthesis and characterization of surfactants via epoxidation of tall oil fatty acid, *J. Surf. Deterg.,* **6**, 47–53, 2003.

Hinze, A.G., Oxidatives Verfahren zur Herstellung kurzkettiger Carbonsäuren aus ungesättigten Fettsäuren (Oxidative procedure for production of short-chain carbon acids from unsaturated fatty acids), *Fat Sci. Technol*., **89**, 339–342, 1987.

Hwang, H.-S. and Erhan, S.Z., Highly selective asymmetric synthesis of 2-hydroxy fatty acid methyl esters through chiral oxazolidinone carboximides, *J. Am. Oil Chem. Soc.,* **78**, 205–211, 2001.

Klein, H.P., Large-scale production and application of highly concentrated ozone, *J. Am. Oil Chem. Soc*., **61**, 306–312, 1984.

Knothe, G. et al. Hydroxy fatty acids through hydroxylation of oleic acid with selenium dioxide/*tert.*-butylhydroperoxide, *J. Am. Oil Chem. Soc.* **70**, 401–404, 1993.

Knothe, G. et al. Allylic mono- and dihydroxylation of isolated double bonds with selenium dioxide/*tert.*-butylhydroperoxide. NMR characterization of long-chain enols, allylic and saturated 1,4-diols, and enones, *J. Chem. Soc. Perkin Trans.,* **2**, 401–404, 1994.

Knothe, G. et al. Reaction of isolated double bonds with selenium dioxide/hydrogen peroxide: formation of novel selenite esters, *Synthesis,* 57–60, 1997.

La Scala, J. and Wool, R.P., Effect of FA composition on epoxidation kinetics of TAG, *J. Am. Oil Chem. Soc.,* **79**, 373–378, 2002.

Lie Ken Jie, M.S.F. and Cheung, Y.-K., The use of a microwave oven in the chemical transformation of long chain fatty acid esters, *Lipids*, **23**, 367, 1988.

Lie Ken Jie, M.S.F. and Kalluri, P., Ultrasound-assisted oxidative cleavage of acetylenic and ethylenic bonds in unsaturated fatty esters with potassium permanganate, *Lipids,* **31**, 1299–1301, 1996.

Lie Ken Jie, M.S.F. and Pasha, M.K., Epoxidation reactions of unsaturated fatty esters with potassium peroxomonosulfate, *Lipids,* **33**, 633–637, 1998.

Lie Ken Jie, M.S.F. et al. Oxidation reactions of acetylenic fatty esters with selenium dioxide/*tert.*-butylhydroperoxide, *Lipids,* **32**, 1119–1123, 1997.

Lie Ken Jie, M.S.F. et al. Epoxidation of a conjugated linoleic acid isomer, *Eur. J. Lipid Sci. Technol.,* **105**, 391–396, 2003.

Mittelbach, M. and Poklukar, N., Ozonolysis of olefins, IV. Ozonolysis of polyunsaturated fatty esters in HCl/methanol, *Chem. Phys. Lipids*, **55**, 67, 1990.

Omar, M.N. et al. Asymmetric Sharpless epoxidation of 13(*S*)-hydroxy-9(*Z*),11(*E*)-octadecadienoic Acid (13S-HODE), *Eur. J. Lipid Sci. Technol.,* **105**, 43–44, 2003.

Orellana-Coca, C. et al. Chemo-enzymatic epoxidation of linoleic acid: parameters influencing the reaction, *Eur. J. Lipid Sci. Technol.,* **107**, 864–870, 2005.

Pagès-Xatart-Parès, X. et al. Synthesis of new derivatives from vegetable oil methyl esters via epoxidation and oxirane opening, in *Recent Developments in the Synthesis of Fatty Acid Derivatives*, Knothe, G. and Derksen, J.T.P., Eds., AOCS Press, Champaign, IL, pp. 141–156, 1999.

Piazza, G.J., Some recent advances in epoxide synthesis, in *Recent Developments in the Synthesis of Fatty Acid Derivatives*, Knothe, G. and Derksen, J.T.P., Eds., AOCS Press, Champaign, IL, 1999, pp. 182–195.

Piazza, G.J. et al. Epoxidation of fatty acids, fatty methyl esters, and alkenes by immobilized oat seed peroxygenase, *J. Mol. Catal. B: Enzym*., **21**, 143–151, 2003.

Privett, O.S. and Nickell, E.C., Recent advances in the determination of the structure of fatty acids via ozonolysis, *J. Am. Oil Chem. Soc.*, **43**, 393–400, 1966.

Rüsch gen. Klaas, M. and Warwel, S., Chemoenzymatic epoxidation of unsaturated fatty acid esters and plant oils, *J. Am. Oil Chem. Soc.,* **73**, 1453–1457, 1996.

Rüsch gen. Klaas, M., Lipase-catalyzed preparation of peroxy acids and their use in epoxidation. *J. Mol. Catal. A,* **117**, 311–319, 1997.

Rüsch gen. Klaas, M. and Warwel, S., Complete and partial epoxidation of plant oils by lipase-catalyzed perhydrolysis, *Ind. Crops. Prod.,* **9**, 125–132, 1999.

Warwel, S. and Rüsch gen. Klaas, M., Chemo-enzymatic epoxidation of unsaturated carboxylic acids. *J. Mol. Catal. B,* **1** (1), 29–35, 1995.

Watanabe, S. et al. Epoxides of unsaturated fatty acids as antirust additives in water-based cutting fluids, *J. Am. Oil Chem. Soc*., **65**, 1311–1312, 1988.

Wu, M. et al. Separation and spectral data of the six isomeric ozonides from methyl oleate, *Lipids*, **27**, 129, 1992.

Yadav, G.D. and Manjula, K., A kinetic model for the enzyme-catalyzed self-epoxidation of oleic acid, *J. Am. Oil Chem. Soc.,* **78**, 347–351, 2001.

Zaidman, B. et al. Double bond oxidation of unsaturated fatty acids, *J. Am. Oil Chem. Soc*., **65**, 611–615, 1988.

8.4 Halogenation and halohydrins

While not very common, halogenated fatty acids occur naturally to some extent, mainly in marine organisms (Section 1.1.2.11). Halogenation of unsaturated fatty acids is used in standard procedures for determining total unsaturation, i.e., the iodine value (Section 6.33; analytical methods). Since the advent of fatty acid profile analysis by GC, iodine values are less commonly measured, though they are still a useful check on the overall validity of GC results. In the past, brominated vegetable oils were used in soft drinks as dispersing agents for flavoring citrus oils and to impart a cloudy appearance. However, they have been largely banned due to health concerns, including carcinogenicity.

Halogenation — especially bromination — generally occurs in a *trans* manner through a polar addition. Bromination of oleic and elaidic acids gives the *threo* (mp. 28.5°C) and *erythro* (mp 29.5–30°C) racemic diastereomers of 9,10-dibromostearic acid, respectively. Linoleic acid furnishes two tetrabromides: a solid product (mp 115°C; 9*R**,10*R**,12*R**,13*R** racemate) and a liquid isomer (9*R**,10*R**,12*S**,13*S** racemate). Linelaidic acid (9*t*,12*t*) gives a solid tetrabromide (mp 78°C) and linolenic acid gives a solid hexabromide (mp 185°C). The chlorides obtained from oleic, linoleic and linolenic acid melt at 37, 123, and 189°C, respectively.

The normal halogenation reaction can be diverted in its second stage by reaction with a nucleophile other than Br^-. Reaction in alcohol or acid solution, for example, gives alkoxy or acyloxy bromides (Figure 8.8).

Chlorination of linoleic acid in the presence of water gives tetrachloro (49%), trichloromonohydroxy (38%), and dichlorodihydroxy (13%) compounds. These compounds are formed during the bleaching of wood pulp and related products and may be present in chlorine-bleached flour (McKague and Reeve, 1991).

Alternatively, reaction of an olefinic alcohol or acid can furnish a dimeric molecule through interaction of the bromonium ion with a second molecule of reactant acting as a nucleophile. Methoxybromo adducts are easily made by reaction of the alkene with t-butoxy bromide in methanol solution.

Olefinic acids react with $KHCO_3 + I_2 + KI$ to form iodolactones if there is a double bond in the Δ4 (e.g., DHA) or Δ5 position (e.g., EPA, arachidonic acid) (Section 8.9, cyclization). The neutral lactones are easily separated from unreacted acidic material. The Δ4 acids form γ-lactones and Δ5 acids δ-lactones with the iodine located α to the lactone rings.

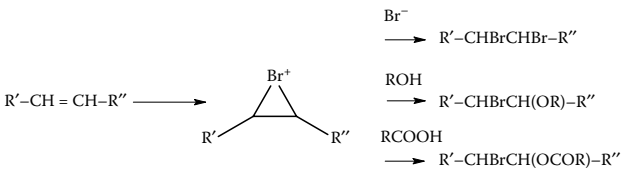


FIGURE 8.8

The former are more stable and more easily formed; the latter are less readily formed and react more quickly. The unsaturated acid can be regenerated from the iodolactone by reaction with trimethylsilyl iodide (or $Me_3SiCl + NaI$).

The rate of formation of iodolactone depends on the choice of solvent, on the reaction temperature, and on the ratio of KI to I_2; the regeneration of alkene from iodolactone depends on the amount of Me_3SiI, since the γ-lactone reacts first. The two iodolactones can be separated by HPLC. This reaction can be manipulated in several ways and has been used in an analytical mode (Imbs et al., 1991). It has also been used as a nonchromatographic method to prepare pure DHA, EPA, and arachidonic acid (Wright et al., 1987; Gaiday et al., 1991).

vic-Dibromo acids are debrominated by reaction with zinc or sodium iodide (or other reagents, such as sodium selenide, thiourea, thiocyanates, chromous sulphate). Both reagents cause *trans* elimination, with NaI being more stereospecific. The resulting alkene has the same configuration as that from which the dibromide was prepared, and bromination-debromination has been used as a method of protecting double bonds:

$$-CH=CH- \xrightarrow{Br_2} -CHBrCHBr- \xrightarrow{NaI} -CH=CH-$$

Dehydrohalogenation of a *vic*-dihalide with base leads to an alkyne or allene depending on which isomer is used. These changes occur by *trans* elimination and the first step occurs easily with both *threo*- and *erythro*-dihalides to form the *Z* and *E*-halides, respectively:

$$\underset{cis}{-CH=CH-} \rightarrow \underset{threo}{-CHBrCHBr-} \rightarrow \underset{Z}{-C=CBr-} \rightarrow -C\equiv C-$$

$$\underset{trans}{-CH_2CH=CH-} \rightarrow \underset{erythro}{-CH_2-CHBrCHBr-} \rightarrow \underset{E}{-CH_2CBr=CH-} \rightarrow -CH=C=CH-$$

Only the *Z* isomer then furnishes the alkyne, the less-reactive *E* isomer giving a mixture of isomeric allenes and alkynes, the latter probably produced by allene rearrangement (Gunstone and Hornby, 1969). Dibromo derivatives of olefinic fatty acids were dehydrobrominated with KOH in 20% aqueous ethanol under ultrasonic irradiation to give the corresponding acetylenic derivatives in 52 to 72% yield (Lie Ken Jie and Kalluri, 1998), vs. 15 to 20% yield reported previously when using sodium in liquid ammonia or DBU (1,5-diazabicyclo(5.4.0)undec-5-ene) (Gunstone and Hornby, 1969).

Monohalogenated long-chain acids are most easily prepared from the appropriate hydroxy acid. Reaction with triphenylphosphine and tetrachloromethane or tetrabromomethane occurs smoothly and with complete inversion. D-(-)-12-Hydroxystearic acid is thereby converted to L-(+)-12-chlorostearic acid. Alternatively, the

alcohol can be converted to its methanesulfonate and then reacted with magnesium bromide etherate in benzene. Fluorine-containing compounds are also prepared from the corresponding alcohols by reaction with 1-chloro-1,2,2-trifluoro-2-diethylaminoethane or DAST (di-ethylaminosulfur trifluoride) (Delgado et al., 1991) or by interaction of the alcohol toluenesulphonate or methanesulphonate with tetrabutylammonium fluoride. α-Chlorocarboxylic acids were prepared from the corresponding dimethyl acylphosphonates (Stevens and Vanderhoydonck, 2001). The phosphonate functionality serves to activate the chain for monochlorination in the α-position. Dehydrohalogenation of the 2-halogeno acid with alcoholic alkali (e.g., KOH-EtOH) gives a mixture of three products: Δ2, 2-hydroxy and 2-ethoxy acids.

Carboxylic acids can be converted to norhalides (RBr or RI) by the Hunsdiecker reaction (silver salt and bromine) or by some modification of this (mercuric oxide and bromine, lead tetraacetate and iodine, or thallium salt and bromine).

Allylic bromination occurs with *N*-bromosuccinimide (NBS) or with *t*-butyloxybromide, but in common with other allylic reactions the product is a mixture of isomers. Work in this area was summarized by Naudet and Ucciani (1971). Heavily brominated and highly unsaturated derivatives of jojoba oil, obtained by allylic bromination (NBS), removal of hydrogen bromide, and bromination, have been described by Shani et al. (1988).

Derivatizing fatty compounds with mixed functional groups, one of them being a halogen, has been explored, too. Hydroxybromination of methyl oleate, oleic acid, and high-oleic sunflower oil was achieved in yields of 90%, 78%, and 40%, respectively, in a one-step reaction using NBS (Eren and Küsefoglu, 2004). The hydroxybromide derivative of sunflower oil can be acrylated and this derivative homopolymerized or copolymerized with styrene. Halgeno-oxo-allenic fatty ester derivatives with chlorine and fluorine as halogens were prepared in a multistep sequence from methyl santalbate (methyl *trans*-11-octadecen-9-ynoate) via halohydrins (Lie Ken Jie et al., 2003).

Methyl ricinoleate and its *trans* isomer were reacted with Cl_2, Br_2, and ICl. Besides the expected dihalide, a halogen-containing tetrahydrofuran or tetrahydropyran derivative was formed by neighboring-group participation between the hydroxyl group and the reaction at the unsaturation (Gunstone and Perera, 1973). The dihalides can be reacted further to give vinyl halides.

References

Delgado, A. et al. Synthesis of potential inhibitors of the biosynthesis of *Spodoptera littoralis*. Part I: Monofluorinated fatty acids, *Chem. Phys. Lipids*, **59**, 127–135, 1991.

Eren, T. and Küsefoglu, S.H., One-step hydroxybromination of fatty acid derivatives, *Eur. J. Lipid Sci. Technol.*, **106**, 27–34, 2004.

Gaiday, N.V. et al. Separation of natural polyunsaturated fatty acids by means of iodolactonization, *J. Am. Oil Chem. Soc.*, **68**, 230–233, 1991.

Gunstone, F.D. and Hornby, G.M., Fatty acids, part 19. The conversion of alkenoic acids to alkynoic acids by bromination-dehydrobromination, *Chem. Phys. Lipids*, **3**, 91–97, 1969.

Gunstone, F.D. and Perera, B.S., Fatty Acids, Part 41. The halogenation of some long-chain hydroxy alkenes with special reference to the possibility of neighbouring group participation leading to cyclic ethers or lactones, *Chem. Phys. Lipids*, **11**, 43–65, 1973.

Imbs, A.B. et al. Application of an analytical modification of the iodolactonization reaction to selective detection of Δ5 (Δ4) unsaturated fatty acids, *Chem. Phys. Lipids*, **60**, 71–76, 1991.

Lie Ken Jie, M.S.F. and Kalluri, P., Ultrasound in fatty acid chemistry: facile dehydrobromination of dibromo fatty esters to acetylenic ester derivatives, *Chem. Phys. Lipids*, **91**, 79–83, 1998.

Lie Ken Jie, M.S.F. et al. Novel halo-oxo-allenic fatty ester derivatives from epoxidized methyl santalbate (methyl *trans*-11-octadecyn-9-ynoate), *Chem. Phys. Lipids*, **125**, 93–101, 2003.

McKague, A.B. and Reeve, D.W., Chlorination of linoleic acid, *Chem. Phys. Lipids*, **58**, 213–218, 1991.

Naudet, M. and Ucciani, E., Allylic halogenation and oxidation of unsaturated esters, in *Topics in Lipid Chemistry*, vol. 2, Gunstone, F.D. (Ed.), Wiley-Interscience, New York, p. **99**, 1971.

Shani, A., Heavily brominated and highly unsaturated derivatives of jojoba oil, *J. Am. Oil Chem. Soc.*, **65**, 1318–1323, 1988.

Stevens, C.V. and Vanderhoydonck, B., Use of acylphosphonates for the synthesis of α-chlorinated carboxylic and α,α'–dichloro dicarboxylic acids and their derivatives, *Tetrahedron* **57**, 4793–4800, 2001.

Wright, S.W. et al. An effective process for the isolation of docosahexaenoic acid in quantity from cod liver oil, *J. Org. Chem.* **52**, 4399–4401, 1987.

8.5 Oxymercuration

The addition of water across multiple bonds, known as hydration, represents one of the most useful methods to functionalize alkenes. Oxymercuration-demercuration is a useful method to accomplish this addition without rearrangement as can happen when performing the hydration reaction using water and acid catalysts (Larock, 1978). The addition of mercuric acetate (or other mercury salts) to alkenes in tetrahydrofuran and water gives a mercury-containing adduct, whereby a hydroxyl group and a –HgOAc group add across the double bond in a *trans* fashion. When water is replaced by an alcohol solvent, such as methanol (R′= Me), the corresponding β-methoxy mercury-containing adducts are formed (Figure 8.9).

The intermediate oxymercury compounds can be treated with a variety of nucleophiles to give nonmercury compounds. For example, treatment with $NaBH_4$ gives high yields of the alcohol or ether addition products

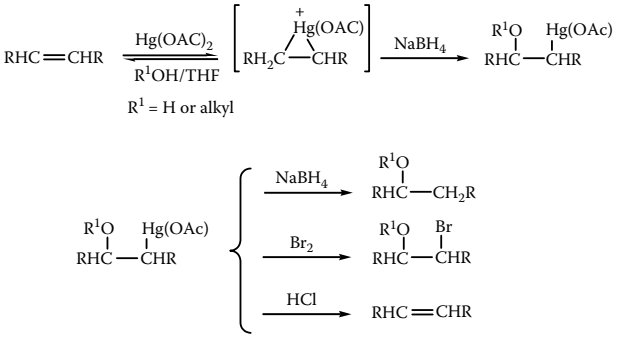


FIGURE 8.9 Oxymercuration-demecuration reactions of alkenes.

(Markovnikov orientation) under mild conditions (Brown and Geoghegan, 1967). Alternatively, the mercury group can also be reacted with bromine to give the bromohydrin or vicinal bromo ether. Finally, reaction of this oxymercury intermediate with hydrocholoric acid furnishes the alkene in its original stereoisomeric form. Mercuric acetate reagent is poisonous and care must be taken, even when working with small amounts, during the reaction and in disposal of the residues.

The oxymercuration reaction has been used by lipid chemists in a number of ways and is probably best known as an aid to isolating individual acids from natural mixtures. The nonreactive saturated esters are easily separated from the more polar mercury derivatives by chromatography. After separation, the latter are converted back to the original monoene, diene, etc., by treatment with hydrochloric acid. Sebedio (1993) has reviewed this topic. Although now overshadowed by silver-ion chromatography, where the adducts do not have to be prepared nor subsequently decomposed, the method has been utilized by Stearns and coworkers (1962) as a preparative procedure to isolate pure linoleate from safflower oil and pure linolenate from linseed oil and for the study of complex mixtures present in partially hydrogenated fish oils (Sebedio and Ackman, 1981). The methoxymercury adducts from oleate (*cis*-double bond) and elaidate (*trans*-double bond) differ slightly in their ^{1}H NMR spectrum, and the -OCH$_3$ signals can be distinguished and used as a basis for measuring the *cis*/*trans* ratio of oleate elaidate mixtures (Schaumburg, 1970).

The oxymercuration reaction can also be used to give a variety of interesting products. For example, Gunstone and Inglis (1973) found that oleate reacts with mercuric acetate in the presence of different nucleophiles, such as H_2O, MeOH, EtOH, AcOH, CH_3CN, or *t*-BuOOH to give the respective 9- and 10-substituted stearate mixtures containing -OH, -OMe, -OEt, -OAc, -NHAc, and -OOBu^t after $NaBH_4$ reduction. Interesting intramolecular reactions occur with hydroxyl alkenols and unsaturated hydroxy esters as starting materials or when they are formed as intermediates during the reaction of polyenes (Gunstone, 1999; Gunstone and Inglis, 1973a; 1973b) (Figure 8.10).

More recently, the reactive C=N bond of 1-pyrroline fatty acid esters were shown to react with mercuric acetate

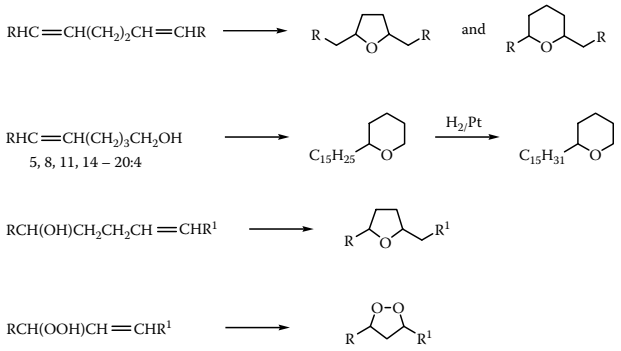


FIGURE 8.10 Intramolecular reactions can occur during the oxymercuration-demercuration reaction.

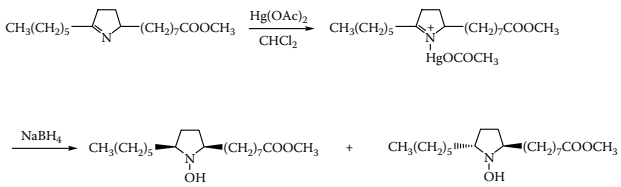


FIGURE 8.11 Oxymercuration-demurcuration of 1-pyrroline fatty acid ester.

to give the corresponding N-mercurial compound in high yield (Lie Ken Jie and Syed Rahmatullah, 1995). Subsequent demercuration using $NaBH_4$ gave a diastereomeric mixture of the corresponding N-hydroxy-substituted pyrrolidine derivatives in high yield (Figure 8.11).

Oxymercuration of unsaturated fatty acids using mercuric acetate and methanol followed by $NaBH_4$ demercuration gives a mixture of isomeric methoxy acids, which can be used to determine double-bond position by mass spectrometry (Christie, 1989). Oleate, for example, gives an easily identified mixture of 9- and 10-methoxystearate (Minnikin, 1978; Gunstone and Inglis, 1973). The method is less satisfactory for polyenes because of the increasing complexity of the spectra of the polymethoxy esters, but good results have been claimed for partial reactions (Platner et al., 1976). After incomplete reaction and subsequent hydrogenation, linolenate gives a mixture of six monomethoxystearates (9-, 10-, 12-, 13-, 15- and 16-), which can be identified in the mass spectrum.

Oxymercuration of acetylenic compounds is not reversible and yields oxo or hydroxyl compounds depending on the experimental procedure employed (Kleiman et al., 1976; Lam and Lie Ken Jie, 1976; Lie Ken Jie and Lam, 1977). Appropriate diynes yield dioxo compounds, which have been used to prepare furan derivatives.

References

Brown, H.C. and Geoghegan, P., Jr., The oxymercuration-demercuration of representative olefins. A convenient, mild procedure for the Markovnikov hydration of the carbon-carbon double bond, *J. Am. Chem. Soc.*, **89**, 1522–1524, 1967.

Christie, W.W., *Gas Chromatography and Lipids*, Oily Press, Ayr, U.K., 1989, pp. 80, 141, 176.

Gunstone, F.D., Novel long-chain compounds produced through neighboring group participation, in *Recent Developments in the Synthesis of Fatty Acid Derivatives*, Knothe, G. and Derksen, J. T. P., Eds., AOCS Press, Champaign, IL, 1999, pp. 1–19.

Gunstone, F.D. and Inglis, R.P., Fatty acids, part 37. Application of the oxymercuration-demercuration reaction to long-chain unsaturated esters, *Chem. Phys. Lipids*, **10**, 73–88, 1973.

Gunstone, F.D. and Inglis, R P., Fatty acids, part 38. Neighbouring group participation in the oxymercuration-demercuration reaction. Another route to 1,4- and 1,5-epoxides, *Chem. Phys. Lipids*, **10**, 89–104, 1973a.

Gunstone, F.D. and Inglis, R.P., Fatty acids, part 39. Oxymercuration demercuration of unsaturated alcohols. a new procedure for the identification, isolation, and estimation of Δ3*t*, Δ4, and Δ5 alkenols and of substances reducible thereto, *Chem. Phys. Lipids*, **10**, 105–113, 1973b.

Kleiman, R. et al. Mass spectra of acetylenic fatty acid methyl esters and derivatives, *Lipids*, **11**, 599–603, 1976.

Lam, C.J. and Lie Ken Jie, M.S.F., Fatty acids, part 5: A study of the oxymercuration-demercuration reaction of some C_{11} -unsaturated fatty esters and methyl octadec-cis-10-en-5-ynoate. *Chem. Phys. Lipids*, **16**, 181–194, 1976.

Larock, R.C., Organomercury compounds in organic synthesis, *Angew. Chem., Int. Edn.*, **17**, 27–37, 1978.

Lie Ken Jie, M.S.F. and Lam, C.H., Fatty acids part 12. The synthesis of furanoid, α,β-unsaturated oxocyclopentenyl and oxocyclohexenyl esters from methyl octadecadiynoates. *Chem. Phys. Lipids*, **19**, 275–287, 1977.

Lie Ken Jie, M.S.F. and Syed Rahmatullah, M.S.K., Further chemical reactions of 1-pyrroline fatty ester: n-substituted pyrrolinium and pyrrolidine derivatives, *Chem. Phys. Lipids*, **77**, 179–186, 1995.

Minnikin, D.E., Location of double bonds and cyclopropane rings in fatty acids by mass spectrometry, *Chem. Phys. Lipids*, **21**, 313–347, 1978.

Plattner, R.E. et al. Double bond location in polyenoic fatty esters through partial oxymercuration, *Lipids*, **11**, 222–227, 1976.

Schaumburg, K., NMR spectra of mercury adducts of unsaturated fatty acids: quantitative determination of cis-trans ratio, *Lipids*, **5**, 505–508, 1970.

Sebedio, J.L., Mercury adduct formation in the analysis of lipids, in *Advances in Lipid Methodology, 2nd ed.*, Christie, W.W., Ed., Oily Press, Dundee, U.K., 1993, pp. 139–155.

Sebedio, J.L. and Ackman, R.G., Application of methoxybromomercuri-adduct fractionation to the analysis of fatty acids of partially hydrogenated marine oils, *Lipids*, **16**, 461–467, 1981.

Stearns, E.M., Jr. et al. Use of mercuric acetate addition to prepare unsaturated fatty ester concentrates, *J. Am. Oil Chem. Soc.*, **39**, 61–62, 1962.

8.6 Metathesis

Olefin methathesis is an equilibrium process whereby alkyl groups attached to olefinic carbon atoms are exchanged and can be most simply represented by the equation (Figure 8.12).

The metathetical transformation of unsaturated compounds discovered and developed during the past 40 years (Mol, 2004; Grubbs and Chang, 1998) offers considerable potential to convert oleochemical feedstocks into specialty chemicals (Mol, 2002; Marvey et al., 2003; Mol, 1994). The importance of the olefin metathesis reaction is underscored by the awarding of the 2006 Nobel Prize in chemistry to Yves Chauvin, Richard Schrock, and Robert Grubbs for their pioneering efforts in the development and use of the metathesis reaction by the chemical industry.

Olefin metathesis reactions can be subdivided into two main reaction categories, namely, self-metathesis (between two identical alkene-containing molecules) and cross-metathesis (between two different alkene-containing molecules). Van Dam and coworkers were the first to demonstrate the self-metathesis of methyl oleate, which gives 9-octadecene and dimethyl 9-octadecene-1,18-dioate for which the latter compound was subsequently used to synthesize the unsaturated C_{17} macrocyclic ketone, civetone (Van Dam et al., 1972) (Figure 8.13).

In a similar manner, the cross-metathesis reaction between ethene and methyl oleate (termed ethenolysis) gives shorter chain compounds, methyl 9-decenoate and 1-decene (Figure 8.14).

As the number of unsaturated centres increases, a greater number of products result. For example, self-metathesis reaction of methyl linoleate (Marvey, 2003; Ast et al., 1976) gives a complex mixture of three groups of products consisting of *cis*- and *trans*-alkenes (12:1, 15:2, 18:3, 21:4, 24:5), monoesters (16:1, 19:2, 22:3, 25:4, 28:5), and diesters (20:1, 23:2, 26:3, 29:4), as well as cyclohexa-1,4-diene. Metathesis of unsaturated oils, such as olive oil, soybean oil palm oil and linseed oil, leads to a complex viscous product mixture that has good drying properties (Refvik et al., 1999; Nicolaides et al., 1990; Marvey et al., 2003; Boelhouwer and Mol, 1985; Nordin et al., 1991; Yarmo et al., 1992; Erhan et al., 1997). Metathesis also occurs with fatty olefinic alcohols when the alcohol moiety

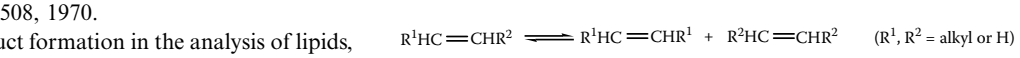


FIGURE 8.12 General equation for olefin metathesis between two alkenes.

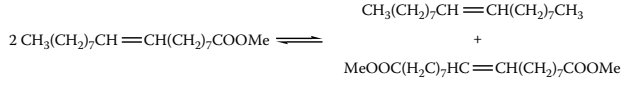


FIGURE 8.13 Self metathesis of methyl oleate to give 9-octadecene and dimethyl 9-octadecene-1,18-dioate.

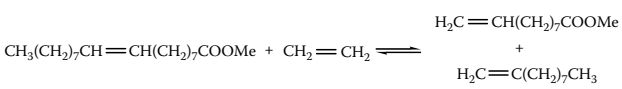


FIGURE 8.14 Ethenolysis reaction of methyl oleate.

is suitably protected such as in the trimethylsilyl ethers (Warwel et al., 1987).

Although the metathesis of simple alkenes such as ethene and 1-butene are commercial processes much used in the petrochemical industry, the application of olefin metathesis to functionalized alkenes, such as unsaturated fatty acid esters and oils, is not currently commercially practiced (Sibeijn et al., 1994). Thus far, the main obstacle to implementing metathesis technology on a commercial scale using oleochemical feedstocks has been the catalyst's expense in conjunction with its poor activity. However, with the advent of improved metathesis catalysts these limitations are being overcome and the olefin metathesis of oleochemical feedstocks is becoming more attractive.

The large number of catalysts that initiate olefin metathesis can be classified as homogeneous and heterogeneous (Ivin and Mol, 1997). These catalysts are typically based upon transition metal compounds such as tungsten hexachloride, titanium, rhenium heptaoxide with a Lewis acid or organometallic compound, such as ethylaluminum chloride, tetramethyl- or tetrabuyltin or triethylboron. More recently, well-defined molybdenum and ruthenium systems have received much interest (Grubbs and Chang, 1998). These latter catalysts are long-lived, highly active, perform under mild conditions, and do not require co-catalysts or promoters in contrast to earlier systems. Further improvements in metathesis catalyst technology are the key driver to making this reaction economically viable.

References

Ast, W. et al. Oligomerisierungsprodukte bei der Olefin Metathese von mehrfach ungesättigten aliphatischen Carbonsäureestern, *Die Makromolekulare Chemie*, **177**, 39–47, 1976.

Boelhouwer, C. and Mol, J. C., Metathesis reactions of fatty acid esters, *Prog. Lipid Res.*, **24**, 243–267, 1985.

Erhan, S.Z. et al. Drying properties of metathesized soybean oil, *J. Amer. Oil Chem. Soc.*, **74**, 703–706, 1997.

Grubbs, R.H. and Chang, S., Recent advances in olefin metathesis and its applications to organic synthesis, *Tetrahedron*, **54**, 4413–4450, 1998.

Ivin, K.J. and Mol, J.C., in *Olefin Metathesis and Metathesis Polymerization*, 2nd ed., Academic Press, San Diego, 1997.

Marvey, B.B. et al. Metathesis of unsaturated fatty acid esters derived from South African sunflower oil in the presence of a 3 wt.% Re_2O_7/SiO_2-$Al_2O_3/SnBu_4$ catalyst, *J. Mol. Catal. A: Chem.*, **201**, 297–308, 2003.

Mol, J.C., Industrial applications of olefin metathesis, *J. Mol. Catal. A: Chem*. **213**, 39–45, 2004.

Mol, J.C., Application of olefin metathesis in oleochemistry: an example of green chemistry, *Green Chem*., **4**, 5–13, 2002.

Mol, J.C., Metathesis of unsaturated fatty acid esters and fatty oils, *J. Mol. Catal.*, **90**, 185–199, 1994.

Nicolaides, C.P. et al. Metathesis of fatty esters derived from South African sunflower oil, *J. Amer. Oil Chem. Soc.*, **67**, 362–363, 1990.

Nordin, N.A.M. et al. Metathesis of palm oil, *J. Mol. Catal.*, **65**, 163–172, 1991.

Refvik, M.D. et al. Ruthenium-catalyzed metathesis of vegetable oils, *J. Amer. Oil Chem. Soc.*, **76**, 93–98, 1999.

Sibeijn, M. et al. Technological and economical aspects of the metathesis of unsaturated esters, *J. Amer. Oil Chem. Soc.*, **71**, 553–561, 1994.

Van Dam, P.B. et al. Metathesis of unsaturated fatty acid esters by a homogenous tungsten hexachloride-tetramethyltin catalyst, *J. Chem. Soc., Chem. Commun.*, 1221–1222, 1972.

Van Dam, P.B. et al. Homogeneous catalytic metathesis of unsaturated fatty esters: new synthetic method for preparation of unsaturated mono- and dicarboxylic acids, *J. Amer. Oil Chem. Soc.*, **51**, 389–392, 1974.

Warwel, S. et al. The influence of protecting groups in the metathesis of oleochemicals, *Fat Sci. Technol.*, **89**, 335–338, 1987.

Yarmo, M.A. et al. Ghani, M., Omar, H. and Malek, A., Transesterification products from the metathesis reaction of palm oil, *J. Mol. Catal.*, **76**, 373–379, 1992.

8.7 Stereomutation

Stereomutation is a term used to describe the interchange (isomerization) of *cis (Z)* and *trans (E)* isomers and has been well studied. Natural unsaturated acids are almost entirely *cis* isomers, and because the *cis* to *trans* isomerization of double bonds is a thermodynamically favoured process, their conversion to *trans* compounds can occur inadvertently during processing (deodorization, hydrogenation, etc.) of fats and oils. Because the *trans* isomer is thermodynamically more stable, double bond migration is generally accompanied by stereomutation. Isomerization of unsaturated acids can also be chemically promoted utilizing appropriate reagents that convert individual stereoisomers to an equilibrium mixture of the *cis* and *trans* isomers.

Isomerization of *cis*-triene acids occurs during steam deodorization (above 230°C) of oils containing these acids and upwards of 25% of these acids may be isomerized during the deodorization process. Deodorization of linolenic acid produces a number of configurational isomers, among which the 9*t*,12*c*,15*c* and 9*c*,12*c*,15*t* isomers predominate, while the 9*t*,12*c*,15*t* and 9*c*,12*t*,15*c* represent minor products (Wolff, 1993, 1993a). Linoleic acid is approximately 13 to 14 times less reactive than linolenic acid under the same conditions and gives predominantly 9*t*,12*c* and 9*c*,12*t* dienes (Wolff et al., 1994, 1996).

In addition to the formation of *trans* fatty acids through deodorization, *trans* fatty acid isomers of this type are also present in processed oils and low-fat spreads produced during partial hydrogenation (Section 4.2). The *trans* content in these materials has been extensively reported (Aro et al., 1998; Wijesundera et al., 1989) and the proportion of *trans* fatty acids currently found in typical soft margarines and low-fat spreads ranges between 0.1 to 17% of total fatty acids (Hunter, 2005). There are many concerns

about the safety and health aspects of edible fats and oils containing *trans* fatty acids at the present time (Kodali and List, 2005).

Cis and *trans* isomers can be interconverted by a sequence of stereospecific reactions that add up to stereomutation of which the following are typical (Larock, 1999) (Figure 8.15).

More commonly, the readily available *cis* isomer is treated with a reagent that brings about isomerization and establishes the *cis*/*trans* equilibrium. The product mixture is then separated by silver-ion chromatography (Section 6.4.2.5) or by crystallization (Section 5.9.3) to isolate the desired *trans* isomer, which is higher melting and less soluble than its *cis* isomer. The reagent utilized to effect this conversion should promote stereomutation without double-bond migration or hydrogen transfer. Nitrogen dioxide, generated from sodium nitrite-nitric acid (Jiang et al., 1999; Litchfield et al., 1965), iodine and light (Chipault and Hawkins, 1960), or a variety of thiol and disulfide compounds (Chatgilialoglu et al., 2002; Chatgilialoglu et al., 2000; Ferreri et al., 2004, 2002, 2001, 1999; Klein et al., 2001; Sprinz et al., 2000; Weber et al., 2000) are commonly used isomerization reagents. The kinetics of thiyl radical-induced isomerization of monounsaturated esters has been well studied (Chatgilialoglu et al., 2002a). Gamma irradiation has also been shown to promote *cis* to *trans* isomerization in barley grains through the formation of thiyl radicals generated *in situ* during irradiation (Geiler et al., 2003). Isomerization utilizing *p*-toluenesulphinic acid also seems to give satisfactory results (Snyder and Scholfield, 1982). More recently, tris(trimethylsilyl)silyl radical [$(TMS)_3Si\cdot$] was shown to effectively isomerize methyl oleate to give and equilibrium mixture containing 78% methyl elaidate (Chatgilialoglu et al., 1995). Reaction with selenium at 190 to 200°C is not used that much now because some double-bond migration also occurs during this reaction. The *cis*/*trans* equilibrium for monoenes and nonconjugated polyenes is such that each *cis* double bond is converted to the *trans* form in 75 to 80% yield. Conjugated polyenes isomerize more readily and give a higher proportion of the all-*trans* isomer.

Recently, the *cis* to *trans* isomerization of unsaturated fatty acids has been shown to occur in living cells and is proposed to be a new adaptive mechanism enabling bacteria to change their membrane fluidity and adapt to environmental changes (Heipieper et al., 2003).

The proportion of *trans* isomer in a reaction mixture can be determined by gas chromatography, infrared spectroscopy, ^{13}C NMR, infrared attenuated total reflectance spectrometry, or preparative silver-ion chromatography (Section 6.4 and Section 6.5). This topic has recently been discussed by Seppanen-Laakso et al. (2002), Christie (1997) and Lanser and Emken (1988).

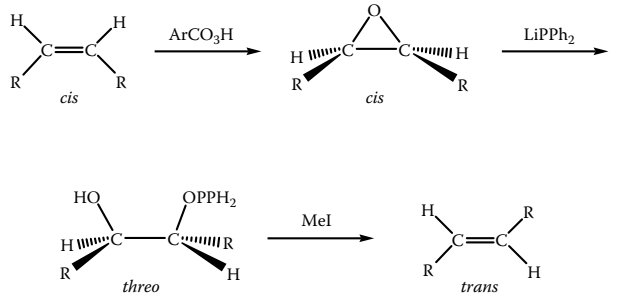


FIGURE 8.15 Stereomutation can be accomplished through a sequence of stereospecific reactions.

References

Aro, A. et al. Trans fatty acids in dietary fats and oils from 14 European countries: the TRANSFAIR study, *J. Food Comp. Anal*., **11**, 137–149, 1998.

Chatgilialoglu, C. et al. Geometrical isomerism of monounsaturated fatty acids: thiyl radical catalysis and influence of antioxidant vitamins, *Free Radic. Biol. Med*., **33**, 1681–1692, 2002.

Chatgilialoglu, C. et al. The kinetics of thiyl radical induced reactions of monounsaturated fatty acid esters, *J. Am. Chem. Soc*., **124**, 12816–12823, 2002a.

Chatgilialoglu, C. et al. (Z)-*(E)* interconversion of olefins by the addition-elimination sequence of the $(TMS)_3Si\cdot$ radical, *J. Org. Chem*., **60**, 3826–3831, 1995.

Chatgilialoglu, C. et al. *Cistrans* isomerization of monounsaturated fatty acid residues in phospholipids by thiyl radicals, *J. Am. Chem. Soc.*, **122**, 4593–4601, 2000.

Chipault, J.R. and Hawkins, J.M., Conversion of conjugated *cis-trans* octadecadienoates to the *trans-trans* isomers, *J. Am. Oil Chem. Soc*., **37**, 176–182, 1960.

Christie, W.W., Structural analysis of fatty acids, in *Advances in Lipid Methodology — Four*, Christie, W. W., Ed., Oily Press, Dundee, U.K., 1997, pp. 119–169.

Ferreri, C. et al. Arachidonate geometrical isomers generated by thiyl radicals: the relationship with *trans* lipids detected in biological samples, *Free Radic. Biol. Med.,* **33**, 1516–1526, 2002.

Ferreri, C. et al. The thiyl radical-mediated isomerization of cis-monounsaturated fatty acid residues in phospholipids: a novel path of membranes damage? *Chem. Commun*., 407–408, 1999.

Ferreri, C. et al. *Cis-trans* isomerization of polyunsaturated fatty acid residues in phospholipids catalyzed by thiyl radicals, *J. Am. Chem. Soc.*, **123**, 4459–4468, 2001.

Ferreri, C. et al. Regioselective cis-trans isomerization of arachidonic double bonds by thiyl radicals: the influence of phopholipid supramolecular organization, *J. Am. Chem. Soc.*, **126**, 1063–1072, 2004.

Geiler, C. et al. Cis-trans-isomerization of unsaturated fatty acids during [gamma]-irradiation of barley grains, *Rad. Phy. Chem*., **67**, 105–113, 2003.

Heipieper, H.J. et al. The *cis-trans* isomerase of unsaturated fatty acids in pseudomonas and vibrio: biochemistry, molecular biology and physiological function of a unique stress adaptive mechanism, *FEMS Microbiol. Lett*., **229**, 1–7, 2003.

Hunter J.E., Dietary levels of trans-fatty acids: basis for health concerns and industry efforts to limit use, *Nutrition Res.,* **25**, 499–513, 2005.

Jiang, H. et al. Nitrogen dioxide induces *cis-trans* isomerization of arachidonic acid within cellular phospholipids, *J. Biol. Chem.*, **274**, 16235–16241, 1999.

Klein, E. and Weber, N., *In vitro* test for the effectiveness of antioxidants as inhibitors of thiyl radical-induced reactions with unsaturated fatty acids, *J. Agric. Food Chem.*, **49**, 1224–1227, 2001.

Kodali, D.R. and List, G.R., *Trans Fats Alternatives*, AOCS Press, Champaign, IL, 2005.

Lanser, A.C. and Emken, E.A., Comparison of FTIR and gas chromatographic methods for quantitation of *trans* unsaturation in fatty acid methyl esters. *J. Am. Oil Chem. Soc.*, **65**, 1483–1487, 1988.

Larock, R.C., *Comprehensive Organic Transformations. A Guide to Functional Group Preparations*, 2nd ed., Wiley-VCH, New York, 1999, pp. 219–250.

Litchfield, C. et al. *Cis-trans* isomerization of oleic acid by nitrous acid, *J. Am. Oil Chem. Soc.*, **42**, 73–78, 1965.

Seppanen-Laakso, T., et al. Analysis of fatty acids by gas chromotography, and its relevance to research on health and nutrition, *Analytica Chimica Acta.,* **465**, 39–62, 2002

Snyder, J.M. and Scholfield, C.R., *Cis-trans* isomerization of unsaturated fatty acids with *p*-toluenesulfinic acid, *J. Am. Oil Chem. Soc.*, **59**, 469–470, 1982.

Sprinz, H. et al. Mechanism of thiyl radical catalyzed isomerization of unsaturated fatty acid residues in homogeneous solution and in liposomes. *Biochim. Biophys. Acta.,* **1483**, 91–100, 2000.

Weber, N. et al. Antioxidants eliminate steromutation and thioether formation during lipase-catalyzed thioesterification and trans thioesterification for the preparation of uniform *cis*-and *trans*-unsaturated thioesters, *Chem. Phys. Lipids*, **105**, 215–223, 2000.

Wijesundera, R.C. et al. Eicosapentaenoic acid geometrical isomer artifacts in heated fish oil esters, *J. Am. Oil. Chem. Soc.*, **66**, 1822–1830, 1989.

Wolff, R.L., Trans-polyunsaturated fatty acids in French edible rapeseed and soybean oils, *J. Am. Oil Chem. Soc.*, **69**, 106–110, 1992.

Wolff, R.L., Heat-induced geometrical isomerization of α-linolenic acid: effect of temperature and heating time on the appearance of individual isomers, *J. Am. Oil Chem. Soc.*, **70**, 425–430, 1993a.

Wolff, R.L., Further studies on artificial geometrical isomers of α-linolenic acid in edible linolenic acid-containing oils, *J. Am. Oil Chem. Soc.*, **70**, 219–224, 1993b.

Wolff, R.L., *Cis-trans* isomerization of octadecatrienoic acids during heating. Study of pinolenic (*cis*-5,*cis*-9,*cis*-12 18:3) acid geometrical isomers in heated pine seed oil, *J. Am. Oil Chem. Soc.*, **71**, 1129–1134, 1994.

Wolff, R.L. et al. Participation of the *cis*-12 ethylenic bond to *cis-trans* isomerization of the *cis*-9 and *cis*-15 ethylenic bonds in heated α-linolenic acid, *J. Am. Oil Chem. Soc.*, **73**, 327–332, 1996.

8.8 Double-bond migration and cyclization

The movement of double bonds in long-chain unsaturated acids occurs with basic or acidic catalysts and earlier literature has been reviewed by Cowan (1949), Rheineck (1958), and Johnson and Pryde (1979). The reaction occurs more easily with methylene-interrupted polyene acids, to give products with conjugated unsaturation. The migration of cis double bonds are usually accompanied by stereomutation (Section 8.7).

Currently, interest in the health benefits attributed to conjugated linoleic acids (CLA) has sparked efforts to isomerize fatty acid double bonds by both chemical (Yang et al., 2002) and biological methods (Griinari and Bauman, 1999) to produce CLA. Saebo (2001) has recently reviewed methods to prepare CLA. Ogawa and coworkers used lactic acid bacteria to produce a mixture of 9*c*,11*t* and 9*t*,11*t* CLA from linoleic acid at 40 mg/ml under the optimized conditions (Ogawa et al., 2005). The CLA-producing reaction was found to consist of two successive reactions consisting of linoleic acid hydration to 10-hydroxy-12-octadecenoic acid followed by dehydration/isomerization of the hydroxy fatty acid to give 9*c*,11*t* and 9*t*,11*t* CLA. Ricinoleic acid and castor oil were also found to be substrates to produce a mixture of 9*c*,11*t* and 9*t*,11*t* CLA by this method.

Isomerization of unsaturated fatty acids has been accomplished under a range of alkaline conditions, including potassium hydroxide in ethanediol at 170 to 180°C (AOCS method Cd 7-58, method approved in 1958), potassium methoxide in small amounts of butanol (Baltes et al. 1961), potassium *t*-butoxide in *t*-butanol at 70°C or in diethyl ether at 25°C (Sreenivasan and Brown, 1956; Sreenivasan and Brown, 1958), the potassium salt of the methyl ether of triethylene glycol at 25 or 60°C, dimsylsodium or dimsylpotassium in dimethylsulphoxide and/or tetrahydrofuran at 25°C (DeJarlais et al., 1973), the potassium salt of 1,3-diaminopropane at 50°C and tetramethylammonium hydroxide (Bittner et al., 1971). Under basic conditions, migration occurs via a resonance-stabilized carbanion (Mounts et al., 1970), to give products with conjugated unsaturation. For example, linoleic acid (9*c*,12*c*), for example, gives mainly a mixture of the 9*c*,11*t* and 10*t*,12*c* dienes (Figure 8.16).

Linolenic acid gives a mixture of trienes with diene or triene conjugation of the double bonds.

$$\text{linolenate } \Delta 9c12c15c \longrightarrow 10t12c15c,\ 9c11t,15c,\ 9c13t15c,\ 9c12c14t$$

and conjugated trienes, such as 10*t*12*c*14*t*

Recently, several photoactive conjugated tetraene fatty acids were prepared from several naturally occurring fatty acids by sequential iodolactonization and treatment with excess 1,8-diazabicyclo[5.4.0] undec-7-ene (Kuklev and Smith, 2004). Isomers of parinaric acid (9,11,13,15-18:4) were prepared from α-linolenic acid in high yields by preparing a conjugated tetraene system (1,3,5,7-octatetraene) by bromination and sequential dehydrobromination reactions (Kuklev and Smith, 2004a).

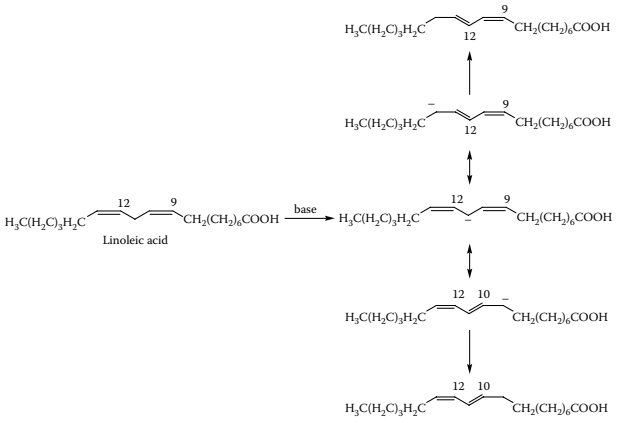


FIGURE 8.16 Base catalyzed isomerization of linoleic acid.

The conjugated products are easily detected by ultraviolet spectroscopy, and alkali isomerization was the basis of a method for the quantitative measurement of linoleate and linolenate prior to the general use of gas chromatography (Jamieson and Reid, 1965; Johnson and Pryde, 1979). The reaction also provided some information about double bond position. For example, linolenic acid (Δ9,12,15) gives conjugated dienes (10*t*12*c*15*c*, 9*c*11*t*,15*c*, 9*c*13*t*15*c*, 9*c*12*c*14*t*) and trienes (such as 10*t*12*c*14*t*), while the less familiar Δ3,9,12 and Δ5,9,12 isomers give only conjugated dienes (AOCS method Cd 7-58). The absence of conjugated triene indicated that one double bond was separated from a methylene-interrupted diene unit by more than one methylene group.

Under acidic conditions, double bond migration occurs through a sequence of protonation-deprotonation processes. Sulfuric acid-catalyzed isomerization of oleic acid to yield γ-stearolactone dates back to the early 1900s and was reexamined by Clutterbuck in 1924. With perchloric acid, oleic acid gives isomeric octadecenoic acids along with mixtures of the γ-stearolactone (thermodynamic product) and δ-stearolactone (kinetic product) resulting from interaction of carboxyl group and carbonium ion on C-4 (Showell et al., 1968; Isbell and Cermak, 2001; Cermak and Isbell 2000; Isbell and Plattner, 1997). The ratio between the γ- and δ-stearolactone is dependent upon acid choice and reaction conditions. The use of polyphosphoric acid also gives cyclic enones.

Hydrogenation (Section 4.2) and reactions occurring at allylic sites such as oxidation (Section 8.2) are generally accompanied by double-bond migration. Organometallic catalysts such as iron and cobalt carbonyls (Frankel and Metlin, 1967), rhodium complexes (DeJarlais and Gast, 1971), and Ru/Al_2O_3 complexes (Bernas et al., 2004) effect double-bond migration, as do some metals and their oxides, sulfur dioxide, and iodine.

Polyene acids undergo cyclization (without dimerization) at elevated temperatures (and may occur during frying) (Johnson and Pryde, 1979; Dobson, 1999). This

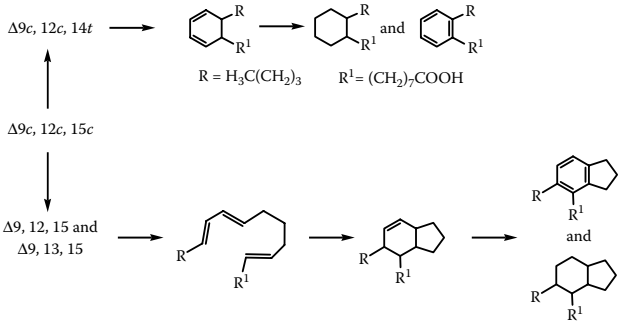


FIGURE 8.17 Some typical cyclization reaction products.

reaction requires double bonds in appropriate positions and of correct configuration, and heat in addition to reagents, such as alkali (to promote double-bond migration) and sulfur or iodine (to promote stereomutation) are frequently used. Cyclization of linoleate has been observed (Dobson et al., 1997), though three or more double bonds are usually required to generate the appropriate intermediates. It is for this reason that linolenic acid (or linseed oil) (Dobson et al., 1995), eleostearic acid (or tung oil) (Paschke and Wheeler, 1955), and fish oils have been most extensively studied. Recently, Dobson and Sebedio (1999) looked at the cyclic products formed from γ-linolenic acid in evening primrose oil heated at 275°C. The cyclized products obtained from these different oils contained mixtures of cyclohexane, cyclohexene, and cyclohexadiene products with all possible combinations of configurational isomers in addition to benzene, indane, or indene systems and acyclic compounds.

The bicyclic acids resulting from intramolecular diene synthesis require a Δ1,3,8-triene unit. Bicyclic acids of this type in tall oil probably result from the Δ5,9,12 acids via a 5,10,12-triene formed under the alkaline conditions of wood pulping. Some typical reaction products are shown in Figure 8.17, but other products of similar kind are also present since double-bond migration does not give only the isomeric trienes in the sequence. Heat treatment of oils containing linoleic and/or linolenic acid (sunflower, linseed, partially hydrogenated soybean) gives rise to several monomeric cyclic acids (Dobson et al., 1996, 1996a). There are *cis* and *trans* isomers of 1,2-disubstituted cylcohexanes and cyclopentanes. Double bonds may still be present in the ring systems or the side chains. Because of their complexity, they have generally been identified only after hydrogenation of the unsaturated centers. The unsaturated cyclic acids may be used in surface coatings, while the hydrogenated products (cyclohexane derivatives) remain liquid at low temperature, and may be useful as low-temperature lubricants. These types of compounds have been identified in used frying oils (Christie et al., 1993; Dobson et al., 1995). The topic has been reviewed by Sebedio and Grandgirard (1989).

References

Bernas, A. et al. Influence of ruthenium precursor on catalytic activity of ru/al$_2$o$_3$ catalyst in selective isomerization of linoleic acid to *cis*-9,*trans*-11- and *trans*-10,*cis*-12-conjugated linoleic acid, *Appl. Catal. A: Gen*., **267**, 121–133, 2004.

Bittner, E.D. et al. Alkali isomerization — gas chromatography with the microreactor, *J. Amer. Oil Chem. Soc*., **48**, 663–635, 1971.

Cermak, S.C. and Isbell, T.A., Synthesis of δ-stearolactone from oleic acid, *J. Am. Oil Chem. Soc.,* **77**, 243–248, 2000.

Christie, W.W. et al. Silver ion chromatography and gas chromatography mass spectrometry in the structural analysis of cyclic monoenoic acids formed in frying oils, *Chem. Phys. Lipids*, **66**, 143–153, 1993.

Clutterbuck, P.W. γ-hydroxystearic acid, *J. Chem. Soc.*, **125**, 2330–2333, 1924.

Cowan, J.C., Isomerization reaction of drying oils, *Ind. Eng. Chem*., **41**, 294–304, 1949.

DeJarlais, W.J. et al. Conjugation of polyunsaturated fats with dimsylsodium, *J. Amer. Oil Chem. Soc*., **50**, 108–109, 1973.

DeJarlais, W.J. and Gast, L.E., Conjugation of polyunsaturated fats: methyl linoleate with tris(triphenylphosphine) chlororhodium, *J. Am. Oil Chem. Soc.,* **48**, 21–24, 1971.

Dobson, G., Cyclic fatty acids in heated vegetable oils: structures and mechanisms, in *Recent Developments in the Synthesis of Fatty Acid Derivatives*, Knothe, G. and Derksen, J.T.P., Eds., American Oil Chemists' Society, Champaign, IL, 1999, pp. 196–212.

Dobson, G. et al. Saturated bicyclic fatty acids formed in heated sunflower oils, *Chem. Phys. Lipids*, **87**, 137–147, 1997.

Dobson, G. et al. Silver ion chromatography and gas chromatography-mass spectrometry in the structural analysis of cyclic dienoic acids formed in frying oils, *Chem. Phys. Lipids,* **75**, 171–182, 1995.

Dobson, G. et al. Monocyclic saturated fatty acids formed from oleic acid in heated sunflower oils, *Chem. Phys. Lipids*, **82**, 101–110, 1996a.

Dobson, G. et al. The nature of cyclic fatty acids formed in heated vegetable oils, *Grasas y Aceites*, **47**, 26–33, 1996b.

Frankel, E.N., Homogeneously catalyzed reactions, in *Fatty Acids*, Pryde, E. H., Ed., American Oil Chemists' Society, Champaign, IL, 1979, pp. 433–437.

Frankel E.N. and Metlin, S., Conjugation of soybean oil by decomposition of its iron tricarbonyl complex with carbon monoxide, *J. Am. Oil Chem. Soc.,* **44**, 37–39, 1967.

Griinari, J.M. and Bauman, D.E., Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants, in *Advances in Conjugated Linoleic Acid Research, Vol. 1,* Yurawecz, M.P. et al. Eds. American Oil Chemists' Society, Champaign, IL, 1999, pp. 180–200.

Isbell, T.A. and Cermak, S.C., Synthesis of δ-eicosanolactone and δ-docosanolactone directly from meadowfoam oil, *J. Am. Oil Chem. Soc.,* **78**, 527–531, 2001.

Isbell, T.A. and Plattner, B.A., A Highly Regioselective synthesis of δ-lactones from meadowfoam fatty acids, *J. Am. Oil Chem. Soc.,* **74**, 153–158, 1997.

Jamieson, G.R. and Reid, E.H., The analysis of oils and fats by gas chromatography. ii. The alkaline isomerization of linoleic acid, *J. Chromatog*., **20**, 232–239, 1965.

Johnson, R.W. and Pryde, E.H., Isomerization, conjugation, and cyclization, in *Fatty Acids*, Pryde, E.H., Ed., American Oil Chemists' Society, Champaign, IL, 1979, pp. 319–342.

Kuklev, D. and Smith, W.L., Synthesis of long chain n-3 and n-6 fatty acids having a photoactive conjugated tetraene group, *Chem. Phys. Lipids*, **130**, 145–158, 2004a.

Kuklev, D. and Smith, W.L., Synthesis of four isomers of parinaric acid, *Chem. Phys. Lipids*, **131**, 215–222, 2004b.

Mounts, T.L. et al. Conjugation of polyunsaturated acids, *Lipids*, **5**, 997–1005, 1970.

Ogawa, J. et al. Production of conjugated fatty acids by lactic acid bacteria, *J. Biosci. Bioeng*., **100**, 355–364, 2005.

Paschke, R.F. and Wheeler, D.H., Cyclization of eleostearic acid, *J. Am. Oil Chem. Soc.,* **32**, 473–478, 1955.

Rheineck, A.E., Recent advances in the technology of drying oils, *Prog. Chem. Fats Other Lipids,* **5**, 165–262, 1958.

Saebo, A., Commercial production of conjugated linoleic acid (CLA), *Lipid Technol. Newsl.*, **7**, 9–13, 2001.

Sebedio, J.L. and Grandgirard, A., Cyclic fatty acids: natural sources, formation during heat treatment, synthesis and biological properties, *Prog. Lipid Res.*, **28**, 303–336, 1989.

Showell, J.S. et al. Perchloric acid isomerization of oleic acid, *J. Org. Chem.,* **33**, 2697–2704, 1968.

Sreenivasan, B.S. and Brown, J.B., Isomerization of linoleic, linolenic, and other polyunsaturated acids with potassium tertiary butoxide, *J. Amer. Oil Chem. Soc*. **33**, 521–526, 1956.

Sreenivasan, B.S. and Brown, J.B., Further studies on the isomerization of polyunsaturated fatty acids by potassium tertiary butoxide, *J. Amer. Oil Chem. Soc*. **35**, 89–93, 1958.

Von Baltes, J. et al. Die katalytische Alkali-Isomerisierun von Polyen-Verbindungen I. Ein neues Verfahren zur Umwindlung von Isolenfettsäuren in Konjeunfettsäuren, *Fette Seifen Anstrichm*., **63**, 413–420, 1961.

von Mikusch, J.D. and Sagredos, A.N., Zur chemie der cyclischen fettsauren, *Fette Seifen Anstrichm*., **73**, 384–393, 1971.

Yang, L. et al. Production of conjugated linoleic aids through KOH-catalyzed dehydration of ricinoleic acid, *Chem. Phys. Lipids*, **119**, 23–31, 2002.

8.9 Cyclisation

Cyclic fatty acids can be obtained from various natural sources (Section 1.1.2.9; Sebedio and Grandgirard, 1989). Synthetically, cyclic acids can be obtained either through inter- or intramolecular cyclization. The synthesis of three-, five-, six-membered carbocyclic and furanoid fatty acids has been reviewed by Lie Ken Jie (1979).

8.9.1 Formation of carbocyclic compounds

Heating of fatty compounds. Various cyclic fatty acids were isolated from evening primrose oil that had been heated to 275°C (Dobson and Sebedio, 1999). These species, formed from γ-linolenic acid in the oil, included eight cyclopentenyl fatty acids (with rings from either C-7

to C-11 or C-8 to C-12 in the original chain; one double bond was lost during cyclization and the others were located at C-9 and C-12, and C-6 and C-9, respectively) and eight cyclohexenyl fatty acids (with rings from either C-7 to C-12 and double bonds at C-5 and C-9 or C-9 and C-13) in the original chain. The double bonds in the chain and the chains around the rings resulted in all possible combinations of configurational isomers. The structures were similar to those reported for α-linolenic acid. A mixture containing bicyclic hexahydroindenoic esters as main components was obtained by heating the mixture present in alkali-isomerized methyl linolenate (Matikainen et al., 2003). Isomerization reactions with model compounds confirmed the results obtained for the alkali-isomerized methyl linolenate. By compiling information from various literature sources on the formation of cyclic and bicyclic fatty acid monomers formed in heated oils, new information on the mechanisms was obtained (Destaillats and Angers, 2005). Concerted routes are proposed. Polyunsaturated fatty acids are more prone to cyclization than monounsatured ones, such as oleic acid.

Diels-Alder reaction. The Diels-Alder reaction forms the chemical basis for the diene number (no longer used). The reactions of a variety of dienophiles, such as nitroethylene, β-nitrostyrene, acryclic acid, acrylonitrile, acrolein, methacrolein, methyl vinyl ketone, and others, with pure isomers of conjugated 9,11-octadecadienoic acid were reported (Teeter et al., 1957).

Isolated double bonds in fatty acid chains are not very reactive in Diels-Alder reactions. However, the conjugated methyl 10(*E*),12(*Z*)-octadecadienoate easily undergoes room-temperature Diels-Alder reactions in the presence of 1 to 1.8 equivalents of a Lewis acid and catalytic amounts of iodine. Methyl 12-oxo-10(*E*)-octadecenoate can serve as a dienophile (aus dem Kahmen and Schäfer, 1998). High pressure and temperature as well as the presence of BHT (butylated hydroxytoluene) was used as the method for norbornylization, using cyclopentadiene, of the fatty acids in linseed oil (Chen et al., 2002). The residual double bond in the norbornyl moiety was epoxidized with hydrogen peroxide and quarternary ammonium tetrakis-(diperoxotungsto)phosphate(3-) as a catalyst (Chen et al., 2002; Figure 8.18 for epoxidized norbornyl structure). Epoxidation of the norbornyl moiety produces a more reactive epoxy group than an epoxy group located directly on the fatty acid chain. The epoxidized norbornylized linseed oil was then cured with vinyl ethers. This is more reactive than epoxidized linseed oil, but less reactive than cycloaliphatic epoxides.

Transition-metal trifluoromethane-sulfonates (triflates; $M(OTf)_x$) were used as recyclable catalysts in the Diels-Alder reaction of conjugated linoleic acid ethyl ester with different quinones and α,β-unsaturated aldehydes and ketones (Behr et al., 2000; see Figure 8.19 for one regioisomer of the Diels-Alder product of conjugated linoleic acid ethyl ester with methyl vinyl ketone and Figure 8.20 for that with naphthoquinone). With $Sc(OTf)_3$ or $Cu(OTf)_2$, the reactions occur at 25 to 40°C. Only 10 mol% catalyst is necessary.

Another possibility for accelerating Diels-Alder reactions may be the presence of ionic liquids (Behr et al., 2003). 1,3-Dialkylimidazolium compounds were used, in which butyl methyl imidazolium and octyl methyl imidazolium served as cations of the ionic liquid. The best yield in a neat ionic liquid for the reaction of conjugated ethyl linoleate with methyl vinyl ketone was 46% with scandium triflate as the catalyst at 40°C under the conditions used. When using the ionic liquid in a nonpolar solvent, such as dichloromethane, reaction times are reduced. Strong temperature-dependent phase behavior is observed with acetonitrile as additional solvent.

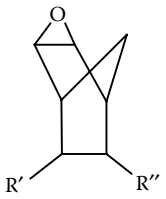
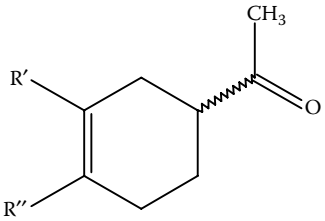
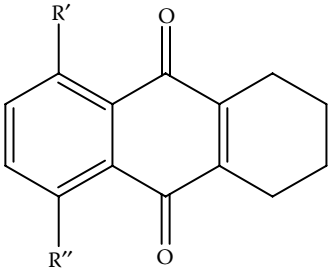


FIGURE 8.18



$R' = (CH_2)_{7/8} - COOEt$
$R'' = (CH_2)_{5/4} - CH_3$

FIGURE 8.19



$R' = (CH_2)_{7/8} - COOEt$
$R'' = (CH_2)_{5/4} - CH_3$

FIGURE 8.20

8.9.2 Cyclopropane, -propene, and -pentane derivatives

Generally, cyclopropane compounds can be prepared by the Simmons-Smith reaction from the corresponding olefinic compounds using di-iodomethane and zinc-copper couple; see, for example, the report by Christie and Holman (1966) in which nearly quantitative conversion was obtained. Ultrasound was applied to the Simmons-Smith reaction using methyl oleate, methyl linoleate, methyl ricinoleate, and furanoid fatty esters as substrates (Lie Ken Jie and Lam, 1988). Cyclopropanation was reported when using *in situ*-generated diazomethane in the presence of palladium (II) actetate with terminal unsaturated, α,β-unsaturated, and cyclopentene fatty esters being more reactive than methyl elaidate, which was more reactive than methyl oleate (Gangadhar et al., 1988). Six cyclopropene homologs of methyl sterculate were synthesized from the corresponding alkynoic esters in three steps (Williams and Sgoutas, 1972).

Petroselinic acid was cyclized via methyl 2-iodopetroselinate as intermediate to give methyl *trans*- and *cis*-2-dodecyl-1-cyclopentanecarboxylate (Figure 8.21) using tributyltin hydride (Mahler and Metzger, 1993). Employing hexabutylditin yielded bicyclic γ-lactones and methyl *trans*-2-(1 N-iodododecyl)-1-cyclopentanecarboxylate. The initiator system $SnCl_2$/AgOAc afforded the same products.

8.9.3 Formation of heterocyclic compounds

Lactonization. Manganese (III) acetate mediation can be used in the synthesis of γ-lactones by adding short-chain carboxylic acids to methyl oleate (Metzger and Linker, 1991). The γ-lactone moiety is located at the former double bond position in the fatty acid chain. The copper-initiated radical addition of alkyl α-iodocarboxylates leads to γ-lactones in high yields (Metzger and Mahler, 1995). For example, the addition of ethyl iodoacetate to methyl 10-undecenoate yields the terminal lactone depicted in Figure 8.22, while the addition of methyl 2-iodopalmitate to methyl 10-undecenoate afford the corresponding product shown in Figure 8.23. The alkyl 2-bromocarboxylates also give similar products. More examples are available from the literature (Metzger and Mahler, 1995). Alkyl 2-iodoalkanoates, such as 2-iodopalmitate, reacted with various alkenes in the presence of copper powder afforded lactones disubstituted in the lactone ring (Metzger et al., 1997).

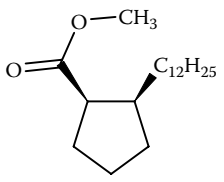


FIGURE 8.21

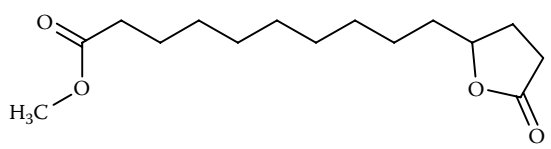


FIGURE 8.22

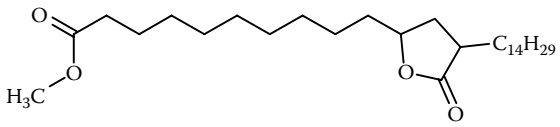


FIGURE 8.23

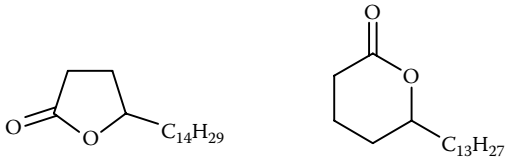


FIGURE 8.24

δ-Stearolactone (see Figure 8.24 for structure of strearolactones) was formed from oleic acid in the presence of sulfuric acid using methylene chloride as solvent (Cermak and Isbell, 2000). Ratios of δ/γ lactone as high as 15:1 were achieved, with lower temperatures giving higher δ/γ ratios (Cermak and Isbell, 2000). Other acids can also be used in this cyclization. In the synthesis of δ-lactones from meadowfoam fatty acids in the presence of perchloric or sulfuric acids, methylene chloride as the solvent increased regioselectivity to the δ-lactone with solvents of higher dielectric strength generally increasing δ/γ-lactone ratios and yields (Isbell and Plattner, 1997). A direct synthesis of δ-eicosanolactone and δ-docosanolactone from meadowfoam oil triacylglycerols was again achieved with perchloric and sulfuric acids, with perchloric acid-mediated reactions requiring less acid and the use of polar solvents having little effect on the δ/γ-ratio and yield (Isbell and Cermak, 2001). Lipase-catalyzed epoxidation of meadowfoam fatty acids in presence of hydrogen peroxide yielded some 5,6-epoxy fatty acids, which were lactonized to 6-hydroxy δ-eicosanoic and docosanoic lactones with sulfuric acid as catalyst (Frykman and Isbell, 1997).

Iodolactonization, which employs $KHCO_3$, I_2, and KI, is a means for separating naturally occurring polyunsaturated fatty acids, such as docosahexaenoic acid and other Δ4 or Δ5 acids from fatty acid concentrates (Gaiday et al., 1991; Imbs et al., 1991; see Figure 8.25 for structure of iodolactones). Macrolactonization of ricinoleic acid and 12-hydroxystearic acid in the presence of Boc_2O (t-butyl dicarbonate) and *Candida cylindrica* lipase was reported (Nagarajan 1999). Unsaturated fatty compounds, such as oleic acid in combination with 1,ω-diols, gave macrolides

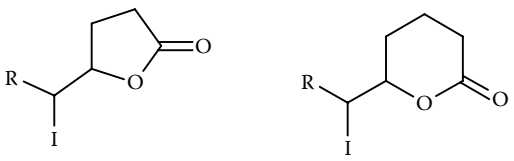


FIGURE 8.25

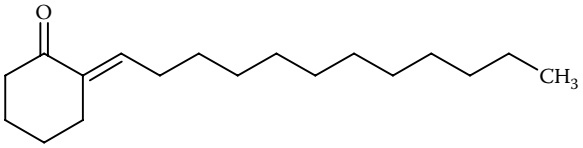


FIGURE 8.26

(macrolactones) suitable as musk fragrances in a sequence of reactions (Rodefeld et al., 1998).

Acylations. Petroselinic acid gave the cyclic ketone with an exocyclic *E* double bond depicted in Figure 8.26 through $EtAlCl_2$-induced intramolecular acylation, with subsequent hydrogenation leading to 2-dodecylcyclohexanone (Metzger and Biermann, 1993). Acylation of unsaturated fatty compounds, such as oleic acid, methyl oleate, and 10-undecenoic acid with α,β-unsaturated acyl chlorides induced by the Lewis acid $EtAlCl_2$ afforded corresponding allyl vinyl ketones, which could undergo Nazarov cyclizations, to substitute 2-cyclopentenones (Metzger and Biermann, 1998).

Furanization. The synthesis of the complete series of isomeric furanoid C_{18} fatty acids with the furan moiety "migrating" from one of the chains to the other was reported using furan, furfural, or methyl octadecadiynoate as starting materials (Lie Ken Jie and Ahmad, 1978). Linoleic acid was converted to C_{18} furanoid acids substituted in the ring with methyl groups as found in fish oils (Lie Ken Jie and Ahmad, 1981). A procedure for the synthesis of methyl and dimethyl substituted furanoid fatty esters involves methylation of the dioxo stearate derivatives (Lie Ken Jie and Ahmad, 1983) and another procedure for introducing methyl groups into the 3,4-position of 2,5-disubstituted C_{18} furanoid fatty esters (Figure 8.27) was reported later (Lie Ken Jie and Wong, 1991). C_{18} fatty esters with a 3,4-disubstituted furan moiety were synthesized by reacting a furan fatty acid with dimethyl acetylenedicarboxylate to give a Diels-Alder-type product (Lie Ken Jie and Wong, 1992). Further reaction steps, including a retro-Diels-Alder reaction, yielded the desired product. Epoxyoxoene fatty esters are intermediates in the synthesis of fatty pyrrole esters, in which furanoid fatty esters were identified (Hidalgo and Zamora, 1995). Oxidative ring opening of 2,5-disubstituted furanoid fatty esters leads to 2(*E*)-ene-1,4-diones and 2,3-epoxy-1,4-diones (Lie Ken Jie et al., 1997). Halogen-containing cyclic ethers (tetrahydrofuran or tetrahydropyran derivatives) were reported in some halogenations of long-chain hydroxyl alkenes (Gunstone and Perera, 1973).

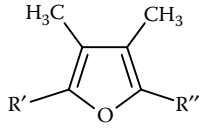


FIGURE 8.27

Formation of other heterocyclic compounds. Various heterocyclic compounds derived from fatty acids have been reported. In a procedure related to click chemistry (Kolb et al., 2001), terminal tetrazoles derived from fatty nitriles were reported, which could be reacted further to give 1,3,4-oxadiazoles and bis(oxadiazoles) (Fürmeier and Metzger, 2003a). Similarly, methylene-interrupted triaziridine compounds were synthesized from linoleic and linolenic acids (Metzger and Fürmeier, 1999). 4,5-Dihydro-oxazoles, oxazolidines, imidazoles and oxazoles were obtained by different procedures (Fürmeier and Metzger, 2003a). 3,5-Disubstituted isoxazoles derived from fatty esters by reaction with the lithiated anion of *N*-(isopropylidene)isopropylamine and subsequent dehydrative cyclization (Kenar and Wetzel, 2003) serve as intermediates in the synthesis of β-enaminones and β-diketones (Kenar, 2003). Fatty isoxazoline compounds (Kenar and Erhan, 2001) are intermediates in the preparation of fatty esters containing the β-hydroxy ketone moiety using a reductive hydrogenolysis-hydrolysis procedure employing Raney nickel (Kenar, 2002).

Aziridines were ultimately derived from vernolic and ricinoleic acids by a reaction series including reaction of epoxides with sodium azide and NH_4Cl to give azido hydroxy compounds, which upon treatment with polymer-bound-triphenylphosphine gave the aziridines (Fürmeier and Metzger, 2003b). Pyrazole fatty esters were synthesized in a multistep sequence from methyl ricinoleate via a keto-allenic ester intermediate with ultrasound irradiation being applied in the last step (Lie Ken Jie and Lau, 1999). Ultrasound assistance was also used in the synthesis of the six, seven, and eight-membered heterocyclic 2,3-dihydropyrazine, 2,3-dihydro-1*H*-1,4-diazepine, and 4-tetrahydro-1,4-diazocine derivatives from methyl 9,10-, 10,12-, and 9,12-dioxostearates with diaminomethane (Lie Ken Jie et al., 2001).

Isoquinolines with long-chain substituents were derived from dopamines reacted with long-chain aldehydes in Pictet-Spengler condensations or fatty acids reacted with homoveratrylamine in a sequence with Bischler-Napieralski cyclization as key step (Matuszewska et al., 2005). Such compounds are of interest for their neurochemical activity (Matuszewska et al., 2005).

References

aus dem Kahmen, M. and Schäfer, H.J., Conversion of unsaturated fatty acids — cycloadditions with unsaturated fatty acids, *Fett/Lipid,* **100**, 227–235, 1998.

Behr, A. et al. Transition-metal trifluoromethane-sulphonates — recyclable catalysts for the synthesis of branched fatty derivatives by *Diels-Alder* reactions at unsaturated fatty esters, *Eur. J. Lipid Sci. Technol.,* **102**, 342–350, 2000.

Behr, A. et al. Use of ionic liquids for the Diels-Alder reaction of oleochemicals, *Eng. Life Sci.,* **3**, 325–327, 2003.

Cermak, S.C. and Isbell, T.A., Synthesis of δ-stearolactone from oleic acid. *J. Am. Oil Chem. Soc.,* **77**, 243–248, 2000.

Chen, J. et al. Synthesis and photopolymerization of norbornyl epoxidized linseed oil, *Polymer,* **43**, 5379–5389, 2002.

Christie, W.W. and Holman, R.T., Mass spectrometry of lipids. I. Cyclopropane fatty acid esters, *Lipids,* 1 (**3**), 176–182 (1966).

Coxon, G.D. et al. Facile synthesis of (*Z*)-tetracos-5-enoic acid and racemic *cis*-4-(2-octadecylcyclopropane-1-yl)-butanoic acid, *Chem. Phys. Lipids,* **126**, 49–53, 2003.

Destaillats, F. and Angers, P., On the mechanisms of cyclic and bicyclic fatty acid monomer formation in heated edible oils, *Eur. J. Lipid Sci. Technol.,* **107**, 767–772, 2005.

Dobson, G., Cyclic fatty acids in heated vegetable oils: structures and mechanisms, in *Recent Developments in the Synthesis of Fatty Acid Derivatives*, Knothe, G. and Derksen, J.T.P., Eds., AOCS Press, Champaign, IL, 1999, pp. 196–212.

Dobson, G. and Sebedio, J.-L. Monocyclic dienoic fatty acids formed from linolenic acid in heated evening primrose oil, *Chem. Phys. Lipids,* **97**, 105–118, 1999.

Frykman, H.B. and Isbell, T.A., Synthesis of 6-hydroxy δ-lactones and 5,6-dihydroxy eicosanoic/docosanoic acids from meadowfoam fatty acids *via* a lipase-mediated self-epoxidation, *J. Am. Oil Chem. Soc.,* **74**, 719–722, 1997.

Fürmeier, S. and Metzger, J.O., Synthesis of new heterocyclic fatty compounds, *Eur. J. Org. Chem.*, 885–893, 2003a.

Fürmeier, S. and Metzger, J.O., Fat-derived aziridines and their N-substituted derivatives: biologically active compounds based on renewable raw materials, *Eur. J. Org. Chem*., 649–659, 2003b.

Gaiday, N.V. et al. Separation of natural polyunsaturated fatty acids by means of iodolactonization, *J. Am. Oil Chem. Soc.,* **68**, 230–233, 1991.

Gangadhar, A. et al. Cyclopropanation of unsaturated fatty acid methyl esters using diazomethane and palladium (II) acetate, *J. Am. Oil Chem. Soc.,* **65**, 601–606, 1988.

Gunstone, F.D. and Perera, B.S., Fatty acids, Part 41. The halogenation of some long-chain hydroxy alkenes with special reference to the possibility of neighbouring group participation leading to cyclic ethers or lactones, *Chem. Phys. Lipids,* **11**, 43–65, 1973.

Hidalgo, F.J. and Zamora, R., Epoxyoxoene fatty esters: Key intermediates for the synthesis of long-chain pyrrole and furan fatty esters, *Chem. Phys. Lipids,* **77**, 1–11, 1995.

Imbs, A.B. et al. Application of an analytical modification of the iodolactonization reaction to selective detection of Δ 5(Δ 4) unsaturated fatty acids, *Chem. Phys. Lipids,* **60**, 71–76, 1991.

Isbell, T.A. and Cermak, S.C., Synthesis of δ-eicosanolactone and δ-docosanolactone directly from meadowfoam oil, *J. Am. Oil Chem. Soc.,* **78**, 527–531, 2001.

Isbell, T.A. and Plattner, B.A., A highly regioselective synthesis of δ-lactones from meadowfoam fatty acids, *J. Am. Oil Chem. Soc.,* **74**, 153–158, 1997.

Kenar, J.A., Reduction of fatty ester Δ^2-isoxazoline heterocycles. Preparation of fatty esters containing the β-hydroxy ketone moiety, *J. Am. Oil Chem. Soc.,* **79**, 351–356, 2002.

Kenar, J.A., Preparation of long-chain β-enaminones and β-diketones from long-chain 3,5-disubstituted isoxazole compounds, *J. Am. Oil Chem. Soc*., **80**, 1027–1032, 2003.

Kenar, J.A. and Erhan, S.Z., Synthesis of Δ^2-isoxazoline fatty acid ester heterocyles. *J. Am. Oil Chem. Soc.,* **78**, 1045–1050, 2001.

Kenar, J.A. and Wetzel, A.R., Preparation of fatty 3,5-disubstituted isoxazole compounds from FA esters, *J. Am. Oil Chem. Soc.,* **80**, 711–716, 2003.

Kolb, H.C. et al. Click chemistry: diverse chemical function from a few good reactions, *Angew. Chem.,* **40**, 2004, 2001.

Lie Ken Jie, M.S.F. and Lam, C.H., Fatty Acids, Part XVI. The synthesis of all isomeric C_{18} furan-containing fatty acids, *Chem. Phys. Lipids,* **21**, 275–287, 1978.

Lie Ken Jie, M.S.F., Synthesis of three-, five-, six-membered carbocyclic and furanoid fatty acids, *Chem. Phys. Lipids,* **24**, 407–430, 1979.

Lie Ken Jie, M.S.F. and Ahmad, F., Conversion of linoleic and latex furanoid acid to fish C18 dimethyl furanoid isomers, *J. Chem. Soc., Chem. Commun*., 1110–1111, 1981.

Lie Ken Jie, M.S.F. and Lam W.L.K., Cyclopropanation of unsaturated fatty esters and triglycerides, *J. Am. Oil Chem. Soc.,* **65**, 118–121 (1988).

Lie Ken Jie, M.S.F. et al. Fatty acids: Part 25. Chemical transformations of C_{18} furanoid fatty esters, *J. Am. Oil Chem. Soc.,* **60**, 1777–1782, 1983.

Lie Ken Jie, M.S.F. and Wong, K.P, A novel method for the introduction of a methyl group into the furan ring of a 2,5-disubstituted C_{18} furanoid fatty ester *via* a malonic acid function, *Lipids,* **26**, 837–841, 1991.

Lie Ken Jie, M.S.F. and Wong, K.P., Synthesis of dimethyl-substituted C_{18} furanoid fatty ester, *J. Am. Oil Chem. Soc.,* **69**, 485–487, 1992.

Lie Ken Jie, M.S.F. et al. Ultrasonically stimulated oxidation reactions of 2,5-disubstituted C_{18} furanoid fatty ester, *Chem. Phys. Lipids,* **85**, 101–106, 1997.

Lie Ken Jie, M.S.F. and Lau, M.M.L., Ultrasound assisted synthesis of pyrazole fatty ester derivatives from a key C_{18} keto-allenic ester, *Chem. Phys. Lipids,* **101**, 237–242, 1999.

Lie Ken Jie, M.S.F. et al. Cyclodehydration reactions of methyl 9,10-; 10,12-; and 9,12-dioxostearates under ultrasonic irradiation, *Lipids,* **36**, 201–204, 2001.

Mahler, R. and Metzger, J.O., Radical cyclization of petroselinic acid (Radikalische Cyclisierung von Petroselinsäure), *Fat Sci. Technol.,* **95**, 337–339, 1993.

Matikainen, J. et al. A study of 1,5-hydrogen shift and cyclization reactions of an alkali isomerized methyl linolenate, *Tetrahedron,* **59**, 567–573, 2003.

Matuszewska, I. et al. Synthesis of a novel class of fatty acids-derived isoquinolines, *Chem. Phys .Lipids,* **135**, 131–145, 2005.

Metzger, J.O. and Biermann, U., Alkylaluminum dichloride induced Friedel-Crafts acylation of unsaturated carboxylic acids and alcohols, *Liebig's Ann. Chem.*, 645–650, 1993.

Metzger, J.O. and Biermann, U., Lewis acid induced additions to unsaturated fatty compounds IV: Synthesis of cyclopentenones from Friedel-Crafts acylation products of

unsaturated fatty compounds with α,β-unsaturated acyl chlorides, *Fett./Lipid,* **100**, 2–6, 1998.
Metzger, J.O. and Fürmeier, S., New type of skipped oligoaziridines: synthesis of new fatty acid derivatives containing aziridine functions, *Eur. J. Org. Chem.*, 661–664, 1999.
Metzger, J.O. and Linker, U., New results on free radical additions to unsaturated compounds, *Fat Sci. Technol.,* **93**, 244–249, 1991.
Metzger, J.O. et al. Radical additions of activated haloalkanes to alkenes initiated by electron transfer from copper in solvent-free systems, *Angew. Chem. Int. Ed. Engl,.* **34**, 902–904, 1995.
Metzger, J.O. et al. Radical additions of alkyl 2-haloalkanoates and 2-haloalkanenitriles to alkenes initiated by electron transfer from copper in solvent-free systems, *Liebig's Ann./Recueil*, 2303–2313, 1997.
Nagarajan, M., Boc_2O mediated macrolactonisation: syntheses of *R*-(+)-ricinoleic acid lactone and (±)-12-OH-stearic acid lactone, *Synth. Commun.,* **29**, 2467–2475, 1999.
Rodefeld, L. et al. Synthesis and olfactory properties of new macrolides from unsaturated fatty acids and 1,T-diols, *Tetrahedron,* **54**, 5265–5286, 1998.
Sebedio, J.-L. and Grandgirard, A., Cyclic fatty acids: natural sources, formation during heat treatment, synthesis and biological treatment, *Prog. Lipid Res.,* **28**, 303–336, 1989.
Teeter, H.M. et al. Reactions of conjugated fatty acids. IV. Diels-Alder adducts of 9,11-octadecadienoic acid, *J. Org. Chem.,* **22**, 512–514, 1957.
Williams, J.L. and Sgoutas, D.S., Synthesis of six lower homologs of methyl sterculate, *Chem. Phys. Lipids,* **9**, 295–308, 1972.

8.10 Dimerisation

Heating fatty acids in the presence of radical, oxidative, and cationic sources produces dimer fatty acids (Guillaumin, 1970; Cowan, 1962; Berman and Loeb, 1975; Figge, 1971). Commercially, manufacture of dimer fatty acids is accomplished thermally at 230°C for 4 to 8 hours, using montmorillonite clay catalyst (Berman and Loeb, 1975a). Molecular distillation of the resulting product mixture gives enriched fractions of monomer (isostearic acid) and dimer containing some trimer (Berman and Loeb, 1975). The mechanism and products structures are not completely understood, but they are branched and/or cyclic compounds with 36 carbon atoms and two carboxyl groups. Rajadhyaksha and coworkers have described the use of superacid catalysts based on zirconia, and claim that these catalysts give higher dimer/trimer ratios (Rajadhyaksha et al., 1988). Koster et al. (1998) have recently examined the importance of active sites as well as structural and textural parameters of montmorillonite clay in the dimerization reaction of oleic acid.

Historically, interest in dimer acids is a result of work to thermally polymerize (heat body) vegetable oils. In the late 1920s and early 1930s, Scheiber (1929) and Kappelmeier (1933) postulated that conjugated dienes are formed by hydrogen transfer and/or rearrangement of monounsaturated or polyunsaturated fatty acids before dimerization can occur. Diels-Alder type reactions between the conjugated polyunsaturated fatty acid and a dieneophile subsequently gives the monocyclic dimer acid products as 1,2,3,4-tetrasubstituted cyclohexene ring compounds. Complex mixtures of head-to-head and head-to-tail monocyclic structural isomers are formed. Finally, through hydrogen transfer reactions the cyclohexene ring is converted to cylcohexane and benzene derivatives (Figure 8.28).

In addition to the monocyclic dimers formed by Diels Alder-type reactions during the clay catalyzed process, acyclic dimers and bicyclic dimers, such as those formulated in Figure 8.29, are also formed. For example, oleic acid dimerization gives mixtures of acyclic and cyclic dimers as well as large amounts of saturated materials by hydrogen transfer, isomerization, and rearrangement reactions (Koster et al. 1998; den Otter, 1970, 1970a, 1970b; Sen Gupta, 1967).

Linoleate reacts in a similar manner and studies using soybean oil have provided information about aromatic compounds present in both monomer and dimer fractions (Christopoulou et al., 1989; Figge, 1971a; Sen Gupta, 1968; Sen Gupta and Scharmann, 1968; Sen Gupta and Scharmann, 1968a; Möhring and Spiteller, 1990; Adelhardt et al., 1991).

Monoene acids produce mainly acyclic (40%) and monocyclic (55%) dimers, but diene acids furnish monocyclic (55%) and bicyclic (40%) dimers. Tall oil fatty acids, containing both oleic and linoleic acid, are frequently used to produce dimers on an industrial scale. A typical

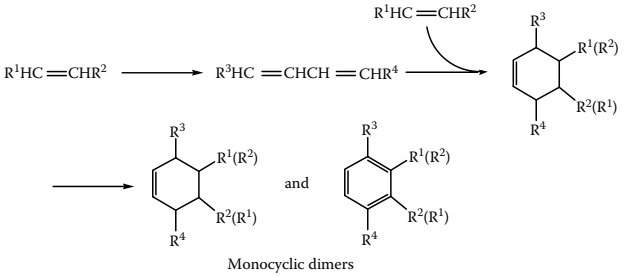


FIGURE 8.28 Formation of dimer acids from a monounsaturated fatty acid, such as oleic acid. This reaction proceeds through desaturation of the monoene acid.

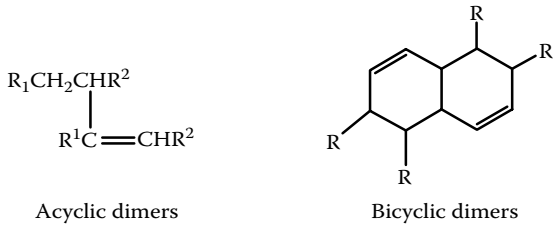


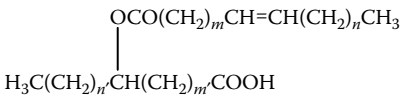
FIGURE 8.29 Typical acyclic and bicyclic dimers formed by Diels Alder-type reactions during the clay catalyzed process.

product contains 80% dimer acids and 20% trimer acids, with only a trace of monomer (Berman and Loeb, 1975). Meadowfoam fatty acids also give a high-quality dimer (Burg and Kleiman, 1991). Standard procedures common to fats and oils analyses are used to measure dimer acid properties, such as acid value, saponification value, Gardner color, viscosity, specific gravity, and pour point. Chromatographic methods to analyze dimer acid components have been reported (Rao et al., 1989; Zhao and Olesik, 2001).

Dimer acids are used mainly as polyamides (Fury, 1975; Heidarian et al., 2004). Nonreactive polyamides, produced from dimer acids and diamines in stoichiometric amounts, are flexible and tough and have excellent adhesive properties (Vedanayagam and Kale, 1992, Chen et al., 2002). They are used as shoe adhesives, in printing inks, for surface coatings, and in textile applications. Reactive polyamides, with free amine groups that can react further, are used as components of thermosetting epoxy systems in surface coatings (Frihart, 2004; Vijayalakshmi et al., 1992). Dimer acids have also been used to prepare segmented block copolymers (Guo et al., 2004; Maniar et al., 1994, 1993a, 1993b). Dimer acids are also used for corrosion inhibition in petroleum-producing equipment (as imidazoles) and as lubricants (as esters) (Fury et al., 1975).

Dimerization is typically carried out in the presence of a small amount of water (1 to 2%). If the water is increased (9 to 10%), and particularly with monoene rather than polyene acids, the yield of dimer declines and is replaced by estolides (linear oligomeric polyesters of hydroxyl fatty acids) of the type shown (Wheeler and Godfrey, 1975; Lyons et al., 1969; Dilworth et al., 1959; Isbell et al., 1994) (Figure 8.30).

Estolides have also been reported to occur in nature (Aitzetmuller et al., 1992; Hayes et al., 1995a, 1995b). Estolides find use as lubricants and functional fluids, and extensive work by Cermak and Isbell have shown these materials to possess good pour points and oxidative stability (Cermak and Isbell, 2003, 2002a, 2003b, 2004; Isbell et al., 2001). The preparation of estolides using lipase-catalyzed reactions has also been reported (Yoshida et al., 1997; Hayes and Kleiman, 1995; Bodalo-Santoyo et al., 2005).



estolide ($n' = n$ or $n + 1 = m' = m + 1$ or m)

FIGURE 8.30 General chemical structure of estolides (the upper and lower halves of this structure have the same number of carbon atoms).

References

Adelhardt, R. et al. Produkte der dimerisierung ungesättigter fettsauren V: Die aromatenfraktion dimerer fetsäuren, *Fat Sci. Technol.*, **93**, 277–282, 1991.

Aitzetmuller, K. et al. High-performance liquid chromatographic investigations of stillingia oil, *J. Chromatog. A,* **603**, 165–173, 1992.

Berman, L.U. and Loeb, M.L., Structure and properties of dimer acids, in *The Dimer Acids. The Chemical and Physical Properties, Reactions, and Applications of Polymerized Fatty Acids*, Leonard, E.C., Ed., Humko Sheffield Chemical, Memphis, TN, 1975a, pp. 9–26.

Berman, L.U. and Loeb, M.L., The general characteristics of dimer acids, in *The Dimer Acids. The Chemical and Physical Properties, Reactions, and Applications of Polymerized Fatty Acids*, Leonard, E.C., Ed., Humko Sheffield Chemical, Memphis, TN, 1975b, pp. 1–8.

Bodalo-Santoyo, A. et al. Enzymatic biosynthesis of ricinoleic acid estolides, *Biochem. Eng. J.*, **26**, 155–158, 2005.

Burg, D.A. and Kleiman, R., Preparation of meadowfoam dimer acids and dimer esters, and their use as lubricants, *J. Am. Oil Chem. Soc.*, **68**, 600–603, 1991.

Cermak, S.C. and Isbell, T.A., Improved oxidative stability of estolide esters, *Ind. Crops Prod.*, **18**, 223–230, 2003.

Cermak, S.C. and Isbell, T.A., Physical properties of saturated estolides and their 2-ethylhexyl esters, *Ind. Crops Prod.,* **16**, 119–127, 2002.

Cermak, S.C. and Isbell, T.A., Synthesis and physical properties of estolide-based functional fluids, *Ind. Crops Prod.*, **18**, 183–196, 2003.

Chen, X. et al. Polyamides derived from piperazine and used for hot-melt adhesives: synthesis and properties, *Int. J.Adhes.*, **22**, 75–79, 2002.

Christopoulou, C.N. and Perkins, E G., Isolation and characterization of dimers formed in used soybean oil, *J. Am. Oil Chem. Soc.*, **66**, 1361–1370, 1989.

Cowan, J.C., Dimer acids, *J. Am. Oil Chem. Soc.*, **39**, 534–545, 1962.

Dilworth, J.P. et al. Stabilized calcium fatty acid base grease, U.S. Patent 2,877,181, 1959.

den Otter, M.J.A.M., The Dimerization of oleic acid with a montmorillonite catalyst I: important process parameters; some main reactions, *Fette Seifen, Anstrichm.*, **72**, 667–673, 1970a.

den Otter, M.J.A.M., The dimerization of oleic acid with a montmorillonite catalyst ii: glc analysis of the monomer; the structure of the dimer; a reaction model, *Fette Seifen, Anstrichm.*, **72**, 875–883, 1970b.

den Otter, M.J.A.M., The dimerization of oleic acid with a montmorillonite catalyst III: Test of the reaction model, *Fette Seifen, Anstrichm.*, **72**, 1056–1066, 1970c.

Figge, K., Dimeric fatty acid[1-^{14}C] methyl esters. I. Mechanisms and products of thermal and oxidative-thermal reactions of unsaturated fatty acid esters-literature review, *Chem. Phys. Lipids*, **6**, 159–177, 1971a.

Figge, K., Dimeric fatty acid[1-^{14}C] methyl esters. II. Preparation and rate of formation; thermal isomerisation of monomers, *Chem. Phys. Lipids*, **6**, 178–199, 1971b.

Frihart, C.R., Specific adhesion model for bonding hot-melt polyamides to vinyl, *Int. J. Adhes. Adhes.*, **24**, 415–422, 2004.

Fury, L.A. et al. Commercial applications of dimer acids and their derivatives, in *The Dimer Acids. The Chemical and Physical Properties, Reactions, and Applications of Polymerized Fatty Acids*, Leonard, E.C., Ed., Humko Sheffield Chemical, Memphis, TN, 1975, pp. 49–97.

Guillaumin, R., Quelques aspects actuels de la lipochemie: obtention de dérivés bifonctionnels par dimérisation, *Rev. Fr. Cops Gras*, **17**, 441–454, 1970.

Guo, W.-X. and Huang, K.-X. Preparation and properties of poly(dimer acid-dodecanedioic acid) copolymer and poly(dimer acid-tetradecanedioic acid) copolymer, *Poly. Degrad. Stabil.*, **84**, 375–381, 2004.

Hayes, D.G. et al. Occurrence of estolides in processed dimorphotheca pluvialis seed oil, *Ind. Crops Prod.*, **4**, 295–301, 1995a.

Hayes, D.G. et al. The triglyceride composition, structure and presence of estolides in the oils of lesquerella and related species, *J. Am. Oil Chem. Soc.,* **72**, 559–569, 1995b.

Hayes, D.G. and Kleiman, R., Lipase-catalysed synthesis and properties of estolides and their esters, *J. Am. Oil Chem. Soc*., **72**, 1309–1316, 1995.

Heidarian, J. et al. Study on kinetics of polymerization of dimer fatty acids with ethylenediamine in the presence of catalyst, *Chem. Eng. J.*, **100**, 85–93, 2004.

Isbell, T.A. and Cermak, S.C., Purification of meadowfoam monoestolide from polyestolide, *Ind. Crops Prod.*, **19**, 113–118, 2004.

Isbell, T.A. et al. Physical properties of estolides and their ester derivatives, *Ind. Crops Prod.*, **13**, 11–20, 2001.

Isbell, T.A. et al. Acid-catalyzed condensation of oleic acid into estolides and polyestolides, *J. Am. Oil Chem. Soc.,* **71**, 169–174, 1994.

Kappelmeier, C.P.A. Chemical processes of stand-oil formation, *Farben-ZTG.*, **38**, 1018–20, 1933.

Koster, R.M. et al. Active sites in the clay catalysed dimerisation of oleic acid, *J. Mol. Catal. A: Chem*., **134**, 159–169, 1998.

Lyons, J.F. et al. Power steering process and lubricating composition, U.S. Patent 3,429,820, 1969.

Maniar, M. et al. Controlled release of a local anesthetic from fatty acid dimer based polyanhydride, *J. Control. Rel.*, **30**, 233–239, 1994.

Manuel, H.J. and Gaymans, R.J., Segmented block copolymers based on dimerized fatty acids and poly(butylene terephthalate), *Polymer,* **34**, 636–641, 1993a.

Manuel, H.J. and Gaymans, R.J., Segmented block copolymers based on poly(butylene terephthalate) and telechelic polyesters and polyamides of dimerized fatty acids, *Polymer*, **34**, 4325–4329, 1993b.

Möhring, H. and Spiteller, G., produkte der dimerisierung ungesättigter fettsauren II: Die monomerfraktion der dimerisierung reiner linosäure 1. aromatenfraktion, *Fat Sci. Technol*., **92**, 126–131, 1990.

Rajadhyaksha, R.A. et al. Superacid catalyzed dimerization of fatty acids derived from safflower oil and dehydrated castor oil, *J. Am. Oil Chem. Soc*., **65**, 793–797, 1988.

Rao, T.C., Kale, V. et al. Chromatographic methods for the determination of monomer, dimer and trimer fractions in dimer fatty acids, *J. Chromatog. A*, **466**, 403–406, 1989.

Scheiber, J., Reactions in the formation of stand oils, *Farbe u Lack*., 585–587, 1929.

Sen Gupta, A.K., Untersuchungen über die Struktur Dimerer Fettsäuren I: Struktur dimerer Ölsäure-Methylester, *Fette Seifen Anstrichm*., **69**, 907–913, 1967.

Sen Gupta, A.K., Untersuchungen über die Struktur Dimerer Fettsäuren III: Zur Frage der Diels-Alder-Reaktion bei der thermischen Polymerisation von Linolsäure-Methylester, *Fette Seifen Anstrichm*., **70**, 153–155, 1968.

Sen Gupta, A.K. and Scharmann, H., Untersuchungen über die Struktur Dimerer Fettsäuren II: Herstellung und Strukturaufklärung von Diels-Alder-Dimeren aus 9*t*,11*t*-Octadecadiensäure-Methylester, *Fette Seifen Anstrichm*., **70**, 86–90, 1968a.

Sen Gupta, A.K. and Scharmann, H., Untersuchungen über die Struktur dimerer Fettsäuren IV: Struktur Dimerer 9*c*,11*c*-Octadecadiensäure-Methylester, *Fette Seifen Anstrichm*., **70**, 265–272, 1968b.

Sen Gupta, A.K., Untersuchungen über die Struktur Dimerer Fettsäuren V: Thermische und Peroxidkatalysierte Dimerisierung von Linolensäure-Methylester, *Fette Seifen Anstrichm*., **71**, 873–876, 1969.

Vedanayagam, H.S. and Kale, V., Non-reactive polyamides from C_{36} dimer acids: preparation and properties, *Polymer*, **33**, 3495–3499, 1992.

Vijayalakshmi, P. et al. Reactive polyamides from cycloaliphatic C_{21} dicarboxylic acid and their evaluation as epoxy curing agents, *Polymer*, **33**, 3252–3256, 1992.

Wheeler, C.C. and Godfrey, A.W., Lubricating oil composition, U.S. Patent 3,909,423, 1975.

Yoshida, Y. et al. Enzymatic synthesis of estolides by a bioreactor, *J. Am. Oil Chem. Soc*., **74**, 261–267, 1997.

Zhao, J. and Olesik, S.V., Separation of dimer acids using enhanced-fluidity liquid chromatography, *Analy. Chim. Acta*, **449**, 221–236, 2001.

Zoleski, B.H. and Gaetani, F.J., Low foaming railway diesel engine lubricating oil compositions, U.S. Patent 4,428,850, 1984.

8.11 Chain branching and extension

Branched fatty compounds are of commercial significance because the branching can cause some advantageous changes in physical properties while retaining the same or nearly the same number of carbon atoms. For example, they have a broader liquidity range, making them of interest for low-temperature applications and their low surface tension causes good spreadability. They are used in surfactants, lubricants, cosmetics, and as polymer additives, defoamers, and wood protecting agents (Behr and Laufenberg, 1991).

Various reactions yield branched fatty compounds. In some cases, especially if the fatty starting material possesses a terminal double bond, chain extension instead of branching may occur. Therefore, both kinds of products, branched and chain-extended fatty compounds, are discussed here. When branching is introduced, the reaction may occur with retention of a double bond, often with change of configuration, or with saturation of the double bond.

8.11.1 Products containing C and O in the new moieties

Isomerization. Acidic zeolites, especially species with large pores, catalyze the isomerization of unsaturated long-chain fatty acids to methyl- and ethyl-branched

species with the same number of carbons via carbocationic intermediates (Zhang et al., 2004).

Friedel-Crafts acylation. Friedel-Crafts acylation of alkenes can be induced by Lewis acids, such as $AlCl_3$, $SnCl_4$, and $ZnCl_2$. With acyl chlorides in dichloromethane, β,γ-unsaturated ketones are obtained and two regioisomers 9(10)-acetyl-10(8)-(*E*)-octadecenoic acid (see Figure 8.31, in which 10-acetyl-8-(*E*)-octadecenoic acid is shown) are formed from oleic acid (Biermann and Metzger, 1992). The conditions for acylation of methyl oleate were identical to those of oleic acid. Oleyl alcohol gave corresponding products with $EtAlCl_2$ as catalyst. With 10-undecenoic acid, chain extension gave 12-oxo-9-tridecenoic acid with an *E*/*Z* isomer ratio of 3.2:1. Acylation of 10-undecenoic acid with heptanoyl chloride and subsequent $NaBH_4$ reduction afforded ricinelaidic acid. Anhydrides can also be used in this reaction in place of the acyl halide (Biermann and Metzger, 1992). Thus, reaction of oleic acid with succinic anhydride yielded regioisomers 9(10)-(1-oxo-3-carboxy-propyl)-10(8)-(*E*)-octadecenoic acid (Figure 8.32, 10-(1-oxo-3-carboxy-propyl)-8-(*E*)-octadecenoic acid is shown). Acylation of 10-undecenol with glutaric anhydride leads to chain-extended 5-oxo-16-hydroxy-8(*E*)-palmitic acid (Metzger and Biermann, 1993).

Ene and ane reactions. The Diels-Alder reaction usually yields cyclic products. Related reactions that do not afford cyclic products are the ene and the ane reaction. The ene reaction is the "indirectly substituting addition" of an alkene (the "enophile") to an alkene with allylic hydrogen (the "ene") (Metzger and Leisinger, 1988). The reaction proceeds stereospecifically as *cis* addition like the Diels-Alder reaction. Unsaturated fatty compounds, for example, esters of oleic acid, can serve as ene component in the reaction. Similarly, alkanes can be added to olefinic compounds by means of the ane reaction.

Ene reaction. The addition of maleic anhydride to unsaturated fatty compounds is an example of the ene reaction. However, in a study of the addition of maleic anhydride to ethyl oleate and ethyl elaidate, an addition reaction (an ane reaction) with position retention of the double bond competed with the ene reaction (Holmberg and Johansson, 1982), in which the double bond shifts. Figure 8.33 depicts one of the regiosiomers formed in the ene reaction. When methyl oleate reacted with maleic anhydride at 190°C, the two regioisomers of the ene reaction were formed in 68% yield (Metzger and Biermann, 1994). The double bond not only shifts, but the configuration also inverts from *Z* to *E*. Diastereomers are formed, with *threo* being the preferred configuration. Reaction of methyl 10-undecenoate with maleic anhydride afforded the chain-extended product as geometric isomers with an *E*/*Z* ratio = 3:1 (Metzger and Biermann, 1994). Different transition metal catalysts were tested in the addition of maleic anhydride to oleic acid (Behr and Handwerk, 1992a). Their effectiveness was found to depend on several parameters, including the nature of the ligands attached to the catalyst, in tests conducted at a molar ratio of oleic acid/catalyst = 40:1. For example, among rhodium catalysts, rhodium bis(acetate) ($Rh(OAc)_2$) was most effective and, among platinum catalysts, it was H_2PtCl_6. Catalysts based on palladium, ruthenium, and indium were less effective. Methyl esters gave lower yields than the free acids. An ene reaction was also carried out between ethyl oleate and glyoxalic acid ethyl ester or mesoxalic acid diethyl ester with $SnCl_4$ as catalyst (Behr and Fiene, 2000). δ-Lactones were also formed in the reaction of ethyl oleate with mesoxalic acid diethyl ester. Stoichiometric amounts of catalyst were needed to achieve high yields, but shorter reaction times and lower temperatures are benefits.

In 50% xylene medium, the reaction of high-oleic sunflower oil with maleic anhydride afforded alkenyl succinic anhydrides (ASA; compounds in which the maleic anhydride moiety is attached to the fatty acid chain) obtained as a mixture of two positional isomers, one with the anhydride unit at C9 and a Δ7 double bond, one with the anhydride unit at C7 with a Δ9 double bond (Candy et al., 2005). The highest yields (>70%) were attained at temperatures of 240 to 250°C with a molar ratio of maleic anhydride to methyl esters 1.5 to 1.7. However, for industrial applications the best conditions were given as T = 220 to 235°C and a molar ratio of 1.2 to 1.35 due to a minimum of side products. For the reaction of methyl oleate in rapeseed oil methyl esters (RME) with maleic anhydride, the optimal reaction conditions were determined to be 210 to 220°C at a molar ratio maleic anhydride/RME = 1.5 at about 80% yield (Quesada, 2003). The product had a high content of undesirable side products, high viscosity and dark color. A molar ratio <1.5 gave a clearer, less viscous product with lower ASA content.

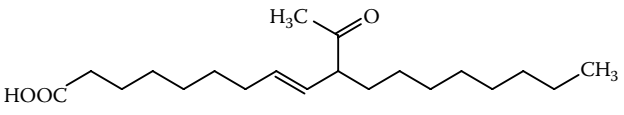


FIGURE 8.31

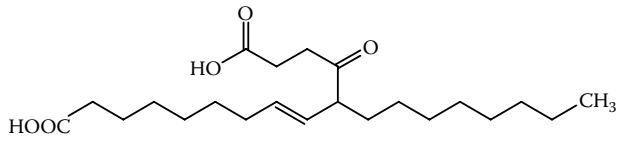


FIGURE 8.32

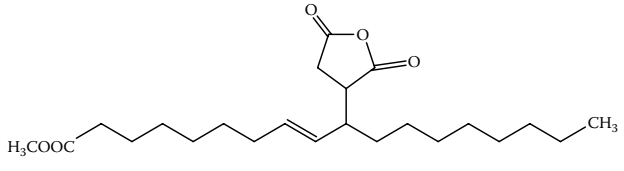


FIGURE 8.33

ASAs are of interest as curing agents in the production of thermosetting unsaturated polyester resins (Quesada, 2003). The products of the reaction of MA with oleic acid or linoleic acid were evaluated in corrosion and lubrication tests as cutting fluid additives (Watanabe et al., 1986). The triethanolamine salts of these compounds had effective rust-inhibiting and antiwear properties for water-based cutting fluids. Ring opening of the addition products with maleic anhydride can lead to tricarboxylic acids.

Formaldehyde (as paraformaldehyde) can also be used as an enophilic starting material in the ene reaction with Me_2AlCl and EtAl-sesquichloride ($Et_3Al_2Cl_3$) being suitable catalysts (Biermann and Metzger, 1991). Oleic acid and 10-undecenoic acid afforded (*E*)-9(10)-(hydroxymethyl)-10(8)-octadecenoic acid and the chain-extended 12-hydroxy-9-dodecenoic acid, respectively. With oleyl alcohol, (*E*)-9(10)-(hydroxymethyl)-10(8)-octadecen-1-ol was obtained. With fatty esters as enes, the addition of formaldehyde is catalyzed by $EtAlCl_2$, a stronger Lewis acid. A more commercially viable procedure requiring less catalyst, with, for example, $RuCl_3$, H_2PtCl_6, BF_3, or $SnCl_4$ as catalysts, was also investigated (Behr and Handwerk, 1992b). Besides the formation of regioisomers comprising the formal addition of HCHO or substitution with $-CH_2OH$, minor amounts of larger adducts were observed in the reaction of formaldehyde with oleic acid. Propylene carbonate was the most suitable solvent and the optimum temperature range was 70 to 90°C. Compounds with multiple double bonds were also suitable as starting materials.

Ane reaction. Various alkanes (cyclohexane, *n*-heptane) and alkylaromatics (toluene) were added to methyl 11-undecenoate (Metzger and Bangert, 1995). The reaction conditions were 2 minutes at 450°C and 250 bar pressure for the formation of 11-cyclohexylundecanoate. Regioisomers can be formed although the terminal addition is favored as in the synthesis of 11-cyclohexylundecanoate accompanied by only 5% of 10-cyclohexyllundecanoate. When adding n-heptane, the regioisomers methyl stearate and methyl 12-methylheptadecanoate were formed. With toluene, methyl 12-phenyldodecanoate was formed, although the yield of 30% was lower. The reaction proceeds through a free radical chain process.

Reaction with manganese(III)acetate. A variety of compounds can be added to fatty compounds, such as methyl oleate via manganese(III)acetate mediation (Metzger and Linker, 1991). The oxidation of enolizable compounds with manganese(III)acetate generates electrophilic radicals for addition to electron-rich alkenes. For example, acetone can be added to methyl oleate to give the regioisomers methyl 9(10)-acetonylstearate in about equal amounts (Metzger and Linker, 1991). Other materials that can be added to methyl oleate in a similar fashion include malonic esters to give addition of the malonic ester moiety via its C2 carbon and cyanoacetic

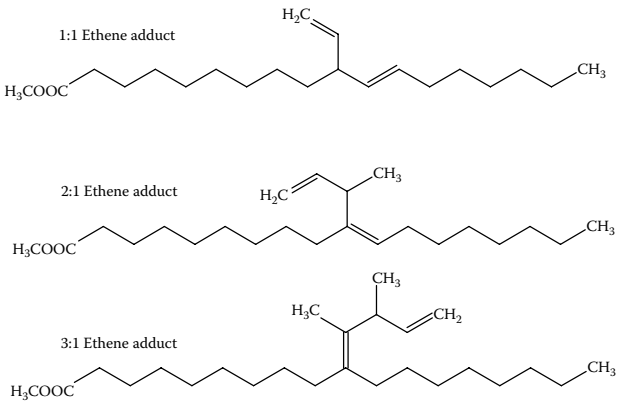


FIGURE 8.34

acid to give formal addition of acetonitrile (Metzger and Linker, 1991). Azide radicals, obtained from NaN_3, were also added. Adding stoichiometric amounts of cupric acetate to the mixture leads to stereospecific formation of two regioisomeric (*E*)-alkenes.

Other procedures. $RhCl_3 \cdot 3H_2O$ has been used as a catalyst for obtaining branched compounds (Behr and Laufenberg, 1990). For example, from conjugated methyl linoleate (methyl 9,11- and 10,12-octadecadienoate) adducts with ethene or propene can be formed. Multiple adducts can be obtained (see Figure 8.34 for adducts formed with ethene). The ratio of the various adducts depends on the solvent, temperature, pressure, and other parameters.

Conjugated methyl linoleate was also co-oligomerized with ethene using a rhodium-catalyzed ($RhCl_3 \cdot 3H_2O$) system in temperature-dependent solvent systems as a new catalyst recycling concept and in presence of organic chlorides (such as cinnamoyl chloride, which have labile C-Cl bonds, thereby increasing catalytic activity), leading to products similar to those depicted in Figure 8.34 (Behr and Fängewisch, 2003). The turnover rate was enhanced 100-fold from 2 to 220 h^{-1}. The products may be useable as biodegradable lubricants.

Acrylation of fatty epoxides leads to materials, such as acrylated methyl oleate (Bunker and Wool, 2002). These compounds can be polymerized to give materials of interest as pressure-sensitive adhesives.

8.11.2 Products with heteroatoms in the new moieties

Ritter reaction. This reaction consists of the addition of nitriles to alkenes in a strongly acidic medium to give substituted amides and was applied to oleic acid with acetonitrile, propionitrile, acrylonitrile, and other nitrile substrates, yielding subsituted amidostearic acids (Roe and Swern, 1953). In an $SnCl_4$-catalyzed Ritter reaction, nitriles were added to the double bonds of unsaturated fatty compounds, affording *N*-acylamino fatty esters (Biermann and Metzger, 1990, 1993; see Figure 8.35 for

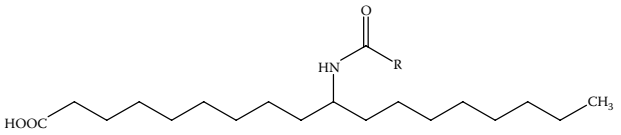


FIGURE 8.35

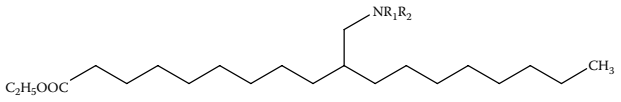


FIGURE 8.36

one regioisomer). Acrylonitrile was added nearly quantitatively to methyl oleate and ethyl undecenoate at 50°C in the presence of water and stoichiometric quantities of $SnCl_4$. Similarly, acetonitrile was added to methyl oleate to give methyl *N*-acetyl-9(10) stearate regioisomers. Lower temperatures in this reaction, while slowing the reaction, suppress the formation of regioisomers.

Other nitrogen compounds. With a catalyst such as $[Rh(cod)Cl]_2$ at 140°C, ethyl oleate was hydroaminomethylated (see Figure 8.36 for the C10 regioisomer) using synthesis gas (CO and H_2) and various amines, such as hexylamine, benzylamine, morpholine, and others (Behr et al., 2000a). Solvents were toluene or 1,4-dioxan. The sterically hindered diisopropylamine gave poor yields. Oleyl alcohol gave lower yields than ethyl oleate when reacting with morpholine. With a two-fold excess of the ester component, two molecules of ethyl oleate could be linked when using a primary amine.

Halogenated compounds. Reacting perfluorohexyl iodide and methyl 10-undecenoate in the presence of azo-*iso*-butyronitrile (AIBN) extended the fatty acid chain by the perfluorinated moiety with addition of iodine (Metzger and Linker, 1991; see Figure 8.37). For addition to nonterminal double bonds, such as in methyl oleate, the $SnCl_2$/AgOAc system is suitable (Metzger and Linker, 1991), yielding regioisomeric methyl 9(10)-perfluorohexylstearates.

The copper-initiated addition of halonitriles leads again to chain-extended or branched compounds depending on the starting fatty material (Metzger and Mahler, 1995). Thus, the addition of 2-bromohexanenitrile to methyl 10-undecenoate yielded methyl 10-iodo-12-cyanopalmitate and the addition of iodoacetontrile to methyl erucate afforded methyl 13-iodo-14-(CH_2CN)-erucate.

Lead powder and catalytic amounts of copper(II)acetate in methanol can be used to add perfluoroalkyl iodides to double bonds as in petroselinic acid (Metzger et al., 1996). The products are regioisomers (1-iodo-2-perfluoroalkyl and 1-perfluoroalkyl-2-iodo moieties in the 6,7

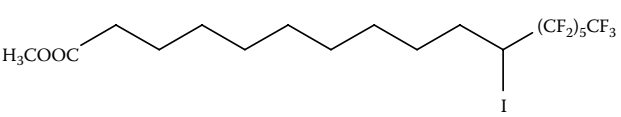


FIGURE 8.37

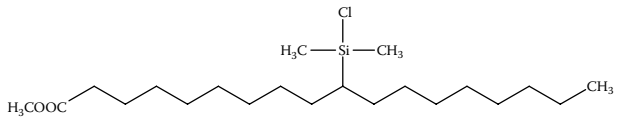


FIGURE 8.38

positions) of the starting perfluoroalkyl iodides added at the double bond. The reaction of perfluoroalkyldiiodides with the terminal double bond in methyl 10-undecenoate leads to the chain extended mono- and diaddition products. These products can be catalytically hydrogenated to substitute the residual iodine with H.

Hydrosilylation. A variety of silicon hydrides, such as trichlorosilane, methyldichlorosilane, dimethyldichlorosilane, phenyl dichlorosilane and methyl phenyl chlorosilane, were catalytically added to esters of oleic, linoleic, and 10-undecenoic acid (Saghian and Gertner, 1974). In these products, the branching occurs as a C-Si bond (see Figure 8.38 for one regioisomer from the addition of dimethylchlorosilane to methyl oleate). Chloroplatinic acid (H_2PtCl_6) and Pt on C are effective catalysts. With methyl 10–undecenoate as starting material, the silyl moiety added exclusively to the terminal carbon (Saghian and Gertner, 1974). Using a biphasic liquid-liquid reaction system, H_2PtCl_6 could be used under mild conditions and the catalyst could be recycled (Behr et al., 2002). For the more reactive methyl 10-undecenoate, alkoxy, or alkylhydrosilanes could be used, while for methyl linoleate and methyl α-linolenate, more reactive chlorohydrosilanes were necessary. With methyl α-linolenate, a product mixture was obtained including a 1:1 adduct, hydrogenated 1:1 adduct, 2:1 adduct, and oligomeric materials. Ethyl oleate did not give desired products, or significant amounts thereof, under the conditions used (Behr et al., 2002a). In another study, it was found that a broad range of Pt(IV), Pt(II), and Pt(0) compounds can be used in the facile (10 minutes, up to 88% yield) hydrosilylation of methyl 10-undecenoate (Behr et al., 2002b). Hydrosilylation of methyl linoleate occurred only with Pt(IV) and Pt(II) with labile ligands or with Karsted solution (Pt(0)-divinyltetramethyldisiloxane complex).

References

Behr, A. and Laufenberg, A., Synthesis of new branched fatty acids by rhodium catalyzed homogeneous oligomerization (Synthese neuer verzweigter Fettsäureester durch homogene Rhodiumkatalyse), *Fat Sci. Technol.,* **93**, 20–24, 1991.

Behr, A. and Handwerk, H.-P., Transition metal catalyzed addition of maleic anhydride to fatty derivatives (Übergangsmetallkatalysierte Addition von Maleinsäureanhydrid an Fettstoffe), *Fat Sci. Technol.,* **94**, 204–208, 1992a.

Behr, A. and Handwerk, H.-P., Catalytic addition of formaldehyde to unsaturated oleochemicals (Katalytische Verknüpfung ungesättigter Fettstoffe mit Formaldehyd), *Fat Sci. Technol.,* **94**, 443–447 1992b.

Behr, A. and Fängewisch, C., Rhodium-catalyzed synthesis of branched fatty compounds in temperature-dependent solvent systems, *J. Mol. Catal. A.,* **197**, 115–126, 2003.

Behr, A. and Fiene, M., Lewis-acid catalyzed ene reaction of electron-deficient aldehydes and ketones at unsaturated fatty acid derivatives, *Eur. J. Lipid Sci. Technol.,* **102**, 212–217, 2000.

Behr, A. et al. Hydroaminomethylation of fatty acids with primary and secondary amines — a new route to interesting surfactant substrates, *Eur. J. Lipid Sci. Technol.,* **102**, 467–471, 2000.

Behr, A. et al. The synthesis of silicon oleochemicals by hydrosilylation of unsaturated fatty acid derivatives, *Eur. J. Lipid Sci. Technol.,* **104**, 161–166, 2002a.

Behr, A. et al. Platinum-catalysed hydrosilylation of unsaturated fatty acid esters, *Adv. Synth. Catal.,* **344**, 1142–1145, 2002b.

Biermann, U. and Metzger, J.O., Lewis acid catalyzed additions to unsaturated fatty acids I: $SnCl_4$-Catalyzed additions of nitriles to methyl oleate and ethyl 10-undecenoate (Lewis-Säure katalysierte Additionen an ungesättigte Fettstoffe I: $SnCl_4$-katalysierte Addition von Nitrilen an Ölsäuremethylester und 10-Undecensäureethylester), *Fat Sci. Technol.,* **92**, 133–134, 1990.

Biermann, U. and Metzger, J.O, Lewis acid catalyzed additions to unsaturated fatty acids II: Alkylaluminium halide catalyzed ene reactions of unsaturated fatty compounds and formaldehyde, *Fat Sci. Technol.,* **93**, 282–284,1991.

Biermann, U. and Metzger, J.O., Lewis acid induced additions to unsaturated fatty compounds III. Alkylaluminium halide induced Friedel-Crafts acylations of unsaturated fatty compounds, *Fat Sci. Technol.,* **94**, 329–332, 1992.

Biermann, U. and Metzger, J.O., Lewis acid induced additions to unsaturated fatty compounds, *Fat Sci. Technol.,* **95**, 326–328, 1993.

Bunker, S.P. and Wool, R.P., Synthesis and characterization of monomers and polymers for adhesives from methyl oleate, *J. Polym. Sci., Part A: Polym. Chem*., **40**, 451–458, 2002.

Candy, L. et al. Synthesis of alkenyl succinic anhydrides from methyl esters of high oleic sunflower oil, *Eur. J. Lipid Sci. Technol.,* **107**, 3–11, 2005.

Holmberg, K. and Johansson, J.-A., Addition of maleic anhydride to esters of mono-unsaturated fatty acids, *Acta Chem. Scand.,* **B36**, 481–485, 1982.

Metzger, J.O. and Bangert, F., Ane additions to unsaturated fatty compounds: thermally initiated additions of alkanes to methyl 10-undecenoate, *Fat Sci. Technol.,* **97**, 7–9, 1995.

Metzger, J.O. and Biermann, U., Products of the thermal ene reaction of unsaturated fatty compounds and maleic anhydride, *Fat Sci. Technol.,* **96**, 321–323, 1993.

Metzger, J.O. and Leisinger, K.F., The ene reaction and its application to unsaturated oleochemicals (Die En-Reaktion und ihre Anwendung auf ungesättigte Fettstoffe), *Fat Sci. Technol.,* **90**, 1–5, 1988.

Metzger, J.O. and Linker, U., New results on free radical additions to unsaturated compounds, *Fat Sci. Technol.,* **93**, 244–249, 1991.

Metzger, J.O. and Mahler, R., Radical additions of activated haloalkanes to alkenes initiated by electron transfer from copper in solvent-free systems, *Angew. Chem. Int. Ed. Engl.,* **34**, 902–904, 1995.

Metzger, J.O. et al. Electron transfer initiated free radical additions of perfluoroalkyl iodides and diiodides to alkenes, *Liebig's Ann.*, 693–696, 1996.

Quesada, J., Production of alkenyl succinic anhydrides from low-erucic and low-linolenic rapeseed oil methyl esters, *Eur. J. Lipid Sci. Technol.,* **105**, 281–287, 2003.

Roe, E.T. and Swern, D., Fatty acid amides. VI. Preparation of substituted amidostearic acids by addition of nitriles to oleic acid, *J. Am. Chem. Soc.,* **75**, 5479–5481, 1953.

Saghian, N. and Gertner, D., Hydrosilylation of long chain unsaturated fatty acid esters, *J. Am. Oil Chem. Soc.,* **51**, 363–367, 1974.

Watanabe, S. et al. Characteristic properties as cutting fluid additives of the products from the reaction of unsaturated fatty acids with maleic anhydride, *Mater. Chem. Phys.,* **15**, 89–96, 1986.

Zhang, Z.C. et al. New process for the production of branched-chain fatty acids, *J. Surf. Deterg.,* **7**, 211–215, 2004.

8.12 Hydrolysis, alcoholysis, esterification, and interesterification

Some procedures covered in this section are usually well covered in organic chemistry textbooks or are standard procedures in fatty acid chemistry. Experimental details of some related analytical methods, such as formation of methyl esters, are available from standard procedures provided by organizations such as the American Oil Chemists' Society (AOCS) and others. Generally, the reactions discussed here can be carried out both chemically and enzymatically, and are reversible under appropriate conditions. A review on chemical catalysts for most types of reactions discussed here was given by Hoydonckx et al. (2004). A review on enzymatic reactions was provided by Gunstone (1999) and a book on enzymatic modification of lipids was edited by Bornscheuer (2000). The use of enzymatic reactors for the lipid modification was reviewed (Xu, 2003). The industrial application of lipases for producing fatty esters used in the personal care industry was reviewed (Hills, 2003).

8.12.1 Hydrolysis

The hydrolysis of esters or lipids is easily conducted by refluxing with aqueous ethanolic alkali. Acidifying the hydrolysate liberates fatty acids, which can be extracted with hexane or other organic solvents. Nonacidic compounds (unsaponifiables), such as hydrocarbons, long-chain alcohols, sterols and glycerol ethers, are present in the organic extract. Glycerol remains in the aqueous phase. Quantitative hydrolysis serves for determining the saponification equivalent or saponification value (see Section 6.3.2).

The production of soap by alkaline hydrolysis (saponification) is usually carried out around 100°C. Glycerol is recovered as a second commercial product (Section 9.2.5). Sodium and potassium salts are used as soaps (Section

9.2.1). Salts of other metals are used in the polymerization of drying oils, the manufacture of greases and lubricants, and as ingredients in plastics.

Fats can also be hydrolyzed to free acids in what is probably a homogeneous reaction between fat and water dissolved in the oil phase (fat splitting). This process occurs in the presence of sulfonated long-chain alkylbenzenes (24 to 48 h, ~100°C) or of oxides of zinc, magnesium or calcium (2 to 3 h, 250°C, 50 bar). These compounds act partially as emulsifying agents to bring fat and water together and partially as catalysts. Continuous high pressure uncatalyzed countercurrent splitting is conducted at 20 to 60 bar and 250°C. Lower temperatures are recommended for highly unsaturated oils (Sonntag, 1979, 1988).

Partial hydrolysis of lipids occurs during digestion under the influence of lipases. Lipases present in seeds also promote hydrolysis, so that most extracted lipids contain some free acid and some partial glycerides. This is undesirable because removal of free acid during processing is accompanied by some loss of fat. Lipolysis can be minimized by inactivation of the lipase before extraction. Enzymatic deacylation is also the basis of some valuable analytical techniques. Lipolysis on a commercial scale to produce fatty acids and glycerol, which are more easily purified, has been reported (Bilyk et al., 1991).

8.12.2 Esterification

Esters can be prepared by interaction of a carboxylic acid or an acyl derivative with an alcohol or its equivalent (esterification) or by reacting an ester with an alcohol (alcoholysis), an acid (acidolysis) or another ester (ester–ester interchange, interesterification, transesterification). These last three processes requiring at least one ester are collectively referred to as transesterification reactions. In the literature these terms are sometimes used less strictly and interchangeably. Each process usually requires acid, base, or enzymatic catalysis. A brief overview of the preparation of esters is given by Bondioli (2004).

Fatty acids are often studied in the form of their esters (e.g., for chromatographic purposes), usually as methyl esters. Methyl esters are made from acids by reaction with a large excess of methanol containing H_2SO_4 (1 to 2%), HCl (5%), or BF_3 (12 to 14%) as catalyst, or by reaction with diazomethane. Acid-catalyzed procedures are not suitable for acids with cyclopropane, cyclopropene, epoxide, or allylic hydroxy groups, and for conjugated polyene acids. Such acids, however, can be safely esterified with diazomethane or the ester can be obtained directly from the lipid by base-catalyzed transesterification. Care must also be taken with unsaturated acids. Under more vigorous conditions, both BF_3 and H_2SO_4 promote the addition of methanol to a double bond to give a methoxy-substituted acid. An alternative esterification procedure is the interaction of alkyl halide with the anion of the acid as a salt (Ag, Na, K, Ca, $N(CH_3)_4$).

In all of these procedures, it is generally convenient to use an excess of alkylating agent. This is not always so, and in the synthesis of glycerol esters, for example, it is more usual to use equivalent or near equivalent quantities of hydroxy compound and acylating agent, which may be free acid (with appropriate catalyst), acid anhydride, or acid chloride (Section 8.13).

Enzymes can also be used to catalyse the reaction and they have been employed to provide glycerol esters and wax esters (Trani et al., 1991) with more references available from Gunstone (1999) and Bornscheuer (2000). The selectivity of enzymes has been exploited to concentrate acids, such as γ-linolenic, which is esterified less readily than the more common saturated and unsaturated acids (Hills et al., 1990). Some recent examples are the esterification of glycerol and oleic acid with lipase-catalysis with the reaction depending on factors, such as water content and organic solvent (Yesiloglu and Kilic, 2004). The reaction medium, i.e., organic solvents and supercritical fluids, (Cernia et al., 1998) as well as temperature (Pinsidorom et al., 2004) are critical factors in the esterification, with the temperature depending on the melting point of the fatty acid used (Pinsirodom et al., 2004). The fatty alcohol used can affect the reaction rate (Shintre et al., 2002). *Acinetobacter* and coryneform species were used in the synthesis of wax esters, such as oleyl oleate and those derived from soybean oil, which resemble those found in jojoba (Kaneshiro et al., 1996).

8.12.3 Alcoholysis

Alcoholysis is widely employed to convert lipids directly to methyl or other alkyl esters without first isolating the free acids and to prepare partial glycerides through interaction of a triacylglycerol and free glycerol.

Formation of alkyl esters. This reaction has attained additional significance in recent years because of the increased production of biodiesel (Section 9.6), which is defined as the alkyl esters of vegetable oils or animal fats. Fatty acid methyl esters also serve as starting materials for the production of fatty alcohols. The alcoholysis reaction when producing biodiesel is usually referred to as transesterification in the literature. (For a general scheme of alcoholysis/transesterification, see Figure 8.39.)

With the increasing significance of biodiesel, the number of research papers on its production has increased almost exponentially. Numerous reviews discussing the various processes are available (Demirbas, 2003; Fukuda

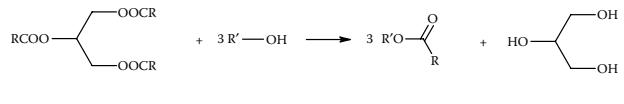


FIGURE 8.39

et al., 2001; Gutsche, 1997; Haas et al., 2002; Haas and Foglia, 2005; Ma and Hanna, 1999; Meher et al., 2006; Mittelbach and Remschmidt, 2004; Schuchardt et al., 1998; Van Gerpen and Knothe, 2005).

Most research on biodiesel production is concerned with the production of methyl esters. These are currently the most common form of biodiesel due to methanol being the least expensive alcohol in most countries. When producing methyl esters, two phases exist at the beginning (alcohol, vegetable oil) and end (methyl esters, glycerol) of the reaction. Reactions with higher alcohols are more prone to formation of emulsions.

The production of vegetable oil alkyl (methyl) esters (biodiesel) can be carried out both chemically and enzymatically, although for cost reasons the latter approach currently is not applied commercially. However, advantages of the enzymatic approach include mild reaction conditions, reduced waste, tolerance for water in the feedstock, and the ability to more easily produce esters using a variety of alcohols.

Methanolysis with an alkaline catalyst is faster than reaction with an acidic catalyst (Freedman and Pryde, 1982; Freedman et al., 1984; Canakci and Van Gerpen, 1999). For maximum yield, the reaction system should be free of moisture and the free fatty acid content of the vegetable should be less than 0.5% (Freedman et al., 1984). At 32°C, transesterification was 99% complete in 4 h when using an alkaline catalyst (NaOH or NaOMe). At 60°C and an alcohol:oil molar ratio of at least 6:1 using fully refined oils, the reaction was complete in 1 h to give methyl, ethyl, or butyl esters. The reaction parameters investigated were molar ratio of alcohol to vegetable oil, type of catalyst (alkaline vs. acidic), temperature, reaction time, degree of refinement of the vegetable oil, and effect of the presence of moisture and free fatty acid. Although crude oils could be transesterified, ester yields were reduced because of the presence of gums and extraneous materials. Recently, the direct use of alkoxides has become more common because water cannot be formed according to the reaction ROH + XOH $\rightarrow$ ROX + H_2O (X = Na or K), which occurs when using hydroxides.

The transesterification of soybean oil with methanol or 1-butanol was reported to proceed with pseudo-first order or second order kinetics, depending on the molar ratio of alcohol to soybean oil (30:1 pseudo-first order, 6:1 second order; NaOBu catalyst) while the reverse reaction was second order (Freedman et al., 1986). The methanolysis of sunflower oil at a molar ratio of methanol:sunflower oil = 3:1 was reported to begin with second-order kinetics, but then the rate decreased due to formation of glycerol (Mittelbach and Trathnigg, 1990). The originally proposed kinetics (Freedman et al., 1986) were reinvestigated (Mittelbach and Trathnigg, 1990; Boocock et al., 1996; Noureddini and Zhu, 1997; Boocock et al., 1998). It was shown that an originally proposed shunt reaction as part of the forward reaction is unlikely, that second-order kinetics are not followed and that miscibility phenomena play a significant role.

Several methods for accelerating the transesterification reaction are discussed in the articles cited here. They include the addition of co-solvents, such as tetrahydrofuran (THF) or methyl *tert*.-butyl ether (MTBE) accelerating the methanolysis of vegetable oils as a result of solubilizing methanol in the oil and to a rate comparable to that of the faster butanolysis as well as microwave and ultrasonic irradiation. Factorial experiment design and surface response methodology have been applied to different production systems. Supercritical technology has also been employed for transesterification.

Various catalysts, including alkaline-earth metal compounds, such as CaO and $Ba(OH)_2$, $CaCO_3$, alkylguanidines attached to modified polystyrene or siliceous MCM-41 encapsulated in the supercages of zeolite Y or entrapped in SiO_2 sol-gel matrices, were used as catalysts. Alternative processes include *in situ* reactions in which the reaction is carried out directly using oil-containing seeds. More details, including information on other catalysts and procedures, are available from the references cited above.

There has also been some emphasis on utilizing low-cost sources for biodiesel production, leading to the question of the quality of these sources. Low-cost sources, such as restaurant greases and soap stock are of lower quality than refined vegetable oils. A major problem associated with these sources is the high content of free fatty acids, which, as indicated above, should be at a minimum level. Thus, processing high free fatty acid feed stocks requires some changes to the overall production process. Pretreatment of the free fatty acids by acid-catalyzed esterification prior to converting the triacylglycerols by alkali-catalyzed transesterification is an effective method for producing biodiesel from high-free fatty acid feed stocks. For the production of biodiesel from soap stock containing some glycereol esters, all ester bonds were first hydrolyzed by alkali catalysis and the resulting fatty acid sodium salts converted to methyl esters by acid catalysis.

Formation of partial glycerides. When a triacylglycerol and glycerol are heated together in the presence of NaOH or $NaOCH_3$ as catalyst the following equilibrium is established:

$$\text{triacylglycerol} + \text{glycerol} \rightleftharpoons \text{monoacylglycerol} + \text{diacylglycerol}$$

This is an important method of preparing mono- and diacylglycerols. The composition of the equilibrium mixture of all four components depends on the relative amounts of triacylglycerol and glycerol dissolved in the lipid phase.

Monoacylglycerols, which can serve as emulsifiers in foods, etc., can be prepared on a commercial scale using enzymes as catalysts. The importance of the reaction tem-

perature, which relates to the melting behavior of the fat being used, has been emphasized (McNeill et al., 1991; McNeill and Yamane, 1991). Lipase-catalyzed alcoholysis of various triacylglycerols to give 2-monoacylglycerols was affected by the alcohol used and the presence of organic co-solvent (Lee et al., 2004).

8.12.4 Acidolysis

This less common process involves the interaction of ester and carboxylic acid in the presence of H_2SO_4, a metal oxide (Zn, Ca, Mg, Al) or mercuric sulfate at about 150°C. Applied to natural glycerides and lauric acid, for example, C_{16} and C_{18} acids are replaced by the C_{12} acid:

$$R^1COOR + R^2COOH \rightleftharpoons R^2coor + R^1COOH$$

Enzymes can be used in this process also. For example, structured lipids can be formed by lipase-catalyzed acidolysis using supercritical CO_2 (Kim et al., 2004).

8.12.5 Interesterification (transesterification) and structured triacylglycerols

Interesterification procedures are used industrially to improve the physical properties of lard, to produce cocoa butter substitutes from cheaper oils (sometimes combined with hydrogenation and fractionation), to produce fats containing acetic acid, and to produce margarine of appropriate melting behavior with a minimum content of *trans* acids and maximum content of polyene acids. This has been achieved, for example, by interesterification of soybean oil (80%) and fully hydrogenated soybean oil (20%).

Natural fats are mixtures of triacylglycerols in which the acyl groups are usually distributed in a nonrandom manner. Under the influence of an appropriate catalyst, the acyl groups are redistributed, first intramolecularly and then intermolecularly, until a wholly random distribution is finally achieved (Hustedt, 1976; Sreenivasan, 1978; Macrae, 1983). Common catalysts are NaOH, $NaOCH_3$, or a Na-K alloy at 0.2 to 0.4% level. Reaction parameters are about 80°C for 30 to 60 min, and, as with other reactions, the oil or fat should be as free as possible of water, free acid, and hydroperoxides, all of which inactivate catalyst. While mechanisms have been proposed for interesterification, recent work questions these and suggests a mechanism based on enolate anions formed by abstraction of a-hydrogen from a fatty acid moiety (Dijkstra et al., 2005). The chemical interesterification reaction using $NaOCH_3$ was monitored by UV-Vis spectroscopy (Liu and Lampert, 1999).

The redistribution of acyl groups as a result of interesterification leads to a change in the physical properties of the triacylglycerol mixture. Thus, the melting point of soybean oil is raised from −7 to +6°C, that of cottonseed oil from 10 to 34°C, but that of hardened palm kernel oil is reduced from 46 to 35°C.

This procedure can also be applied to mixtures of oils and fats. All the acids in the two (or more) oils will then be distributed at random, and this provides a method of transferring saturated fatty acids to predominantly unsaturated glycerol esters and vice versa. Methyl glycosides are conveniently acylated by reaction of the fully acetylated glycoside with appropriate methyl esters (Akoh and Swanson, 1989).

These changes can be further modified if the interesterification process is carried out at a lower temperature (0 to 40°C), so that fully saturated glycerides (S_3) crystallize from the reaction mixture. This is called directed interesterification. Crystallization disturbs the equilibrium in the liquid phase, so that more S_3 forms and again separates, producing, finally, more S_3 and U_3 and less of the mixed glycerides:

$$US_2 + U_2S \rightleftharpoons U_3 + S_3$$

This usually causes an increase in the melting range of the fat. Such reactions are slower and may take up to 24 h or more.

Interesterification yielding triacylglycerols of defined structure can be simulated by a chemical two-step procedure. Thus, the synthesis of symmetrical and nonsymmetrical triacylglycerols of type SSU and SUS consist of reacting a saturated triacylglycerol (tristearin) with glycerol in presence of $NaOCH_3$ with crystallization of the resulting mono- and diacylglycerols and reesterification of these species with the appropriate unsaturated fatty acid (Adlof and List, 2003).

Enzymatic interesterification is finding increasing attention, although the cost of these catalysts is an impediment (Macrae, 1983; Xu, 2000). The advantages are similar to those mentioned above for alcoholysis/biodiesel production, a major advantage in this case being the better control of product composition. The lipase, coated onto a support material (kieselguhr, hydroxyapatite, alumina) in the presence of a little water, supports interesterification at about 40°C and usually requires 16 to 70 h for complete reaction. The time can be shorter depending on catalyst activity. Batch or continuous processes are possible. The substrate is a natural oil or fat, to which a second oil or fat and/or a particular fatty acid or fatty acid mixture may be added. Several types of reactions can be carried out enzymatically. One type of enzyme (from *Candida cylindricae*, *Corynebacterium acnes,* or *Staphylococcus aureus*) is nonspecific and leads to complete randomization of all acids at all positions. The product is the same as that obtained from the ordinary catalytic process. Lipase-based randomization using supercritical CO_2 has also been reported (Jackson et al., 1997). A second type of lipase (from *Aspergillus niger*, *Mucor miehei*, *Rhizopus arrhizus*, *R. delemar,* and *R. niveus*) is specific for the 1 and 3 positions. Random-

ization is confined to acids in these positions and any added free acid. Fatty acids in the 2 position of the glycerol backbone remain unchanged. A third type of lipase (*Geotrichum candidum*) affects only Δ9-unsaturated acids (oleic, linoleic, linolenic). These acids will be randomized independently of their positions. Saturated acids are not affected. An interesting possibility is the formation of chiral triacylglycerols of type XYZ due to the high stereospecificity of some lipases (Xu, 2000).

An example of the value of this technique is the upgrading of palm midfraction in terms of its potential as a cocoa butter substitute. Before interesterification, this contains 58% POP, 13% POS, and 2% SOS. After interesterification with stearic acid in the presence of the 1,3-specific *A. niger* lipase, these values are 19, 32, and 13%. Several other attempts to make cocoa butter-like fats were described (Chang et al., 1990; Sridhar et al., 1991; Chong et al., 1992). A report on the synthesis of structured TAGs with medium chain fatty acids in the *sn*-1 and *sn*-3 positions and long-chain unsaturated fatty acids in the *sn*-2 positions is Soumanou (1998).

Lipase-based syntheses consisting of several steps are also possible. For example, symmetrical TAGs were synthesized by regiospecific ethanolysis of triolein with immobilized *Candida antarctica* lipase (Novozym 435) to give the 2-MAG, which was then reesterified with *Rhizomucor miehei* lipase (Lipozyme IM) (Irimescu et al., 2002). Medium-chain triacylglycerols were produced from copra oil through a two-step process using papain lipase in an alcoholysis step and interesterification with the alkyl esters (Caro et al., 2004).

A product rich in 2-oleodistearin (79%) was made from high-oleic sunflower oil and stearic acid by interesterification followed by fractionation of the neutral product (Rozendaal and Macrae, 1997). The isomerization of mono- and diacylglycerols depends on a similar acyl migration. The 1- and 2-monoacylglycerols isomerize to a 90:10 mixture of the two isomers under acidic, basic, or thermal conditions. Such a change may occur during chromatography, though this is reportedly less serious if the adsorbent is impregnated with boric acid. Acyl migration was studied in enzymatic interesterification reaction by means of response surface design (Xu et al., 1998).

Diacylglycerols, which are intermediates in lipase-catalyzed interesterification (Xu et al., 1999), behave similarly. The equilibrium mixture of 1,2 (40%) and 1,3 (60%) isomers varies somewhat with temperature. 1,2-Diacylglycerols are not thermodymically stable (Xu et al., 1999). Solid-state saturated diacylglycerols isomerize to virtually 100% of the 1,3 isomer.

Oxidative stability can be affected by interesterification. For example, in a comparison of traditional sunflower oil and a randomized lipid and specific structured lipid produced from sunflower oil with tricaprylin/caprylic acid, the structured lipid was the least oxidatively stable (Timm-Heinrich et al., 2003). However, adding antioxidants to the reaction mixture had no positive effect on the reaction itself or the oxidative status of the product (Xu et al., 2005).

References

Adlof, R.O. and List, G.R., Synthesis and analysis of symmetrical and nonsymmetrical disaturated/monounsaturated triacylglycerols, *J. Agric. Food Chem.,* **51**, 2096–2099, 2003.

Akoh, C.C. and Swanson, B.G., Synthesis and properties of alkyl glycoside and stachyose fatty acid polyesters, *J. Am. Oil Chem. Soc.*, **66**, 1295–1301, 1989.

Bilyk, A. et al. Lipase-catalyzed triglyceride hydrolysis in organic solvent, *J. Am. Oil Chem. Soc.,* **68**, 320–323, 1991.

Bondioli, P., The preparation of fatty acid esters by means of catalytic reactions, *Top. Cataly.,* **27**, 77–82, 2004.

Boocock, D.G.B. et al. Fast one-phase oil-rich processes for the preparation of vegetable oil methyl esters, *Biomass Bioenergy*, **11**, 43–50, 1996.

Boocock, D.G.B. et al. Fast formation of high-purity methyl esters from vegetable oils, *J. Am. Oil Chem. Soc.,* **75**, 1167–1172, 1998.

Bornscheuer, U.T. Ed., *Enzymes in Lipid Modification*, Wiley-VCH, Weinheim, Germany, 2000.

Canakci, M. and Van Gerpen, J., Biodiesel production via acid catalysis, *Trans. ASAE*, **42**: 1203–1210, 1999.

Caro, Y. et al. Enzymatic synthesis of medium-chain triacylglycerols and interesterification of copra oil using a crude papain lipase preparation, *Eur. J. Lipid Sci. Technol*., **106**, 503512, 2004.

Cernia, E. et al. The role of the reaction medium in lipase-catalyzed esterifications and transesterifications, *Chem. Phys. Lipids,* **93**, 157–168 (1998).

Chang, M.-K. et al. Production of cocoa butter-like fat from interesterification of vegetable oils, *J. Am. Oil Chem. Soc*., **67**, 832–834, 1990.

Chong, C.N. et al. Fractionation procedures for obtaining cocoa butter-like fat from enzymatically interesterified palm olein, *J. Am. Oil Chem. Soc*., **69**, 137–140, 1992.

Demirbas, A., Biodiesel fuels from vegetable oils via catalytic and non-catalytic supercritical alcohol transesterifications and other methods: a survey, *Energy Conv. Mgmt.,* **44**, 2093–2109, 2003.

Dijkstra, A.J. et al. The base-catalyzed, low-temperature interesterification mechanism revisited, *Eur. J. Lipid Sci. Technol.,* **107**, 912–921, 2005.

Fauconnot, L. et al. Chemical synthesis and NMR characterization of structured polyunsaturated triacylglycerols, *Chem. Phys. Lipids,* **139**, 125–136, 2006.

Freedman, B. and Pryde, E.H., Fatty esters from vegetable oils for use as a diesel fuel, *ASAE Publ. 4-82* (*Veg. Oil Fuels*), pp. 117–122, 1982.

Freedman, B. et al. Variables affecting the yields of fatty esters from transesterified vegetable oils, *J. Am. Oil Chem. Soc.,* **61**, 1638–1643, 1984.

Freedman, B. et al. Transesterification kinetics of soybean oil, *J. Am. Oil Chem. Soc.,* **63**, 1375–1380, 1986.

Fukuda, H. et al. Biodiesel fuel production by transesterification of oils, *J. Biosci. Bioeng.,* **92**, 405–416, 2001.

Gunstone, F.D., Enzymes as biocatalysts in the modification of natural lipids, *J. Sci. Food Agric*., **79**, 1535–1549, 1999.

Gutsche, B., Technologie der Methylesterherstellung — Anwendung für die Biodieselproduktion (Technology of methyl ester production and its application to biofuels), *Fett/Lipid,* **99**, 418–427, 1997.

Haas, M.J. et al. Enzymatic approaches to the production of biodiesel fuels, in *Lipid Biotechnology*, Kuo, T.M. and Gardner, H.W. Eds., Marcel Dekker, New York, Basel, 2002, pp. 587–598.

Haas, M.J. and Foglia, T.A., Alternate feedstocks and technologies for biodiesel production, in *The Biodiesel Handbook*, Knothe, G., Krahl, J., Van Gerpen, J. Eds., AOCS Press, Champaign, IL, 2005, pp. 42–61.

Hills, M.J. et al. Enzymatic fractionation of fatty acids: enrichment of γ-linolenic acid and docosahexenoic acid by selective esterification catalyzed by lipases, *J. Am. Oil Chem. Soc.*, **67**, 561–564, 1990.

Hills, G., Industrial use of lipases to produce fatty acid esters, *Eur. J. Lipid Sci. Technol.,* **105**, 601–607, 2003.

Hoydonckx, H.E. et al. Esterification and transesterification of renewable chemicals, *Top. Cataly.* **27**, 83–96, 2004.

Hustedt, H.H., Interesterification of edible oil, *J. Am. Oil Chem. Soc.*, **53**, 390–392, 1976.

Irimescu, R. et al. Study of TAG ethanolysis to 2-MAG by immobilized *Candida antarctica* lipase and synthesis of symmetrically structured TAG, *J. Am. Oil Chem. Soc.,* **79**, 879–883, 2002.

Jackson, M.A. et al. Lipase-catalyzed randomization of fats and oils in flowing supercritical carbon dioxide, *J. Am. Oil Chem. Soc.,* **74**, 635–639, 1997.

Kaneshiro, T. et al. Oleyl oleate and homologous wax esters synthesized coordinately from oleic acid by *Acinetobacter* and coryneform strains, *Curr. Microbiol.,* **32**, 336–342, 1996.

Kim, I.-H. et al. Production of structured lipids by lipase-catalyzed acidolysis in supercritical carbon dioxide: effect on acyl migration, *J. Am. Oil Chem. Soc.,* **81**, 537–541, 2004.

Lee, G.-C. et al. Lipase-catalyzed alcoholysis of triglycerides for short-chain monoglyceride production, *J. Am. Oil Chem. Soc.,* **81**, 533–536, 2004.

Liu, L. and Lampert, D., Monitoring chemical interesterification, *J. Am. Oil Chem. Soc.,* **76**, 783–787, 1999.

Ma, F. and Hanna, M.A., Biodiesel production: a review, *Bioresour. Technol.,* **70**, 1–15, 1999.

Macrae, A.R., Lipase-catalyzed interesterification of oils and fats, *J. Am. Oil Chem. Soc*., **60**, 291–294, 1983.

McNeill, G.P. and Yamane, T., Further improvements in the yield of monoglycerides during enzymatic glycerolysis of fats and oils, *J. Am. Oil Chem. Soc*., **68**, 6–10, 1991.

McNeill, G.P. et al. High-yield enzymatic glycerolysis of fats and oils, *J. Am. Oil Chem. Soc.*, **68**, 1–5, 1991.

Meher, L.C. et al. Technical aspects of biodiesel production by transesterification — a review, *Renew. Sustain. En. Rev.,* **10**, 248–268, 2006.

Mittelbach, M. and Remschmidt, C., Starting materials for biodiesel production, additional process variables in biodiesel production, in Mittelbach, M. and Remschmidt, C., Eds. *Biodiesel — The Comprehensive Handbook*, M. Mittelbach, Graz, Austria, 2004.

Mittelbach, M. and Trathnigg, B., Kinetics of alkaline catalyzed methanolysis of sunflower oil, *J. Am. Oil Chem. Soc.,* **92**, 145–148, 1990.

Noureddini, H. and Zhu, D., Kinetics of transesterification of soybean oil, *J. Am. Oil Chem. Soc.,* **74**, 1457–1463, 1997.

Pinsirodom, P. et al. Critical temperature for production of MAG by esterification of different FA with glycerol using *Penicillium camembertii* lipase, *J. Am. Oil Chem. Soc.,* **81**, 543–547, 2004.

Rozendaal, A. and Macrae, A.R., Interesterification of oils and fats, in *Lipid Technologies and Applications*, Gunstone, F.D. and Padley, F.B., Eds., Marcel Dekker, New York, **1997**, 223–245.

Schuchardt, U. et al. Transesterification of vegetable oils: a review, *J. Braz. Chem. Soc.,* **9**, 199–210, 1998.

Shintre, M.S. et al. Kinetics of esterification of lauric acid with fatty alcohols by lipase: Effect of fatty alcohol, *J. Chem. Technol. Biotechnol.,* **77**, 1114–1121 (2002).

Sonntag, N.O.V., Fat splitting, *J. Am. Oil Chem. Soc*., **56**, 729A–732A, 1979.

Soumanou, M.M. et al. Synthesis of structured triglycerides by lipase catalysis, *Fett/Lipid* **100**, 156–160 (1998).

Sreenivasan, B., Interesterification of fats, *J. Am. Oil Chem. Soc.*, **55**, 796–805, 1978.

Sridhar, R. et al. Modification of selected Indian vegetable fats into cocoa butter substitutes by lipase-catalyzed ester interchange, *J. Am. Oil Chem. Soc.*, **68**, 726–730, 1991.

Timm-Heinrich, M. et al. Oxidative stability of structured lipids produced from sunflower oil and caprylic acid, *Eur. J. Lipid Sci. Technol.,* **105**, 436–448 (2003).

Trani, M. et al. Lipase-catalyzed production of wax esters, *J. Am. Oil Chem. Soc*., **68**, 20–22, 1991.

Van Gerpen, J. and Knothe, G., Basics of the Transesterification Reaction, in *The Biodiesel Handbook*, Knothe, G., Krahl, J., and Van Gerpen, J. (Eds.), AOCS Press, Champaign, IL, 2005, pp. 26–41.

Xu, X. et al. Production of specific-structured lipids by enzymatic interesterification: elucidation of acyl migration by response surface design, *J. Am. Oil Chem. Soc.,* **75**, 1179–1186, 1998.

Xu, X. et al. Parameters affecting diacylglycerol formation during the production of specific-structured lipids by lipase-catalyzed interesterification, *J. Am. Oil. Chem. Soc*., **76**, 175–181, 1999.

Xu, X., Production of specific-structured triacylglycerols by lipase-catalyzed reactions: a review, *Eur. J. Lipid Sci. Technol.,* **102**, 287–303, 2000.

Xu, X., Engineering of enzymatic reactions and reactors for lipid modification and synthesis, *Éur. J. Lipid Sci. Technol.,* **105**, 289–304, 2003.

Xu, X. et al. Effects of antioxidants on the lipase-catalyzed acidolysis during production of structured lipids, *Éur. J. Lipid Sci. Technol.,* **107**, 464–468 (2005).

Yesiloglu, Y. and Kilic, I., Lipase-catalyzed esterification of glycerol and oleic acid, *J. Am. Oil Chem. Soc.,* **81**, 281–284, 2004.

8.13 Acid Chlorides, Anhydrides, and Ketene Dimers

8.13.1 Acid chlorides

Acid chlorides have been known for a long time, and although they don't find huge commercial value, they are of great use as reactive intermediates to prepare acylated derivatives. Fatty acids can be converted into acid chlorides by reaction with phosphorus trichloride, phosphorus pentachloride, phosphorus oxychloride, phosgene, oxalyl chloride, thionyl chloride or triphenylphosphine, and carbon tetrachloride (Patai, 1972; Gunstone 1996; Grim, 1979; Sonntag, 1979). Phosphorus trichloride and phosgene ($COCl_2$) are probably the most economical reagents for large-scale reaction. Improved yields have been claimed when the reaction is carried out in the absence of oxygen (Hauser, 1970). For many acylation reactions, fatty acids are first converted to their more reactive acid chlorides. The most common laboratory procedures require the acid to stand at room temperature for 3 to 5 days with thionyl chloride ($SOCl_2$) (2.4 mol) for saturated acids and with oxalyl chloride (1.8 mol) for unsaturated acids (Gunstone, 1999). Recently, Kiuru and Wähälä (2006) reported the preparation of steroidal fatty esters using fatty acid chlorides or anhydrides and microwave heating.

8.13.2 Acid anhydrides

Fatty acid anhydrides are prepared by reacting fatty acids or acid chlorides with acetic anhydride at reflux temperature or with dicyclohexylcarbondiimide (DCC) at room temperature (Kumar et al., 2002; Grimm, 1979; Sonntag et al., 1954). The least-volatile component of the equilibrium mixture (acetic acid or acetyl chloride) is continuously removed by distillation to shift the equilibrium in the desired direction (Figure 8.40).

Both the acid chlorides and anhydrides are effective acylating agents used in the synthesis of acylglycerols and phospholipids (Section 7.4 and Section 7.5). Kumar and coworkers have recently reviewed the preparation and the analysis of fatty acid anhydrides and polyanhydrides (Kumar et al., 2002).

Mixed anhydrides with methanesulphonic or toluene-4-sulphonic acid are also reported to be good acylating agents. They are made as indicated in the following equations (Figure 8.41).

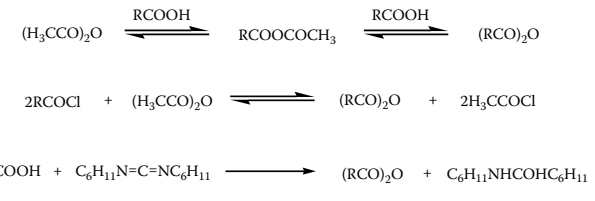


FIGURE 8.40 Preparation of acid anhydrides from carboxylic acids or acid chlorides.

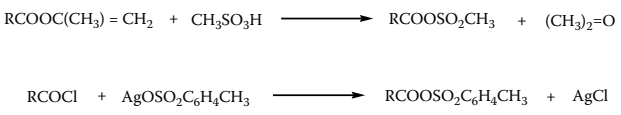


FIGURE 8.41 Preparation of mixed anhydrides using methansulfonic or toluene-4-sulfonic acids.

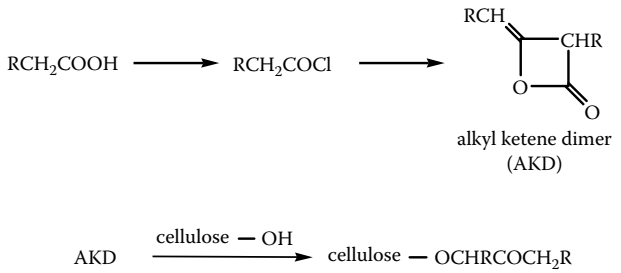


FIGURE 8.42 Preparation of ketene dimer and reaction with cellulose.

8.13.3 Ketene dimers

Ketene dimer made from hydrogenated tallow and, therefore, mainly a saturated C_{16}/C_{18} mixture, is used for sizing paper (Garnier et al., 1998). The dimer is made from the acid chloride. This reacts very slowly with water so that its aqueous dispersion is stable for at least 1 month. The association with paper (cellulose) is partly physical and partly chemical. The latter is thought to result in the formation of a β-oxo ester (Mohlin et al., 2006) (Figure 8.42).

References

Garnier G. et al. Wetting mechanism of alkyl ketene dimers on cellulose films, *Colloids and Surfaces A: Physicochem. Eng. Aspects*, **145**, 153–165, 1998.

Grimm, R.A., Salts, acid chlorides and anhydrides, in *Fatty Acids*, Pryde, E.H., Ed., American Oil Chemists' Society, Champaign, IL, 1979, pp. 218–235.

Gunstone, F.D., The preparation of derivatives for lipid analysis, in *Lipid Synthesis and Manufacture*, Gunstone, F.D., Ed., CRC Press, Boca Raton, FL., 1999, pp. 327–328.

Gunstone, F.D., *The Chemistry of Oils and Fats, Sources, Composition, Properties and Uses,* Blackwell Publishing, Oxford, U.K., 2004, pp. 199–200.

Hauser, C.F., Catalysts for the preparation of acid halides, German patent 1,931,074, 1970.

Kiuru, P.S. and Wähälä, K., Microwave-assisted synthesis of deuterium labeled estrogen fatty acid esters, *Steroids*, **71**, 5460, 2006.

Kumar, N. et al. Analysis of fatty acid anhydrides and polyanhydrides, *Anal. Chim. Acta*, **465**, 257–272, 2002.

Mohlin K. et al. Use of cleavable surfactants for alkyl ketene dimer (akd) dispersions, *Colloids and Surfaces A: Physicochem. Eng. Aspects*, **274**, 200–210, 2006.

Patai, S., *The Chemistry of Acyl Halides*, Interscience, New York, 1972.

Sonntag, N.O.V. et al. Reactions of fatty acid chlorides. I. Preparation of fatty acid anhydrides, *J. Am. Oil Chem. Soc.*, **31**, 151–157, 1954.

Sonntag, N.O.V., Reactions of fats and fatty acids, in *Bailey's Industrial Oil and Fat Products, Vol. 1*, 4th ed., Swern, D., Ed., Wiley-Interscience Publishers, New York, 1979, pp. 108–110.

8.14 Peroxy acids and related compounds

The acyl derivatives of hydrogen peroxide include the peroxy acids (also known as peracids), peroxy esters, and diacyl peroxides (Figure 8.43).

Although they have been widely studied (Ando, 1992; Davies, 1961; Swern, 1970, 1971, 1979; Gunstone, 2004) and safely used, even on a large scale, they should always be handled with care. In particular, they should not be ground, heated in a closed container, inhaled as vapour, or ingested as dust. Extensive books and reviews on these compounds have been published by Ando (1992), Davies (1961), and Swern (1970, 1971, and 1979).

Peroxy acids and diacyl peroxides are mono- and diacyl derivatives of hydrogen peroxide (Swern, 1979). Peroxy acids form esters, of which the *t*-butyl compounds are the best known (Silbert and Swern, 1959). In contrast to carboxylic acids, which form intermolecular hydrogen-bonded dimers, the peroxy acids exist as intramolecular hydrogen bonded monomers. This leads to several differences in the properties of these two types of acids.

8.14.1 Peroxy acids

The most important route to the peroxy acids requires establishment of the equilibrium shown in the following equation (Figure 8.44).

For acids insoluble in water or hydrogen peroxide, the reaction may be carried out in strong acids such as concentrated sulfuric acid or methanesulfonic acid, both of which serve as catalyst and solvent (Swern 1970, 1971, 1979; Parker et al., 1955). More recently, peroxy acids have been prepared under mild reaction conditions from carboxylic acids, hydrogen peroxide, and lipases (Rüsch gen.

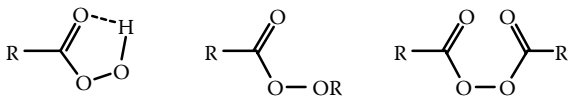


FIGURE 8.43 General structures of peroxy acid, peroxy ester, and diacyl peroxide compounds.

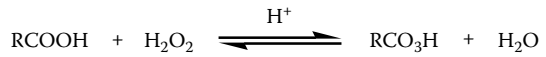


FIGURE 8.44 Preparation of peroxy acids from carboxylic acids and hydrogen peroxide.

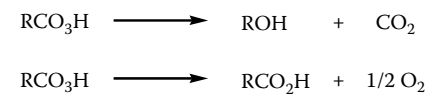


FIGURE 8.45 Thermal decomposition pathways for peroxy acids.

Klaas et al., 2002; Rüsch gen. Klaas and Warwel, 1997; Adelhorst et al., 1990; Björkling et al., 1992).

Peroxy acids decompose on heating, but can be stored for quite long times in the dark at subzero temperatures, although, trace impurities have an adverse effect on their stability (Swern, 1979). Thermal decomposition occurs mainly by a radical process, giving the lower alcohol, but an alternative concerted pathway giving the carboxylic acid is significant when the radical process is inhibited by antioxidants (Davies, 1961a) (Figure 8.45).

As a consequence of their intramolecular hydrogen bonding, the peroxy acids differ from the carboxylic acids in a number of their physical properties (Swern, 1979). For example, their acidity is reduced about 1000-fold (peroxylauric acid pK_a = 10.8; lauric acid pK_a = 7.8), the O-H stretching band appears at 3280 cm^{-1} in the infrared spectrum (3530 cm^{-1} for the unassociated carboxylic acid), and the ^1H NMR signal at δ =11.7–11.0 ppm is upfield compared with that of the carboxylic acid (Swern, 1979).

Peroxy acids are useful oxidizing agents and are generally analyzed on the basis of their ability to oxidize iodide ion to iodine, which can be determined titrimetrically or colorimetrically. Peroxy acids are used to oxidize alkenes to epoxides, ketones to ester and lactones (Baeyer-Villiger oxidation), and in the oxidation of heteroatoms to oxides (sulfides to sulfoxides and sulfones, amines to amine oxides, phosphine to phosphine oxides) (Swern 1979) (Figure 8.46).

Peroxy acids based on monobasic (C_8 and C_9) and dibasic acids (C_{12}) are used as laundry bleaches. The melting points of several peroxy acids are given in Table 8.8.

8.14.2 Peroxy esters

t-Butyl esters of peroxy acids are the most important of the peroxy esters and result from the reaction of *t*-butyl

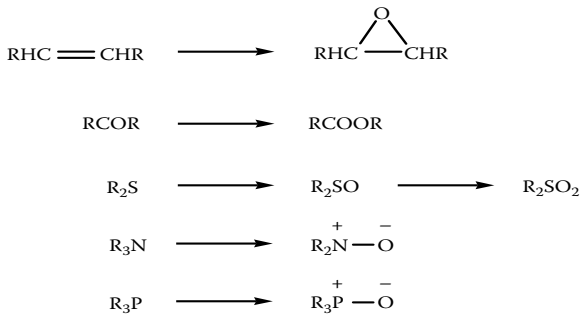


FIGURE 8.46 Typical oxidation reactions of organic compounds using peroxy acids.

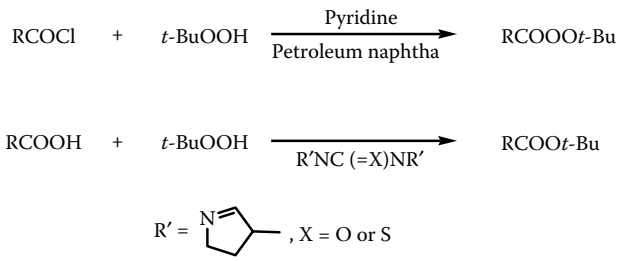


FIGURE 8.47 Preparation of peroxy esters from acyl halides or carboxylic acids and *t*-butyl hydroperoxide.

hydroperoxide with acyl halides (Silbert and Swern, 1959) or with carboxylic acids in the presence of appropriate imidazolide derivatives (Swern, 1979) (Figure 8.47).

These peroxy esters undergo smooth thermal decomposition at temperatures around 75°C and are useful initiators of radical chain reactions (Swern, 1979):

$$RCOO^tBu \rightarrow RCOO\bullet + {}^tBuO\bullet \rightarrow \bullet R + \bullet CH_3 + CO_2 + Me_2CO$$

The melting points of several *t*-butylperoxy esters are given in Table 8.8.

8.14.3 Diacyl peroxides

Diacyl peroxides are obtained by reaction of acyl halides or anhydrides with sodium peroxide or hydrogen peroxide or by reaction of carboxylic acid and hydrogen peroxide in the presence of dicyclohexylcarbodiimide (DCC) (Swern, 1979):

$$2RCOCl + Na_2O_2 \longrightarrow RCOOOCOR + 2NaCl$$

$$2RCOOH + H_2O_2 \longrightarrow RCOOOCOR$$

Diacyl peroxides undergo thermal decomposition by various routes depending upon the substituents and polarity/viscosity of solvents used during the decomposition, of which the most important involved homolysis of the O-O bond to give free radicals. Porter and coworkers have developed important methodology to analyze O-O containing compounds based upon silver ion coordination ionspray mass spectrometry (Yin et al., 2001; Seal et al., 2003). Because diacyl peroxides generate radicals smoothly under controlled conditions (between 20 and 100°C), they are frequently used as radical initiators in polymerization and organic synthetic applications (Fujimori, 1992; Hiatt, 1970; Zhou et al., 1998).

$$RCOOOCOR \longrightarrow 2RCOO\bullet \longrightarrow 2R\bullet + 2CO_2$$

The melting points of several diacyl peroxides are given in Table 8.8.

TABLE 8.8 Melting points of peroxy acids, their *t*-butyl esters, and diacyl peroxides

	Melting points (°C)			
n + 2	Peroxy acids $H_3C[CH_2]_n CO_3H$	Peroxy esters $H_3C[CH_2]_n CO_3Bu^t$	Diacyl peroxides $(H_3C[CH_2]_n COO)$	Diperoxy acids $HO_3C[CH_2]_n CO_3H$
6	15	–	–	117
7	–	–	–	92
8	31	–	22	89
9	35	–	13	90
10	41	−6	41	98
11	48	–	–	104
12	50	8	55	101
13	54	10	49	108
14	56	21	64	106
15	57	–	–	–
16	61	30	72	106
17	62	–	–	–
18	65	39	77	–
19	68.5	–	–	–
20	70.5	–	–	–

Source: Parker, W.E., et al. *J. Am. Chem. Soc*., 77, 4037–4041, 1955; Silbert, L. S. and Swern D., *J. Am. Chem. Soc*., 81, 2364–2367, 1959; and Swern, D., in *Fatty Acids*, Pryde, E.H. (Ed.) American Oil Chemists' Society, Champaign, IL, 1979, pp. 236–259.

References

Adelhorst, K. et al. Enzyme catalysed preparation of 6-O-acyl-glucopyranosides. *Synthesis*, 112–115, 1990.

Ando T., *Organic Peroxides*, John Wiley & Sons, Chichester, U.K., 1992.

Björkling, F. et al. Lipase catalyzed synthesis of peroxycarboxylic acids and lipase mediated oxidations. *Tetrahedron,* **48**, 4587–4592, 1992.

Davies, A.G., Peroxyacids, peroxyesters, and diacyl peroxides, in *Organic Peroxides*, Butterworth and Company, London, 1961, pp. 55–69.

Davies, A.G., Thermally induced O-O homolysis, in *Organic Peroxides*, Butterworth and Company, London, 1961a, pp. 165–173.

Gunstone, F.D., in *The Chemistry of Oils and Fats, Sources, Composition, Properties and Uses,* Blackwell Publishing, Oxford, U.K., 2004, pp. 200–201.

Hiatt, R., Acyl peroxides, in *Organic Peroxides,* vol. 2, Swern, D., Ed, Wiley–Interscience, New York, 1971, pp. 799–929.

Parker, W.E. et al. Peroxides. II. Preparation, characterization and polarographic behavior of longchain aliphatic peracids**,** *J. Am. Chem. Soc*., **77**, 4037–4041, 1955.

Rüsch gen Klaas, M. and Warwel, S., Lipase-catalyzed preparation of peroxy acids and their use for epoxidation, *J. Mol. Catal. A: Chem*., **117**, 311–319, 1997.

Rüsch gen Klaas, M. et al. Biocatalytic peroxy acid formation for disinfection, *J. Mol. Catal. B: Enzym.*, **19**, 20, 499–505, 2002.

Seal, J.R. et al. Analysis of unsaturated compounds by Ag^+ coordination ionspray mass spectrometry: studies of the formation of the Ag^+/lipid complex. *J. Amer. Soc. Mass Spectrom.,* **14**, 872–880, 2003.

Silbert, L. S. and Swern D., Peroxides. VI. Preparation of *t*-butyl peresters and diacyl peroxides of aliphatic monobasic acids. *J. Am. Chem. Soc*., **81**, 2364–2367, 1959.
Swern, D., *Organic Peroxides,* vol. 1., Wiley-Interscience, New York, 1970.
Swern, D., *Organic Peroxides* vol. 2., Wiley-Interscience, New York, 1971.
Swern, D., Peroxy acids, peroxy esters and diacyl peroxides, in *Fatty Acids*, Pryde, E.H., Ed., American Oil Chemists' Society, Champaign, IL, 1979, pp. 236–259.
Yin, H. et al. Analysis of diacyl peroxides by Ag-coordination ionspray tandem mass spectrometry: free radical pathways of complex decomposition. *J. Am. Soc. Mass. Spectrom.,* **12**, 449–455, 2001.
Zhou, G. et al. Synthesis, characterisation and initiator properties of an amphiphilic diperoxide. *Polymers,* **39**, 2677–2682, 1998.

8.15 Nitrogen-containing compounds

Fat-based amides, alkanolamides, nitriles, amines, imidazolines, and compounds derived from these are manufactured on an industrial scale in approximately 350,000 to 500,000 tons per annum and find their uses mainly in surfactant, lubricant, fungicide, and corrosion inhibiting applications.

8.15.1 Amides

The market for refined fatty acid amides is dominated by the unsaturated amides, oleamide (*cis*-9-octadecenamide) and erucamide (cis-13-docosenamide), which are used as antislip and antiblock agents at the 0.1 to 0.5% level for polyethylene film. They are also used in printing inks, as mould-release agents, lubricant additives, and in the production of water repellants (Formo, 1982). Fatty acid amides such as oleamide, anandamide, and N-arachidonoyldopamine are an important new class of lipid signalling molecules that appear to play a role in pain and inflammation (Cravatt et al., 1995; Walker et al., 2005).

Fatty acid amides are typically prepared industrially by the reaction between fatty acids or esters (fatty acid halides, anhydrides, and ammonium salts can also be used) and anhydrous ammonia at 180 to 200°C and 3.5 to 7.0 bar (Sonntag, 1964). Primary and secondary amines, such as monoethanolamine (R^1 = H, R^2 = CH_2CH_2OH; Figure 8.48) and diethanolamine (R^1, R^2 = CH_2CH_2OH; Figure 8.48) can also be utilized to give alkanolamides. Various

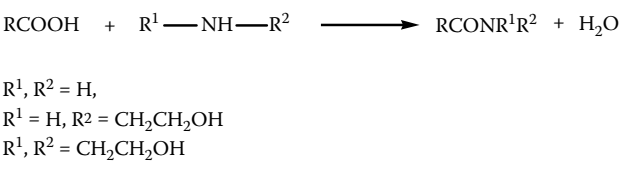


FIGURE 8.48 Preparation of fatty acid amides by reaction between fatty acids or esters and anhydrous ammonia or alkanolamines.

catalysts, such as boric acid, alumina, or zinc alkoxide can optionally be utilized, and urea (at a reaction temperature of 170 to 270°C) may replace ammonia. Recently, long-chain amides prepared by the condensation between fatty acids and amino acids have been of interest as biorenewable surfactants (Infante et al., 2004).

Enzymes, such as those present in *Candida regusa, Rhizomucor miehei*, and porcine pancreas, have also been used to prepare fatty amides by reaction between fatty acids, fatty acid esters, or glycerol esters, and alkylamines. There is some selectivity for the chain length of the acids and amines (Bistline et al., 1991). More recently, lipase enzymes, such as *Candida antarctica* lipase (Novozym 435) have been utilized to catalyze amide formation from lipid compounds, such as tributyrin, trilaurin, olive oil, jojoba wax, and hydroxyl fatty acids (e.g., lesquerolic and ricinoleic acid) (de Zoete et al., 1996; Fernandez-Perez and Otero, 2001; Maugard et al., 1998, 1997, 1997a; Levinson et al., 2005; Levinson et al., 2005a).

Fatty acids can also be condensed with polyamines, such as diethylenetriamine ($NH_2CH_2CH_2NHCH_2CH_2NH_2$) at a temperature of 150 to 170°C without a catalyst to produce fatty amides, such as $RCONHCH_2CH_2NHCH_2CH_2NH_2$. A wide range of polyamines are used commercially, e.g., ethylenediamine, aminoethylethanolamine, aminoethylpiperazine, triethylenetetramine, tetraethylenepentamine, and dimethylaminopropylamine. When the polyamine contains more than one primary or secondary amine group, polyalkylated products can result depending upon reaction conditions and the stoichiometry. For example, diamides can be prepared from fatty acids and ethylenediamine or also from fatty amides and formaldehyde (Figure 8.49). These materials typically find use as lubricants or lubricant additives.

When the polyamines, such as diaminoethane or bisaminoethylamine, are reacted with fatty acids at higher temperatures (190 to 250°C) under vacuum to remove water, cyclization can occur to give imidazoline ring system as shown in Figure 8.49 (Wu and Herrington, 1997). Imidazolines are utilized as fungicides, anticorrosion agents for lubricants, petroleum deemulsifiers, detergents, antistatic agents, and textile softeners. The hydrolysis of imidazolines in alkaline and acidic solution has been reviewed (Watts, 1990).

Ethoxylated amides, useful for their surface active properties, result from reaction between amides and ethylene oxide at 150 to 200°C in the presence of a basic catalyst or from esters (or acids) and diethanolamine at 115 to 160°C and a basic catalyst (Maag, 1984) (Figure 8.50).

Structurally related to the amides are the fatty hydrazide ($RCONHNH_2$), azide $RCON_3$, and hydroxamic acid (RCONHOH) derivatives, which are the acylated fatty acid derivatives of hydrazine, NH_2NH_2, hydrazoic acid (HN_3), and hydroxylamine (NH_2OH), respectively (Sonntag, 1964).

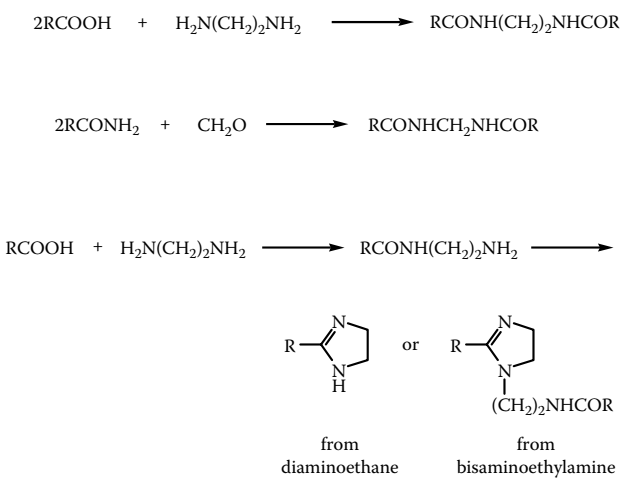


FIGURE 8.49 Preparation of diamides from fatty acids and ethylenediamine or also from fatty amides and formaldehyde.

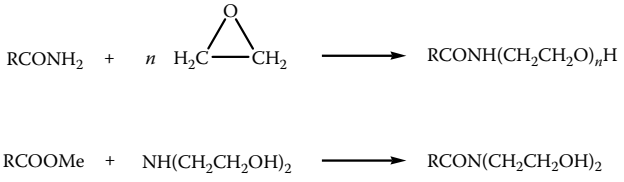


FIGURE 8.50 Preparation of ethoxylated amides.

8.15.2 Nitriles, amines, and their derivatives

Nitriles and primary amines. Nitriles (Figure 8.51) are prepared by heating fatty acids (or methyl esters) with ammonia at 280 to 360°C in the presence of a catalyst, such as bauxite, zinc oxide, manganese acetate, or cobalt salts (James, 1997; Billenstein and Blaschke, 1984). Nitrile preparation directly from fats and oils has also been investigated (Billenstein and Blaschke, 1984). The intermediate fatty amide is not isolated and the resulting nitrile is separated in vapor form from liquid fatty acid and amide. The nitriles have no significant application themselves and are used almost entirely to manufacture the corresponding fatty amines. Hydrogenation in the presence of ammonia (to suppress alkylation products) using a nickel or cobalt catalyst at 120 to 180°C and 20 to 40 bar gives mainly primary amines, although these may be accompanied by secondary and tertiary amines. Double bonds may also be reduced at the same time but conditions are selected to minimize this.

Secondary amines. Secondary amines can be obtained by hydrogenation under slightly different conditions, i.e., higher temperature (approximately 200°C), lower pressure, and liberated ammonia must be removed (Billenstein and Blaschke, 1984). Secondary amines can also be prepared directly by heating primary amines in the presence of catalysts or by reaction of a mixture of alcohol and primary amine under similar reaction conditions. Secondary amines are mainly used as chemical intermediates to

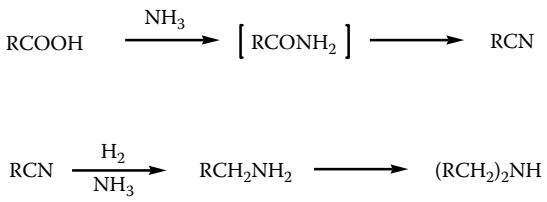


FIGURE 8.51 Nitrile preparation directly from fatty acids and their subsequent reduction to amines.

prepare quaternary ammonium compounds with two long alkyl chains, i.e., $R_2N^+(CH_3)_2X^-$.

Tertiary amines. Tertiary amines may contain three (R_3N), two (R_2NMe), or one ($RNMe_2$) long alkyl chain. Compounds of the type R_3N are made by reduction of nitriles, of nitrile and amine, or of alcohol and amine and ammonia. Methyl groups are introduced when a primary or secondary amine is reacted with formic acid or formaldehyde. Tertiary amines (RCH_2NMe_2) can be made directly from the alcohol (RCH_2OH) and dimethylamine in the presence of thallium sulfate at 360°C. Tertiary amines of another type can also be prepared by reaction of primary or secondary amines with ethylene oxide (Figure 8.52).

Recently, Behr and coworkers applied the hydroaminomethylation reaction to fatty acids using primary and secondary amines (Behr, et al., 2000). Hydroformylation of the fatty acid's unsaturated alkene followed by condensation of the intermediate aldehyde with a primary or secondary amine gave the corresponding secondary or tertiary fatty amine after hydrogenation of the enamine or imine intermediate. Tertiary amines are used as corrosion inhibitors, fuel additives, flotation agents, fungicides, emulsifying agents, foaming agents, and cosmetic ingredients and for the production of quaternary ammonium compounds.

Quaternary ammonium compounds. Quaternary ammonium compounds (commonly called quats) make up about 40% of all cationic and amphoteric surfactants. They are prepared by reaction of primary, secondary, or tertiary amine, a polyamine, or an oxyethylated amine with a short-chain alkylating agent, such as methyl, ethyl, or benzyl chloride (or sulfates), or ethylene oxide. Typical structures include: $RN^+Me_3Cl^-$, $RN^+Me_2(CH_2Ph)Cl^-$, $R_2NMe_2Cl^-$, and R_3NMeCl^-. These quaternary compounds may have one, two, or three long alkyl chains.

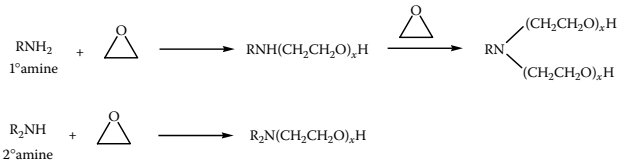


FIGURE 8.52 Reaction of primary and secondary amines with ethylene oxide to give ethoxylated tertiary amines where *n* can have a range of values.

They are used extensively as fabric softeners, as bactericides, for the manufacture of organic-modified drilling mud, and as cationic emulsifiers for asphalt.

Amine oxides. Amine oxides, such as $RN^{+}(O^{-})(CH_3)_2$, are prepared by adding a tertiary amine to aqueous hydrogen peroxide with vigorous agitation at 60 to 65°C (Bognolo, 1997). Water is added to prevent gel formation and obtain a 30 to 35% amine oxide concentration. The resulting amine oxides are widely used in the cosmetics industry and, as cleaners and detergents, can be incorporated into bar soaps (Bognolo, 1997; Smith et al., 1991). They are nontoxic surfactants and are readily metabolized and nonmutagenic.

Polyamines. Polyamines can be prepared by the cyanoethylation-hydrogenation reaction between acrylonitrile and fatty amines (Billenstein and Blaschke, 1984). Adjustment of the amine/acrylonitrile ratio can give branched polyamines. When fatty alcohols are used in place of fatty amines the cyanoethylation-hydrogenation reaction give ether amines and ether polyamines (Figure 8.53). Amphoteric surfactants have recently been reviewed by Uphues (1998).

Polyamines can undergo further reactions, such as ethoxylation, alkylation, and condensation reactions. Polyamines are valuable as flotation agents in mining, fuel-oil additives, adhesive agents for asphalt, and water-repellant agents.

Other nitrogen-containing compounds. Several other industrially important nitrogen-containing compounds have been made and their properties examined. Typical examples, shown in Figure 8.54, include pyrimidines (Takata et al., 1990), pyrrolidones (Tsatsaroni et al., 1987) and derivatives of N-acyldiethanolamine (Zhu et al., 1991). The latter have two (R' = CH_3) or three (R' = C_9H_{19} or $C_{11}H_{23}$) long alkyl chains. On an industrial scale, all these nitrogen-containing compounds are made from mixed fatty acids of natural origin (e.g., coconut, tallow, tall oil) or from individual acids of technical purity.

In addition to the previously mentioned compounds, research to prepare and examine the spectroscopic properties of many other types of interesting acyclic (Biermann et al., 1998; Herron et al., 1997; Lie Ken Jie and Lau, 2000; Kenar, 2003) and heterocyclic (Lie Ken Jie and Cheung, 1999) fatty nitrogen-containing compounds has been performed. Lie Ken Jie and his Hong Kong group and the Metzger group in Germany have done a great deal

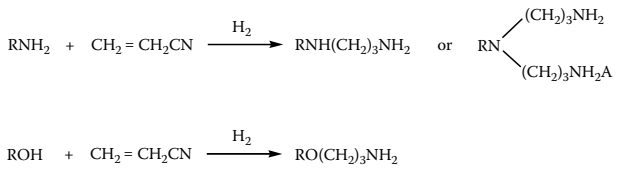


FIGURE 8.53 Polyamine preparation from cyanoethylation-hydrogenation reaction between acrylonitrile and fatty amines or alcohols.

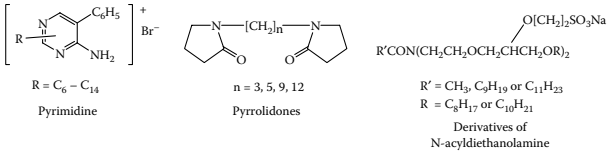


FIGURE 8.54 Other industrially important nitrogen-containing compounds include pyrimidines, pyrrolidones, and N-acyldiethanolamine derivatives.

of work in this area. These N-heterocyclic fatty compounds include: aziridines (Ahmad et al., 1988; Metzger and Fürmeier, 1999; Fürmeier and Metzger, 2003; Lie Ken Jie and Syed-Rahmatullah, 1992), oxazolidones (Javni et al., 2003), isoxazoles (Kenar and Wetzel, 2003), isoxazolines (Kenar and Erhan, 2001), pyrroles (Hildago and Zamora, 1995, 1995a; Lie Ken Jie and Wong, 1993), pyrrolines (Lie Ken Jie and Syed Rahmatullah, 1995, 1991; Lie Ken Jie et al., 1994; Lie Ken Jie and Syed-Rahmatullah, 1991), dihydropyrazines (Lie Ken Jie et al., 2001), azides (Lie Ken Jie and Lao, 1987; Lie Ken Jie and Alam, 2001), pyrazoles (Lie Ken Jie, and Lau, 1999; Lie Ken Jie and Kalluri, 1995), pyrazolines (Agarwal et al., 1989), triazoles (Lie Ken Jie et al., 1998, 1998a), pyridazines (Lie Ken Jie and Kalluri, 1995a), piperidines and pyridines (Lie Ken Jie and Pasha, 1991), and tetrazoles (Biermann et al., 1999; Fürmeier and Metzger, 2003a).

References

Agarwal, R. et al. Preparation of pyrazolines from methyl 4-oxooctadec-2(E)-enoate and their antimicrobial activity, *J. Am. Oil Chem. Soc.*, **66**, 970–971, 1989.

Ahmad, M.B. et al. Aziridination of olefinic and hydroxyolefinic fatty esters, *Ind. J. Chem.*, **27B**, 1140–1141, 1988.

Barrault, J. et al. Hydrogenation and methylation of dodecyl nitrile in the presence of copper catalysts supported on alumina : I: characterization of the catalysts, *Appl. Catal. A: Gen.*, **102**, 137–150, 1993.

Barrault, J. and Pouilloux, Y., Synthesis of fatty amines. selectivity control in presence of multifunctional catalysts, *Catal. Today*, **37**, 137–153, 1997.

Behr, A. et al. Hydroaminomethylation of fatty acids with primary and secondary amines — a new route to interesting surfactant substrates, *Eur. J. Lipid Sci. Technol.*, **102**, 467–471, 2000.

Biermann, U. et al. New chemistry of oils and fats, in *Oleochemical Manufacture and Applications*, Gunstone, F.D. and Hamilton, R.J., Eds., Sheffield Academic Press, Sheffield, U.K., 1999, pp. 290–293.

Biermann, U. et al. Some carbon, nitrogen- and carbon, oxygen-bond forming additions to unsaturated fatty compounds, *Fett Lipid*, **6**, 236–246, 1998.

Billenstein, S. and Blascke, G., Industrial production of fatty amines and their derivatives, *J. Am. Oil Chem. Soc.*, **61**, 353–357, 1984.

Bistline, R.G. et al. Lipase catalyzed formation of fatty amides, *J. Am. Oil Chem. Soc.*, **68**, 95–98, 1991.

Bognolo, G., Nonionic surfactants, in *Lipid Technologies and Applications*, Gunstone, F.D. and Padley, F.B., Eds., Marcel Dekker, New York, 1997, p. 660.

Cravatt, B.F. et al. Chemical characterization of a family of brain lipids that induce sleep, *Science*, **268**, 1506–1509, 1995.

de Zoete, M.C. et al. Lipase-catalysed ammoniolysis of lipids. A facile synthesis of fatty acid amides, *J. Mol. Catal. B: Enzym.*, **1**, 141–145, 1996.

Fernandez-Perez, M. and Otero, C., Enzymatic synthesis of amide surfactants from ethanolamine, *Enzyme Microb. Technol.*, **28**, 527–536, 2001.

Formo, M.W., Miscellaneous oil and fat products, in *Bailey's Industrial Oil and Fat Products*, vol. 2, 4th ed., Swern, D., Ed., John Wiley & Sons, New York, 1982, pp. 343–406.

Fürmeier, S. and Metzger, J. O., Fat-derived aziridines and their N-substituted derivatives: biologically active compounds based on renewable raw materials, *Eur. J. Org. Chem*., 649–659, 2003a.

Fürmeier, S. and Metzger, J. O., synthesis of new heterocyclic fatty compounds, *Eur. J. Org. Chem*., 885–893, 2003b.

Gunstone, F.D., Chemical reactions of fatty acids with special reference to the carboxyl group, *Eur. J. Lipid Sci. Technol*., **103**, 307–314, 2001.

Herron, B.F. et al. Preparation and characterization of methyl 11-amino-(N-*p*-toluenesulfonyl)-9-*E*-octadecenoate and methyl 8-amino-(N-*p*-toluenesulfonyl)-9-*E*-octadecenoate, *J. Am. Oil Chem. Soc*., **74**, 229–234, 1997.

Hidalgo, F.J. and Zamora, R., Epoxyoxoene fatty esters: key intermediates for the synthesis of long-chain pyrrole and furan fatty esters, *Chem. Physics Lipids*, **77**, 1–11, 1995a.

Hildago, F.J. and Zamora, R., *In vitro* production of long chain pyrrole fatty esters from carbonyl-amine reactions, *J. Lipid Res.*, **36**, 725–735, 1995b.

House, J.E., The chemistry and applications of amine derivatives in solvent extractions, *J. Am. Oil Chem. Soc.,* **61**, 357–362, 1984.

Infante, M.R. et al. Amino acid-based surfactants, *Comptes Rendus Chim*., **7**, 583–592, 2004.

James, A.D., Cationic surfactants, in *Lipid Technologies and Applications*, Gunstone, F.D. and Padley, F.B., Eds., Marcel Dekker, New York, 1997, pp. 609–631.

Javni, I. et al. The study of oxazolidone formation from 9,10-epoxyoctadecane and phenylisocyanate, *J. Am. Oil Chem. Soc*., **80**, 595–600, 2003.

Kenar, J.A. and Erhan, S.Z., Synthesis of Δ^2-isoxazolines from unsaturated fatty acid esters, *J. Am. Oil Chem. Soc*., **78**, 1045–1050, 2001.

Kenar, J.A. and Wetzel, A.R., Preparation of fatty 3,5-disubstituted isoxazole compounds from soybean fatty esters, *J. Am. Oil Chem. Soc*., **80**, 711–716, 2003.

Kenar, J.A., Preparation of long-chain β-enaminones and β-diketones from fatty 3,5-disubstituted isoxazole compounds, *J. Am. Oil Chem. Soc*., **80**, 1027–1032, 2003.

Levinson, W.E. et al. Lipase-catalyzed production of novel hydroxylated fatty amides in organic solvent, *Enzyme Microb. Technol*., **37**, 126–130, 2005a.

Levinson, W.E. et al. Characterization of primary fatty amides produced by lipase-catalyzed amidation of hydroxylated fatty acids, *J. Am. Oil Chem. Soc*., **82**, 501–504, 2005b.

Lie Ken Jie, M.S.F. and Cheung, S.W.H., Synthesis of special fatty acids: a review, in *Recent Developments in the Synthesis of Fatty Acid Derivatives*, Knothe, G. and Derksen, J.T.P., Eds., American Oil Chemists' Society, Champaign, IL, 1999, pp. 20–43.

Lie Ken Jie, M.S.F. et al. Cyclodehydration reactions of methyl 9,10-; 10,12-; and 9,12-dioxostearates with 1,2-diaminoethane under ultrasonic irradiation, *Lipids*, **36**, 201–204, 2001.

Lie Ken Jie, M.S.F. and Syed-Rahmatullah, M.S.K., Synthesis and spectroscopic properties of long-chain aza, aziridine and azetidine fatty esters, *J. Am. Oil Chem.*, **69**, 359–362, 1992.

Lie Ken Jie, M.S.F. and Syed-Rahmatullah, M.S.K., Preparation and some spectroscopic properties of N-heterocyclic derivatives of a novel 1-pyrroline C-18 fatty ester, *Lipids*, **26**, 842–846, 1991.

Lie Ken Jie, M.S.F. and Kalluri, P., Synthesis of pyrazole fatty ester derivatives in water: a sonochemical approach, *J. Chem. Soc. Perkin Trans.,* **1**, 1205–1206, 1995.

Lie Ken Jie, M.S.F. and Syed-Rahmatullah, M.S.K., Synthesis and properties of a novel 1-pyrroline fatty acid derivative from methyl isoricinoleate, *J. Chem. Soc. Perkin Trans.,* **1**, 421–424, 1991.

Lie Ken Jie, M.S.F. and Pasha, M.K., Synthesis of novel piperidine and pyridine containing long-chain fatty ester derivatives from methyl isoricinoleate, *J. Chem. Soc. Perkin Trans.,* **1**, 1331–1332, 1991.

Lie Ken Jie, M.S.F. et al. Synthesis of novel triazole fatty acid derivatives from acetylenic fatty esters, *Chem. Phys. Lipids*, **91**, 71–78, 1998.

Lie Ken Jie, M.S.F. and Alam, M.S., Novel azido fatty acid ester derivatives from conjugated C_{18} enynoate, *Chem. Phys. Lipids*, **111**, 29–35, 2001.

Lie Ken Jie, M.S.F. and Lau, M.M.L., Novel lipidic enaminones from a C_{18} keto-allenic ester, *Lipids*, **35**, 1135–1145, 2000.

Lie Ken Jie, M.S.F. and Syed Rahmatullah, M.S.K., Further chemical reactions of 1-pyrroline fatty ester: *N*-substituted pyrrolinium and pyrrolidine derivatives, *Chem. Phys. Lipids*, **77**, 179–186, 1995.

Lie Ken Jie, M.S.F. and Lao, H.B., Fatty acids, part 31. The preparation and some physical properties of azido fatty esters, *Chem. Phys. Lipids*, **45**, 65–74, 1987.

Lie Ken Jie, M.S.F. and Lau, M.M.L., Ultrasound assisted synthesis of pyrazole fatty ester derivatives from a key C_{18} keto-allenic ester, *Chem. Phys. Lipids*, **101**, 237–242, 1999.

Lie Ken Jie, M.S.F. et al. Synthesis of novel triazole fatty acid derivatives from acetylenic fatty esters, *Chem. Phys. Lipids*, **91**, 71–78, 1998.

Lie Ken Jie, M.S.F. et al. Ultrasound in fatty acid chemistry: synthesis of a 1-pyrroline fatty acid ester isomer from methyl ricinoleate, *Lipids*, **29**, 889–892, 1994.

Lie Ken Jie, M.S.F. and Wong, K.P., Synthesis of trisubstituted C_{18} pyrrole fatty ester derivatives, *Lipids*, **28**, 161–162, 1993.

Lie Ken Jie, M.S.F. and Kalluri, P., Synthesis of pyridazine fatty ester derivatives in water: a sonochemical approach, *J. Chem. Soc. Perkin Trans.,* **1**, 3485–3486, 1995.

Linfield, W.M., Fatty oxazolines and imidazolines, *J. Am. Oil Chem. Soc*., **61**, 437–441, 1984.

Maag, H., Fatty acid derivatives: important surfactants for household, cosmetic and industrial purposes, *J. Am. Oil Chem. Soc*., **61**, 259267, 1984.

Maugard, T. et al. Enzymatic amidification for the synthesis of biodegradable surfactants: synthesis of N-acylated hydroxylated amines, *J. Mol. Catal. B: Enzym*., **5**, 13–17, 1998.

Maugard, T. et al. Enzymatic synthesis of glycamide surfactants by amidification reaction, *Tetrahedron*, **53**, 5185–5194, 1997a.

Maugard, T. et al. Lipase-catalysed synthesis of biosurfactants by transacylation of n-methyl-glucamine and fatty-acid methyl esters, *Tetrahedron*, **53**, 7629–7634, 1997b.

Metzger, J.O. and Fürmeier, S., New type of skipped oligoaziridines: synthesis of new fatty acid derivatives containing aziridine functions, *Eur. J. Org. Chem*., 661–664, 1999.

Puchta, R., Cationic surfactants in laundry detergents and laundry aftertreatment aids, *J. Am. Oil Chem. Soc*., **61**, 367–376, 1984.

Schaeufele, P.J., Advances in quaternary ammonium biocides, *J. Am. Oil Chem. Soc*., **61**, 387–389, 1984.

Smith, K.R. et al. High active alkyldimethylamine oxides, *J. Am. Oil Chem. Soc.*, **68**, 619–622, 1991.

Sonntag, N.O.V., Nitrogen derivatives, in *Fatty Acids: Their Chemistry, Properties, Production, and Uses*, 2nd ed., Markley, K.S., Ed., John Wiley & Sons, New York, 1964, pp. 1551–1715.

Takata, T. et al. Surfactant property of linear alkanes with two pyrrolidone moieties in both termini, *J. Am. Oil Chem. Soc*., **67**, 739–742, 1990.

Tsatsaroni, E. et al. Synthesis and properties of new cationic surfactants. II. Odd homologous members, *J. Am. Oil Chem. Soc*., **64**, 1444–1447, 1987.

Uphues, G., Chemistry of amphoteric surfactants, *Fett Lipid*, **100**, 490–497, 1998.

Walker, M.J. et al. Targeted lipidomics: fatty acid amides and pain modulation, *Prostagland. Lipid Med.*, **77**, 35–45, 2005.

Watts, M.W., Imidazoline hydrolysis in alkaline and acidic media—a review, *J. Am. Oil Chem. Soc*., **67**, 993–995, 1990.

Wu, Y. and Herrington, P.R., Thermal reaction of fatty acids with diethylene triamine, *J. Am. Oil Chem. Soc*., **74**, 61–64, 1997.

Zhu, Y.-P., Masuyama, A. et al. Preparation and properties of double-, triple-chain surfactants with two sulfonate groups derived from N-acyldiethanolamines, *J. Am. Oil Chem. Soc*., **68**, 539–543, 1991.

8.16 Other reactions of the carboxyl group

A carboxyl group raises the activity of the adjacent methylene CH_2 hydrogens (pK_a = ~25) above that of other methylene hydrogens in a long-chain acid. This added activity makes it possible to functionalize the α-methylene position in a number of ways. Some of this chemistry is only applicable to saturated fatty acids or esters because the presence of double bonds sometimes promotes similar reactions at their allylic sites leading to a mixture of products. Halogenation of fatty acids in the α-position has been covered (Section 8.4, Halogenation). Herein, sulfonation and the formation and reactions of α-anions is covered. Some of these reactions are accompanied or followed by decarboxylation, so the entire sequence can be summarized as follows (Figure 8.55).

8.16.1 Sulfonation

Sulfonation of saturated fats, oils, fatty acids (or esters) can be effected batch-wise with oleum (stabilized sulfur trioxide), or continuously with dilute vaporized sulfur dioxide or chlorsulfonic acid. Trichloromethane, tetrachloromethane, tetrachloroethylene, and liquid sulfur dioxide have also been used as reaction solvent (Bluestein and Kapur, 1979). If unsaturation is present, sulfation can also occur at the vinyl hydrogens (Inagaki, 1990; Gilbert, 1962) (Figure 8.56).

The sulfonic carboxylic acids (α-sulfo fatty acids) have surface active properties and melting points of 86.5 to 88°C (C_{12}), 85 to 86.5°C (C_{14}), 90 to 91°C (C_{16}), 96 to 97°C (C_{18}), and 96 to 97°C (C_{22}) (Weil et al., 1956). The α-sulfo fatty acids may exist as the corresponding acid salts [$RCH(SO_3Na)COOH$], neutral salts [$RCH(SO_3Na)COONa$], or esters [$RCH(SO_3H)COOR'$] and are used in detergents (Drozd, 1990), and as viscosity-reducing agents, corrosion inhibitors, and ore flotation agents (Bluestein and Kapur, 1979). The sulfonation of stearic and oleic acids, oleic acid dimers, and their esters using sulfur trioxide and chlorosulfonic acid to give anionic surfactants with interesting properties has been studied (Paubert and Canselier, 1995).

Sulfonated fatty acids show high levels of surface activity, are less toxic in aqueous environments than other surfactants, and are biodegradable. Their esters show high hydrolytic stability relative to the free carboxylic acids due to the presence of the sulfo group, which helps stabilize the ester linkage (Bluestein and Kapur, 1979). A mechanism for α-sulfonation of saturated fatty acids with sulfur trioxide proposes a mixed anhydride intermediate ($RCH_2CO_2SO_3H$), which upon heating rearranges to the α-sulfo acid (Bluestein and Kapur, 1979). The sulfonation of fatty acids and alcohols to manufacture anionic surfactants has most recently been reviewed by Roberts (2001).

$$RCH_2COOH \longrightarrow RCHXCOOH \longrightarrow RCH_2X$$

FIGURE 8.55 Products derived from reaction of the α-position of fatty acids.

$$RCH_2COOH(CH_3) \longrightarrow RCH(SO_3H)COOH(CH_3)$$

FIGURE 8.56 α-Sulfonation of fatty acids and their methyl esters.

8.16.2 α-anions

Saturated and monoene acids form dianions when treated with a strong base, such as lithium diisopropylamide (LDA) in hexamethylphosphoramide (HMPA). The α-anion can undergo further reaction with a wide range of reagents (hydroxylation, alkylation, etc.) to give fatty acids substituted at the α-position or products arising from these by decarboxylation (Silbert and Pfeffer, 1979; Petranani and Yonashiro, 1982).

The types of compounds formed in these reactions are listed in Figure 8.57.

More recent work in this area has focused on preparing chiral 2-hydroxy fatty acids using enantioselective chemical (Hwang and Erhan, 2001) and enzymatic (Adam et al., 1998, 1996) approaches.

8.16.3 Polyoxyethylene esters

Carboxylic acids react with ethylene oxide in the presence of an alkaline catalyst (homogeneous and heterogeneous) or with polyoxethylene glycols and an acidic catalyst to give a hydroxyl ester with a range of ethylene oxide units accompanied by the corresponding diester and diol resulting from ester interchange (Kosswig, 1996; Hamid, 2004) (Figure 8.58).

By this reaction carboxylic acids are converted into nonionic surfactants, which are used in various ways (Weil et al., 1979) (see also Chapter 9, Nonfood Uses).

8.16.4 Aldehydes

Esters are readily converted into aldehydes by reaction with lithium aluminum hydride ($LiAlH_4$) in the presence of excess diethylamine in pentane (Cha and Kwon, 1987). Acids give aldehydes through reaction with thexylbromoborane dimethylsulfide in carbon disulfide (Cha et al., 1987) or in a one-pot reaction via the activated pentacoordinated silyl carboxylate (Corriu et al., 1987).

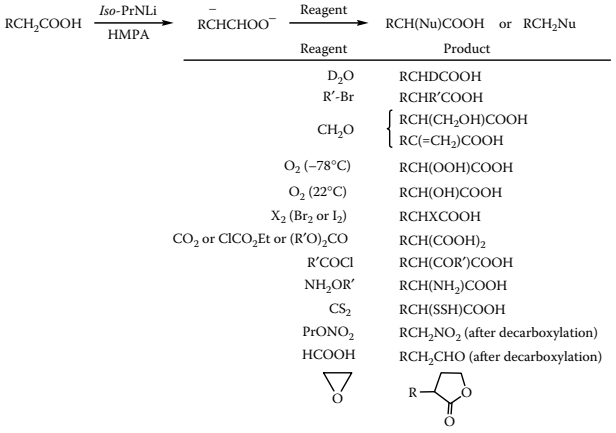


FIGURE 8.57 Formation of fatty acid dianions and some reaction products.

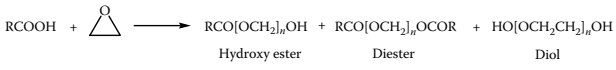


FIGURE 8.58 Reaction of fatty acids with ethylene oxide where *n* can have a range of values.

References

Adam, W. et al. α-Hydroxylation of carboxylic acids with molecular oxygen catalyzed by the α-oxidase of peas (*Pisum sativum*): A novel biocatalytic synthesis of enantiomerically pure (*R*)-2-hydroxy acids. *J. Am. Chem. Soc.,* **120**, 11044–11048, 1998.

Adam, W. et al. Enantioselective α-hydroxylation of carboxylic acids with molecular oxygen catalyzed by the α-oxidation enzyme system of young pea leaves (*Pisum sativum*): A substrate selectivity study. *Tetrahedron: Asymmetry,* **7**, 2287–2292, 1996.

Bluestein, B.R. and Kapur, B.L., Sulfonation. In *Fatty Acids*, Pryde, E.H., Ed., American Oil Chemists' Society, Champaign, IL, 1979, pp. 260–289.

Cha, J.S. and Kwon, S.S., Exceptionally facile reduction of carboxylic acids to aldehydes by lithium aluminum hydride in the presence of diethylamine. *J. Org. Chem.*, **52**, 5486–5487, 1987.

Cha, J.S. et al. Direct conversion of saturated and unsaturated carboxylic acids into aldehydes by thexylbromoborane-dimethyl sulfide. *Tetrahedron Lett.*, **28**, 2389–2392, 1987.

Corriu, R.J.P. et al. The one-pot conversion of carboyxlic acids to aldehydes via activated silyl carboxylates. *Tetrahedron Lett.*, **28**, 3941–3944, 1987.

Drozd, J.C., Use of sulfonated methyl esters in household cleaning products, In *World Conference on Oleochemicals*, Applewhite, T.H., Ed., American Oil Chemists' Society, Champaign, IL, 1990, pp. 257–268.

Gilbert, E.E., The reactions of sulfur trioxide, and its adducts, with organic compounds. *Chem. Rev.*, **62**, 549–589, 1962.

Hamid, S.B.A. et al. Polyoxyethylene esters of fatty acids: An alternative synthetic route for high selectivity of monoesters. *Catal. Today*, **97**, 271–276, 2004.

Hwang, H.-S. and Erhan, S.Z., Highly selective asymmetric synthesis of 2-hydroxy fatty acid methyl esters through chiral oxazolidinone carboximides. *J. Am. Oil Chem. Soc.*, **78**, 205–211, 2001.

Inagaki, T., Development of α-sulfo fatty acid esters. In *World Conference on Oleochemicals*, Applewhite, T.H., Ed., American Oil Chemists' Society, Champaign, IL, 1990, pp. 269–278.

Kossiwig, K., Perspektiven der Fettsäurepolyglykolester als derivate der Fettsaüremethylester. *Tenside, Surfactants, Deterg.*, **33**, 96–100, 1996.

Paubert, M. and Canselier, J.P., Sulfonates derived from dimer acids and esters. Some surfactant properties. *Tenside, Surfact., Deterg.*, **32**, 36–44, 1995.

Petranani, N. and Yonashiro, M., The reactions of dianions of carboxylic acids and ester enolates. *Synthesis*, 521–578, 1982.

Roberts, D.W., Manufacture of anionic surfactants, In *Oleochemical Manufacture and Applications*, Gunstone, F.D., and Hamilton, R. J., Eds., Sheffield Academic Press, Sheffield, U.K., 2001, pp. 55–73.

Silbert, L.S. and Pfeffer, P.E., α-Anions, In *Fatty Acids*, Pryde, E.H., Ed., American Oil Chemists' Society, Champaign, IL, 1979, pp. 272–290.

Weil, J.K. et al. α-Sulfopalmitic acid, In *Organic Synthesis,* vol. 36, Leonard, N.J., Ed., John Wiley & Sons, New York, 1956, pp. 83–86.

Weil, J.K. et al. Nonionic wetting agents, *J. Am. Oil Chem. Soc.*, **56**, 873–879, 1979.

8.17 Oleochemical carbonates

Linear and cyclic carbonates (esters of carbonic acid, H_2CO_3) represent a versatile class of organic compounds (Shaikh and Sivaram, 1996; Buysch, 2003; Parrish et al., 2000) that can be prepared from alcohols, epoxides, or halohydrins by a variety of reagents (Figure 8.59). Oleochemical carbonates, based upon animal- and plant-based feed stocks, are typically prepared from linear and branched fatty alcohols (Kenar et al., 2003). Although known for some time, oleochemical carbonates now find limited commercial use as components in lubricant (Fisicaro and Gerbaz, 1993; Gryglewicz et al., 2003; Westfechtel et al., 1995), cosmetic (Ansmann et al., 2003; Suetake, 2001), and plasticizer (Giolito et al., 1983) formulations. The properties of some oleochemical carbonate compounds have been compiled (Dierker, 2004; Kenar, 2004). Recently, short-chain dimethyl and diethyl carbonate have been utilized as reactive solvents to simultaneously extract and then transesterify oilseed oils (Rüsch gen. Klaas and Warwel, 2001).

8.17.1 Carbonate Preparation

Although the condensation of carbonic acid (H_2CO_3) with an alcohol would appear to be the most direct route to synthesize carbonates, practically speaking, this reaction is not feasible since carbonic acid is insufficiently stable towards condensation. Therefore, a variety of alternative routes have been developed to prepare carbonate compounds (see Figure 8.59).

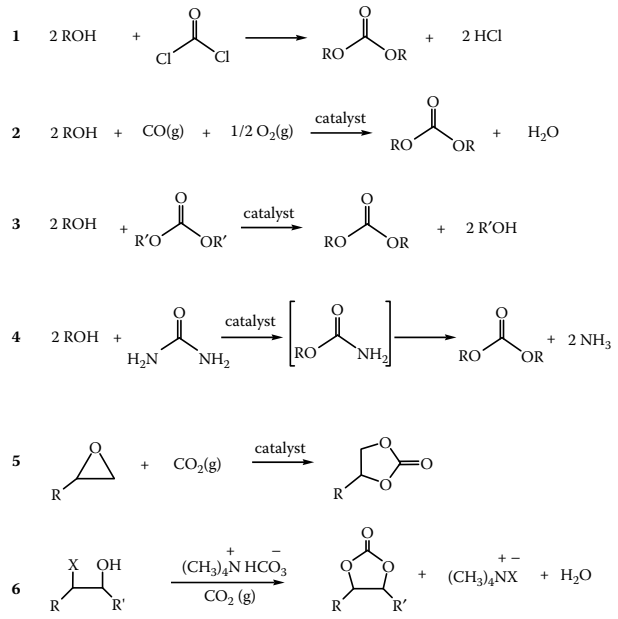


FIGURE 8.59 Synthetic routes to prepare organic carbonates.

Phosgenation Reaction. Phosgene (carbonic acid dichloride, ClCOCl), represents an economical and versatile building block providing the C = O group (Buysch, 2003). Although a highly toxic and corrosive reagent, the reaction between phosgene and hydroxy compounds proceeds readily, can be run continuously, and provides high yields of carbonates (entry 1, Figure 8.59). Currently, the phosgenation reaction is the most important industrial method for producing carbonic acid esters.

Oxidative Carbonylation Reaction. Because oxidative carbonylation, the catalytic reaction of methanol with carbon monoxide and oxygen, represents a "Green" method to prepare carbonates, it is the subject of intensive study (entry 2, Figure 8.59). The reaction is catalyzed by metal salts, most notably copper chloride. This chemistry has been extensively examined for dimethyl carbonate (DMC) synthesis and has been successfully demonstrated on large scale (Delledonne et al., 2001). In fact, since the 1980s, this method has replaced phosgenation as the main method of DMC synthesis, and by eliminating phosgene, dimethyl carbonate has begun to achieve growing importance in the chemical industry (Tundo and Selva, 2002). Application of oxidative carbonylation to longer chain alcohols still faces technical challenges.

Carbonate Interchange Reaction. Transesterification between a low molecular weight dialkyl carbonate and an alcohol produces the corresponding dialkyl carbonate of the alcohol (entry 3, Figure 8.59). This reaction is an equilibrium reaction and adheres to the following basic principles: (1) The more nucleophilic hydroxyl compound displaces the less nucleophilic compound, and (2) the less volatile compound displaces the more volatile one (Shaikh and Sivaram, 1996). The reaction of either dimethyl or diethyl carbonate with linear mid-, long-chain, and branched (Guerbet) oleochemical alcohols in the presence of basic catalysts gives good yields of the corresponding oleochemical carbonates (Kenar et al., 2003).

The carbonate interchange reaction can also be carried out biocatalytically utilizing lipases. The lipase catalyzed reaction between diethyl carbonate and oleyl alcohol gives unsymmetrical carbonate, ethyl oleyl carbonate, as the major product (28% yield) along with minor amounts (5% yield) of the symmetrical oleyl carbonate (Pioch et al., 1991). When a carbonate contains an irreversible leaving group, such as a vinyl moiety, improved reaction yields are achievable. Accordingly, when *n*-octyl vinyl carbonate ($H_2C{=}CHOCOO(CH_2)_7CH_3$) is reacted with octanol, a 61% yield of dioctyl carbonate is obtained. Future work on biocatalyzed reactions to produce oleochemical carbonates could open up exciting new possibilities for oleochemical carbonate syntheses.

Reaction between Alcohols and Urea. Reaction of alcohols with urea at elevated temperatures releases ammonia and produces carbamates as the initially formed reaction products (entry 4, Figure 8.59). The reaction is catalyzed by various metal salts. The intermediate carbamates, which can be isolated in some instances, undergo further reaction to give carbonates in good yields.

Reaction between Epoxides and Carbon Dioxide. Carbon dioxide (CO_2), a low cost, readily available renewable resource, is an attractive reagent for carbonate preparation. However, its direct use is limited because of its low reactivity. Recently, reaction of epoxides with carbon dioxide under pressure in the presence of various Lewis acids and transition-metal catalysts to give five-membered cyclic carbonates has garnered much interest (entry 5, Figure 8.59). This reaction is successfully employed to convert ethylene oxide into ethylene carbonate. Although successful for ethylene carbonate production, work utilizing fats and oils is limited (Doll and Erhan, 2005a). Technical challenges remain to make more efficient catalytic systems and to lower reaction temperatures and pressures.

Reaction between Alkylammonium Hydrogen Carbonates and Halohydrins. Bubbling CO_2 through an alkylammonium hydroxide (R_4N^+OH) solution gives alkylammonium hydrogen carbonate salts ($R_4NHCO^-_3$), an activated form of carbon dioxide, which can be utilized to prepare five-membered cyclic carbonate compounds when reacted with aliphatic fluorinated iodohydrins, aliphatic monosubstituted epoxides, and halohydrins (entry 6, Figure 8.59). Recently, Kenar and Tevis (2004) have prepared fatty acid esters, such as that containing the 5-membered cyclic carbonate group, at the 9,10-position of the alkyl chain (entry 6, Figure 8.59; $R=(CH_2)_7COOR$, $R'=(CH_2)_7CH_3$) in good yields (84 to 90%) under mild conditions from the corresponding 9,10- chlorohydrins using this chemistry. A more direct method recently reported is to eliminate the preparation of halohydrins and directly convert an epoxide group into a 5-membered cyclic carbonate group utilizing tetra-alkylammonium bromides and CO_2 (Doll and Erhan, 2005, 2005a; Tamami et al., 2004).

8.17.2 Carbonate Properties

Aliphatic carbonates are miscible in organic solvents but insoluble in water, except dimethyl and diethyl carbonate, which are sparingly soluble in water. The symmetrical linear carbonates derived from C_9 and lower alcohols are clear colorless liquids with increasing viscosity as chain length increases, whereas the C_{10} and higher alcohol derived carbonates are low melting solids. Introducing unsaturation into the aliphatic carbonate chains lowers the melting point. For example, the melting points of dioctadecyl carbonate and dioleyl carbonate, its unsaturated analog, are 56 to 57°C and 0°C, respectively (Dierker, 2004; Kenar, 2004).

The introduction of branching on the carbonate's alkyl chain effectively lowers the melting point. The branched Guerbet carbonates, 2-ethylhexyl, 2-butyloctyl, and 2-hexyldecyl carbonate all have melting points below −50°C while dioctyl (m.p. −18°C), didodecyl (m.p. 21°C), and dihexadecyl carbonate (m.p. 46 to 47°C), the corresponding linear carbonates of the same molecular formula, melt at much higher temperatures (Kenar et al., 2003).

Commercially available glycerol carbonate, the five membered cyclic carbonate derived from glycerol, can be prepared by carbonate interchange, phosgenation, or reaction with urea, as outlined above. The clear nonvolatile material is liquid below −70°C and has a flash point greater than 204°C. It has characteristics of a polar protic solvent, is more polar than water, and is highly soluble in water. The primary hydroxyl group is reported to be reactive and can react with anhydrides or isocyanates to form ester and urethane linkages, respectively (Mouloungui and Pelet, 2001).

The carbonate moiety is relatively stable to hydrolysis under acidic conditions, but is susceptible to hydrolysis in basic media. Upon hydrolysis, the carbonate gives an alcohol and carbon dioxide without carboxylic acid formation. Hydrolysis becomes increasingly difficult as the chain length and branching of the carbonate's aliphatic functionality increases. Glycerol carbonate is slightly less stable toward hydrolysis than the linear aliphatic carbonates.

8.17.3 Carbonate Applications

The polar nature of the carbonate moiety should enable it to adhere to metal surfaces. Coupled with low pour points, good seal compatibility, good miscibility with hydrocarbon oils, and high thermal-oxidative stability the carbonates are reported to possess tribological properties comparable to or better than mineral oil and traditional synthetic esters with similar viscosities (Fisicaro and Gerbaz, 1993).

More recently, the oily properties of C_8-C_{18} oleochemical carbonates have been exploited as components for personal care products, such as sunscreens, hair care, and cosmetics preparations. Dioctyl carbonate is currently being used as a new type of emollient because of its excellent skin feel and compatibility (Dierker, 2004). It is a good solvent for solid UV filters and can be readily emulsified. This combination of properties makes it unique compared to other commonly used emollients.

An oleochemical-based polycarbonate prepared from dimer-diol, a 36-carbon oleochemical made from the reduction of dimerized fatty acids, has been reported recently (Grützmacher and Westfechtel, 1997). Transesterification of the two alcohol groups present in the dimer-diol with dimethyl carbonate gives an oligomeric structure cross linked by the carbonate functionality. The properties of the oleochemical-based polycarbonate can be tailored by control of the molecular weight and molecular weight distribution. Applications for this interesting

material include the personal care sector and as a building block for polyurethanes (Grützmacher and Westfechtel, 2000).

References

Ansmann, A. et al. Wax based compositions for the preparation of cosmetic tissues and wipes, WO Patent 2003005981, 2003.

Buysch, H.-J., Carbonic Esters, in *Ullmann's Encyclopedia of Industrial Chemistry,* 6th ed., Wiley-VCH, Weinheim, Germany, 2003, pp. 427–455.

Delledonne, D. et al. Developments in the production and application of dimethylcarbonate, *Appl. Catal. A: Gen.*, **221**, 241–251, 2001.

Dierker, M., Oleochemical carbonates—an overview, *Lipid Tech.,* **16**, 130–134, 2004.

Doll, K.M. and Erhan, S.Z., Synthesis of carbonated fatty methyl esters using supercritical carbon dioxide, *J. Agric. Food Chem.*, **53**, 9608–9614, 2005a.

Doll, K.M. and Erhan, S.Z., The improved synthesis of carbonated soybean oil using supercritical carbon dioxide at a reduced reaction time, *Green Chem,*, **7**, 393–395, 2005b.

Fisicaro, G. and Gerbaz G., Dialkylcarbonates, in *Synthetic Lubricants and High-Performance Functional* Fluids, Shubkin, R.L., Ed., Marcel Dekker, New York, 1993, pp. 229–239.

Giolito, S.L. et al. Esters, carbonate ester compositions, and plasticized compositions, U.S. Patent 4,403,056, 1983.

Grützmacher, R. and Westfechtel, A., Herstellung und verwendung hochviskoser Oligocarbonate aus Dimerdiol, German Patent DE 195245406, 1997.

Grützmacher, R. and Westfechtel, A., Verfahren zur herstellung von verbindungen mit terminalen OH-gruppen, German Patent DE 19829593A1, 2000.

Gryglewicz, S. et al. Synthesis of modern synthetic oils based on dialkyl carbonates, *Ind. Eng. Chem. Res.,* **42**, 5007–5010, 2003.

Kenar, J. A., Current perspectives on oleochemical carbonates, *Inform*, **15**, 580–582, 2004.

Kenar, J. A. et al. Synthesis and characterization of dialkyl carbonates prepared from mid-, long-chain, and guerbet alcohols, *J. Am. Oil Chem.,* **81**, 285–291, 2003.

Kenar, J.A. and Tevis, I.D., Convenient preparation of fatty ester cyclic carbonates, *Eur. J. Lipid Sci. Technol.*, **107**, 135–137, 2005.

Mouloungui, Z. and Pelet, S., Study of the acyl transfer reaction: structure and properties of glycerol carbonate esters, *Eur. J. Lipid Sci. Technol.,* **103**, 216–222, 2001.

Parrish, J.P. et al. Perspectives on alkyl carbonates in organic synthesis, *Tetrahedron,* **56**, 8207–8237, 2000.

Pioch, D. et al. First lipase-catalyzed synthesis of fatty carbonate esters, *Biotech. Lett.,* **13**, 633–636, 1991.

Rüsch gen Klaas, M. and Warwel, S., Reactive extraction of oilseeds with dialkyl carbonates, *Eur. J. Lipid Sci. Technol.,* **103,** 810–814, 2001.

Shaikh, A.-A. and Sivaram, S., Organic carbonates. *Chem. Rev.,* **96**, 951–976, 1996.

Suetake, T., Oily solid cosmetics containing dialkyl carbonates, Japanese Patent 20011328918, 2001.

Tamami, B. et al. Incorporation of carbon dioxide into soybean oil and subsequent preparation and studies of nonisocyanate polyurethane networks, *J. Appl. Polym. Sci*, **92**, 883–891, 2004.

Tundo, P. and Selva, M., The chemistry of dimethyl carbonate**,** *Acc. Chem. Res.,* **35**, 706–716, 2002.

Westfechtel, A. et al. Guerbet carbonates, U.S. Patent 5,387,374, 1995.

8.18 Guerbet compounds

The Guerbet reaction is named after Marcel Guerbet (1899), who published extensively on the reaction that bears his name. However, it appears that Markownikov (Markownikoff, 1901) discovered the reaction prior to Guerbet, according to a statement in one of Markownikov's publications.

In summary, the Guerbet reaction is a dimerization of alcohols with liberation of water to give branched alcohols with twice the number of carbons as the starting material (see scheme in Figure 8.60). The "typical" Guerbet alcohols have an even number of carbons with a minimum of six carbon atoms. The number of carbons in the main chain is always greater by four than that of the side chain. The Guerbet reaction of $C_{16\text{-}18}$ fatty alcohols was described in publications dating from the 1950s (Sulzbacher, 1955; Gast et al., 1958). A list of Guerbet compounds with their properties has been compiled (Knothe, 2002).

The Guerbet reaction occurs in the presence of an alkaline catalyst, such as potassium hydroxide, etc. Usually transition metal-containing (Ni, Pd, Cu, Rh, Ir, etc.) components are added to the reaction system to catalyze hydrogenation and can also lead to milder reaction conditions as the reaction is usually conducted at elevated temperature under pressure. Figure 8.60 shows the reaction mechanism. Research has shown that the reaction occurs in several steps (Veibel and Nielsen, 1967; Burk et al., 1985a,b): (1) dehydrogenation of the alcohol that can also be catalyzed by the transition metal; (2) aldol condensation of the carbonyl compounds formed in the first step, followed by liberation of water; (3) hydrogenation of the α, β-unsaturated ketone formed in the previous step with the alcohol being the hydrogen donor; and (4)

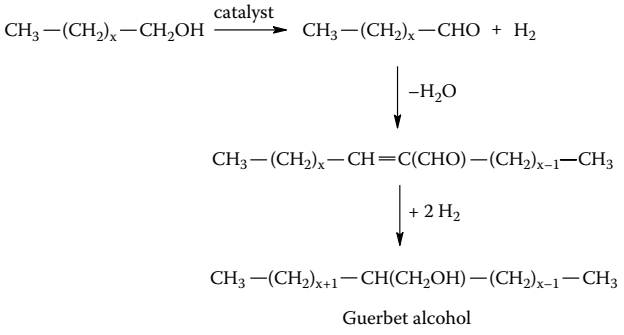


FIGURE 8.60

disproportionation of aldehyde to alcohol and acid, which terminates the chain reaction initiated in the previous step. More details on the mechanism are given in the literature (Veibel and Nielsen, 1967; Burk et al., 1985a). The Guerbet reaction can be applied on a commercial scale, therefore, and due to their interesting properties, Guerbet compounds are of significant commercial interest.

Other Guerbet Compounds. Guerbet alcohols themselves can be used to prepare other derivatives. A straightforward example is Guerbet acids obtained by oxidation of Guerbet alcohols, for example, using Pd, Pt, or Ru catalysts (Behr and Döring, 1992). Guerbet alcohols also can be esterified with a variety of carboxylic acids, such as fatty acids, or by Guerbet acids, the latter being termed di-Guerbet esters (Knothe and Carlson, 1998). Carbonates synthesized from Guerbet alcohols (Kenar et al., 2004, 2005) and esters of fatty and dicarboxylic acids with Guerbet alcohols (Knothe, 2001) have also been reported. The patent literature provides information on various derivatives of Guerbet compounds, including alkoxylated Guerbet alcohols and esters, Guerbet amines, betaines, branched amine oxides, carbonates, esters of meadowfoam oil and ricinoleic acid (castor oil), fluorinated citrate esters, lactams, polyoxyalkylene glycol esters, sorbitan esters, etc. A list of some patents in this area through 2001 is provided in the literature (O'Lenick, 2001).

Alcohols other than the typical ones shown in Figure 8.60 can be obtained from a Guerbet reaction. Secondary alcohols have been condensed under Guerbet conditions (Burk et al., 1985b). Several cross-condensations of mixtures of alcohols in Guerbet reactions have also been reported. These include (1) reaction of methanol with 1-butanol or 1-pentanol in presence of a rhodium catalyst (Burk et al., 1985b); (2) synthesis of 1-propanol and 2-methyl-1-propanol from ethanol and methanol using magnesium oxide (Ueda et al., 1990); and (3) synthesis of *iso*-butanol from methanol and *n*-propanol using various metal-based catalysts (Carlini et al., 2002, 2003a/b, 2004, 2005) and a related system for the condensation of methanol and ethanol or methanol, ethanol, and *n*-propanol (Carlini et al., 2003c). Reactions of fatty alcohols of chain length C_8-C_{14} and benzyl alcohol yielded 2-benzyl fatty alcohols (Krause and Syldatk, 1985).

Other classes of compounds can be subjected to reactions under Guerbet conditions. The reaction of amines under Guerbet conditions gave imines as major product (Miller, 1960).

8.18.1 Properties and Applications

Their wide liquidity range documented by melting points (especially) and boiling points is one reason why Guerbet compounds have significant practical applications. For example, 1-octanol has a melting point of −16.7°C and a boiling point of 194.4°C. The Guerbet alcohol with the same number of carbon atoms, 2-ethylhexanol, has a melting point of −70°C and a boiling point of 184.6°C.

Other properties that make Guerbet compounds of interest are good lubricity, good oxidative stability since they are saturated compounds, and volatility comparable to the straight-chain isomers (the boiling points are only slightly reduced compared to the straight-chain congeners; see the above example).

Numerous patents describe the uses of Guerbet compounds and derivatives. The list given by O'Lenick (2001) also provides related information. The applications for C_{12-36} Guerbet alcohols may be summarized as components of cosmetics and lubricants as well as solvents or solubilizers for printing colors and inks and starting materials for other materials with other applications including surfactants. Higher Guerbet alcohols (C_{32-36}) are useful for the production of specialty waxes and cosmetic uses. Related materials, such as Guerbet acids, or esters of other acids with Guerbet alcohols are also of commercial interest. They have similar applications. The benzyl fatty alcohols are useful as surfactants as are Guerbet sulfates and ether sulfates (O'Lenick, 2001).

2-Ethylhexanol. The derivatization of a Guerbet compound with other compounds and the resulting expansion of utility is demonstrated by what is probably the best-known representative of the typical Guerbet alcohols, 2-ethylhexanol. With a production volume of about 2×10^6 t/a a few years ago, it is the most important industrial alcohol after the lighter C_{1-4} alcohols (Bahrmann et al., 2001). However, for economic reasons, it is now produced industrially not from butanol but from butanal, which in turn is derived from propene (Bahrmann et al., 2001). The main use (>60%) of 2-ethylhexanol is to serve in the production of plasticizers, such as diethylhexyl phthalate (DEHP; dioctyl phthalate) and diethylhexyl adipate (Bahrmann et al., 2001). Other uses of 2-ethylhexanol include the production of 2-ethylhexyl acrylate, which in turn is used in coating materials, adhesives, and inks, 2-ethylhexylnitrate used as cetane improver additives for diesel fuel, and 2-ethylhexylphosphates as an additive for lubricating oils (Bahrmann et al. 2001).

An alternative synthesis of the corresponding acid, 2-ethylhexanoic acid, is achieved by hydrogenating the δ-lactone (depicted in Figure 8.61) in presence of a water-soluble rhodium-phosphine catalyst (Behr and Brehme 2002). The δ–lactone is obtained from a reaction of butadiene with carbon dioxide.

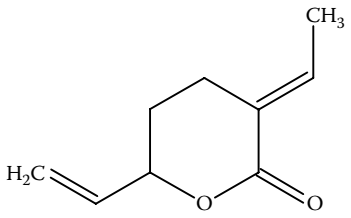


FIGURE 8.61

References

Bahrmann, H. et al. 2-Ethylhexanol, in *Ullmann's Encyclopedia of Industrial Chemistry*, 6th ed., Wiley-VCH, Weinheim, Germany; online version, 2001.

Behr, A. and Brehme, V.A., Homogeneous and heterogeneous catalyzed three-step synthesis of 2-ethylheptanoic acid from carbon dioxide, butadiene and hydrogen, *J. Molec. Catal. A,* **187**, 69–80, 2002.

Behr, A. and Döring, N., Synthesis of branched fatty acids by catalytic oxidation of alcohols. (Herstellung verzweigter Fettsäuren durch katalytische Oxidation von Alkoholen.), *Fat Sci. Technol.,* **94**, 13–18, 1992.

Burk, P.L. et al. The rhodium-promoted Guerbet reaction. Part I. Higher alcohols from lower alcohols, *J. Mol. Catal*., **33**, 1–14, 1985a.

Burk, P.L. et al. The rhodium-promoted Guerbet reaction. Part II. Secondary alcohols and methanol as substrates, *J. Mol. Catal.,* **33**, 15–21, 1985b.

Carlini, C. et al. Selective synthesis of isobutanol by means of the Guerbet reaction. Part 1. Methanol/*n*-propanol condensation by using copper based catalytic systems, *J. Mol. Catal. A: Chem.*, **184**, 273–280, 2002.

Carlini, C. et al. Selective synthesis of isobutanol by means of the Guerbet reaction. Part 3. Methanol/*n*-propanol condensation by using bifunctional catalytic systems based on nickel, rhodium, and ruthenium species with basic components, *J. Molec. Catal. A: Chem.,* **206**, 409–418, 2003a.

Carlini, C. et al. Selective synthesis of isobutanol by means of the guerbet condensation of methanol with *n*-propanol in the presence of heterogeneous and homogeneous palladium-based catalytic systems, *J. Molec. Catal. A: Chem.,* 204–205, 721–728, 2003b.

Carlini, C. et al. Selective synthesis of isobutanol by means of the Guerbet reaction. Part 2. Reaction of methanol/ethanol and methanol/ethanol/*n*-propanol mixtures over copper based / MeONa catalytic systems, *J. Molec. Catal. A: Chem.*, **200**, 137–146, 2003c.

Carlini, C. et al. Guerbet condensation of methanol with *n*-propanol to isobutyl alcohol over heterogeneous copper chromite/Mg-Al mixed oxides catalysts, *J. Molec. Catal. A: Chem.,* **220**, 215–220, 2004.

Carlini, C. et al. Guerbet condensation of methanol with *n*-propanol to isobutyl alcohol over heterogeneous bifunctional catalysts based on Mg-Al mixed oxides partially substituted by different metal components, *J. Molec. Catal. A: Chem.,* **232**, 13–20, 2005.

Gast, L.E. et al. Reactions of unsaturated fatty alcohols. VI. Guerbet reaction of soybean and linseed alcohols, *J. Am. Oil Chem. Soc.*, **35**, 703–707, 1958.

Guerbet, M., Action de l'alcool amylique de fermentation sur dérivé sodé, *Comptes rendus,* **128**, 511–513, 1899.

Kenar, J.A. et al. Synthesis and characterization of dialkyl carbonates prepared from mid-, long-chain, and Guerbet alcohols, *J. Am. Oil Chem. Soc.,* **81**, 285–291, 2004.

Kenar, J.A. et al. Physical properties of oleochemical carbonates, *J. Am. Oil Chem. Soc*., **82**, 201–205, 2005.

Knothe, G. and Carlson, K.D., Synthesis, mass spectrometry, and nuclear magnetic resonance characterization of di-Guerbet esters, *J. Am. Oil Chem. Soc.,* **75**, 1861–1866, 1998.

Knothe, G., Characterization of esters of fatty acids and dicarboxylic acids with Guerbet alcohols, *J. Am. Oil Chem. Soc.,* **78**, 537–540, 2001.

Knothe, G., Synthesis, applications, and characterization of Guerbet compounds and their derivatives, *Lipid Technol.,* **14**, 101–104, 2002.

Krause, H.-J. and Syldatk, A., Neue Tenside aus gemischten Guerbet-Alkoholen, *Fette, Seifen, Anstrichm.,* **87**, 386–390, 1985.

Markownikoff, W. and Zuboff, P., Ueber die Condensation höherer Alkohole: Tricaprylalkohol, *Chem. Ber.,* **34**, 3246–3249, 1901.

Miller, R.E., The Guerbet reaction. I. The reaction of amines under guerbet conditions, *J. Org. Chem.*, **25**, 2126–2128, 1960.

O'Lenick, A.J., Guerbet chemistry, *J. Surfact. Deterg.,* **4**, 311–315, 2001.

Sulzbacher, M., The Guerbet reaction of cetyl alcohol, *J. Appl. Chem.,* **5**, 637–641, 1955.

Ueda, W. et al. A low-pressure Guerbet reaction over magnesium oxide catalyst, *J. Chem. Soc., Chem. Commun.*, 1558–1559, 1990.

Veibel, S. and Nielsen, J.I., On the mechanism of the Guerbet reaction, *Tetrahedron* **23**, 1723–1733, 1967.

9

NONFOOD USES OF OILS AND FATS

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9.1 Introduction

Annual production of commodity oils and fats is now (2004) around 130 million tonnes and the general consensus is that ~ 80% is used for human food, ~ 6% for animal feed, and ~ 14% for the oleochemical industry though these ratios may change through the increasing use of oils and fats for oleochemical purposes, especially biodiesel. This chapter is devoted to the major uses of that last 14% (18 million tonnes). Before mineral oil and gas were used to supply many of our needs, oils and fats were widely used as illuminants and lubricants. Illumination was provided by a wick burning in olive oil in Mediterranean countries in biblical times or in the early days of the railways using rapeseed oil (then a high-erucic variety known as colza oil). At one time, lighthouses used seal oil or whale oil in their lamps. In a book written by T. P. Hilditch in 1927, an entire chapter was devoted to "the use of fats in candles and illuminants." There are suggestions that the axles of early chariots were greased with a mixture of animal fat and lime producing calcium soaps. More importantly, soap made from fat and alkali (wood ash) has been used for centuries. Other uses are based on oxidative drying (hardening) of oil films to form coatings and have been exploited by artists, decorators, and producers of linoleum. With the development of fossil fuels and the inventiveness of chemists in the petrochemical industry, oils and fats ceased to be used as illuminants or as lubricants.

Today there is a limited return to the use of oils and fats for some of these purposes, mainly on environmental grounds. Oil and gas supplies are not known with certainty, but they are finite and will not last indefinitely. Oils and fats can sometimes be used in place of fossil fuels and are even being burnt in electrical power stations to reduce the levels of burdensome stocks, to raise prices by removal of excess oil, or to meet targets for the reduction of carbon dioxide production. In contrast to fossil fuels, oils and fats represent a renewable resource produced by agricultural systems from carbon dioxide and water with sunlight providing the necessary energy. But some numbers should be applied to this issue to maintain a sense of perspective. Annual production of mineral oil, at 3.5 billion tonnes, is almost 30 times that of oils and fats, and the greater part of these latter are essential for food purposes. For example, biodiesel can never replace the demand for conventional fossil fuel — it can only diminish it marginally (Dumelin, 2005). Other environmental reasons are based on the fact that oils and fats are biodegraded more quickly than petrochemical products and, therefore, disappear more easily from the environment when used or spilled. Finally, when fully degraded, these materials liberate carbon dioxide trapped only months earlier and, therefore, do not add to total carbon dioxide, one of the greenhouse gases responsible for global warming. This contrasts with petrochemical products, which are oxidised to carbon dioxide trapped millennia earlier.

Environmental issues are more complex than is generally appreciated. It must not be forgotten that the growing, harvesting, and transporting of oilseeds and their products are achieved only at some environmental cost. Hirsinger (2001) has reviewed the relation between oleochemicals and the environment and Urata et al. (2001) have described the contribution of surfactants and lipids to "Green Chemistry." Yanagawa (2001) has discussed sustainable growth of the Asian-Pacific surfactant and detergent industries and Williams (2005) has reported on European detergent rules.

The use of oils and fats as oleochemicals depends either on the physical properties of fatty acids and esters or on chemical properties relating to the carboxyl group, to olefinic centres, or to the whole molecule (as in its combustion).

Linseed and castor oil are classed as industrial oils. Other oils used for nonfood purposes include significant proportions of the lauric oils (coconut and palm kernel), palm oil and palm stearin, tallow, rapeseed oil (both the high-erucic and the low-erucic oil), and lesser amounts of soybean and other oils. This listing may change when larger volumes of biodiesel are prepared. USDA figures for 2003/04 show the proportion of the commodity food oils used for industrial purposes in EU-15 as rapeseed oil (39%), palm kernel oil (29%), coconut oil (17%), palm oil (10%), soybean oil (7%), sunflower oil (6%), with olive oil, groundnut oil, and cottonseed oil at 1% or less. In the U.S. about 3% of total usage of soybean oil is for industrial purposes. Another important oleochemical feedstock is tall oil (a name based on *tallolja*, the Swedish word for pine oil). Tall oil fatty acids are by-products of the wood pulp industry and result when pine wood chips are digested, under pressure, with an aqueous mixture of sodium hydroxide and sodium sulfide during which the acids are converted to their sodium salts. Tall oil is the cheapest source of fatty acids rich in oleic and linoleic acids.

In volume terms, surface-active compounds dominate among oleochemicals — a proportion of 90% has been reported, but this share is falling with increasing use as a fuel or by production of biodiesel. Surfactant molecules are mainly saturated or monounsaturated and find different uses according to their chain length. Three categories are recognised: C_{12} and C_{14} compounds from the lauric oils competing with identical products from the petrochemical industry, C_{16} and C_{18} compounds mainly from tallow and palm stearin, and C_{20} and C_{22} compounds from fish oils, high-erucic rapeseed oil, and crambe oil. These compounds may be used as acids or salts (soaps), as esters of methanol, other alkanols, or glycerol, as long-chain alcohols, or as nitrogen-containing compounds. Other oleochemical uses exploit the high unsaturation of oils, such as linseed and soybean oil, while castor oil is a unique source of several important chemicals.

Several aspects of this topic have been covered in the sixth edition of Bailey's Industrial Oil and Fat Products with chapters on soaps (Burke, 2005), detergents and detergency (Lynn, 2005), glycerine (Schroeder, 2005), biodiesel (Reaney et al., 2005), lubricants, hydraulic fluids, and inks (Erhan, 2005), polymers and plastics (Narine and Kong, 2005), paints (Lin, 2005), leather and textiles (Kronich and Kamath, 2005), and pharmaceutical and cosmetic uses (Hernandez, 2005). Hauthal and Wagner (2004) have reviewed household cleaning, care, and maintenance products.

References

Burke, M.R., Soaps, in *Bailey's Industrial Oil and Fat Products,* 6th ed., Shahidi, F., Ed., Wiley Interscience, New York, 2005, Chap. 3.

Dumelin, E.E., Biodiesel — a blessing in disguise? *Eur. J. Lipid Sci. Technol.,* **107**, 63–64, 2005.

Erhan, S.Z., Vegetable oils as lubricants, hydraulic fluids, and inks, in *Bailey's Industrial Oil and Fat Products,* 6th ed., Shahidi, F., Ed., Wiley Interscience, New York, 2005, Chap. 7.

Hauthal, H.G. and Wagner, G. Eds., *Household Cleansing, Care, and Maintenance Products*, Ziolkowsky GmbH publishing, Augsberg, Germany, 2004. Translation of the original German volume published in 2003.

Hernandez, E., Pharmaceutical and cosmetic use of lipids, in *Bailey's Industrial Oil and Fat Products,* 6th ed., Shahidi, F., Ed., Wiley Interscience, New York, 2005, Chap. 12.

Hilditch, T.P., *The Industrial Chemistry of Fats and Waxes,* Baillière, Tindall and Cox, London, 1927.

Hirsinger, F., Oleochemicals and the environment, in *Oleochemical Manufacture and Applications,* Gunstone, F.D. and Hamilton, R.J., Eds., Sheffield Academic Press, Sheffield, U.K., 2001, Chap 10.

Kronick, P. and Kamath, Y.K., Leather and textile uses of fats and oils, in *Bailey's Industrial Oil and Fat Products,* 6th ed., Shahidi, F., Ed., Wiley Interscience, New York, 2005, Chap. 10.

Lin, K.F., Paints, varnishes, and related products, in *Bailey's Industrial Oil and Fat Products,* 6th ed., Shahidi, F., Ed., Wiley Interscience, New York, 2005, Chap. 9.

Lynn, Jr., J.L., Detergents and detergency, in *Bailey's Industrial Oil and Fat Products,* 6th ed., Shahidi, F., Ed., Wiley Interscience, New York, 2005, Chap. 4.

Narine, S.S. and Kong Xiaohua, Vegetable oils in production of polymers and plastics, in *Bailey's Industrial Oil and Fat Products,* 6th ed., Shahidi, F., Ed., Wiley Interscience, New York, 2005, Chap. 8.

Reaney, M.J.T., Vegetable oils as biodiesel, in *Bailey's Industrial Oil and Fat Products,* 6th ed., Shahidi, F., Ed., Wiley Interscience, New York, 2005, Chap. 6.

Schroeder, K., Glycerine, in *Bailey's Industrial Oil and Fat Products,* 6th ed., Shahidi, F., Ed., Wiley Interscience, New York, 2005, Chap. 5.

Urata, K. and Takaishi, N., A perspective on the contribution of surfactants and lipids toward "Green Chemistry:" present status and future potential, *J. Surfact. Deterg.,* **4**, 191–200, 2001.

Williams, M., Europe tightens detergent rules, *Oils Fats Int.*, **21**(2), 20–21, 2005.

Yanagawa, Y., Perspectives on the sustainable growth of Asian-Pacific surfactant and detergent industries towards the new millennium, *J. Oleo Sci.,* **50**, 281–393, 2001.

9.2 Basic oleochemicals

The basic oleochemicals are fatty acids, methyl esters, alcohols, amines, and glycerol. Traditionally these have been produced mainly in North America, Western Europe, and Japan from local or imported oils and fats. But this is changing, and countries in South East Asia, particularly Malaysia, have become major producers of

basic oleochemicals using their increasing indigenous supplies of raw material. This is shown in Table 9.1 containing projected figures up to the year 2010. Over the 10-year period, the production of oleochemicals is expected to rise by one-third from 5.76 to 7.75 million tonnes. Although this rise is apparent in all regions, market share will fall in North America and Western Europe, but rise in Asia. It is predicted that by 2010 Asian production will equal production in Western Europe and North America combined. This statement refers particularly to Malaysia and possibly underestimates the contribution likely to be made by China by 2010 (MPOB, 2005).

The materials used in the oleochemical industry and the processes by which oleochemicals are produced are summarised in Table 9.2 and Table 9.3. It is interesting to note in Table 9.3 the wide range of products that contain oleochemicals.

TABLE 9.1 Estimates for 2000, 2005, and 2010 of basic oleochemicals (million tonnes) by region and by commodity (percentage figures are given in parentheses)

	2000	2005	2010
World total	5.76	6.69	7.75
By region			
Western Europe	1.76 (31%)	1.87 (28%)	1.96 (25%)
North America	1.36 (24%)	1.52 (23%)	1.66 (21%)
Asia	2.27 (39%)	2.79 (42%)	3.54 (46%)
Other	0.37 (6%)	0.51 (7%)	0.59 (8%)
By commodity			
Fatty acids	3.05	3.50	4.00
Methyl esters	0.66	0.73	0.80
Alcohols	1.44	1.73	2.07
Amines	0.57	0.62	0.70
Glycerol	0.75	0.86	1.00

Note: For more recent data on glycerol see Tables 9.4 and Table 9.5 and MPOB (2005).
Source: Adapted from Gunstone, F.D., *The Chemistry of Oils and Fats*, Blackwell Publishing, Oxford, 2004.

TABLE 9.2 Basic oleochemicals and downstream products produced from triacylglycerols

Basic Oleochemicals	Downstream Derivatives
Fatty acids	Esters, metal salts (soaps), amides and amines, ketene dimers, anhydrides, acid chlorides, peroxy acids, and esters
Methyl esters	Acids, other esters, alcohols, α-sulfonates
Alcohols	Ethylene oxide adducts, sulfates, Guerbet alcohols and acids
Amines	Various cationic surfactants – Section 9.3.6
Glycerol	Polyglycerol, mono- and diacylglycerols and their acetates, tartates, lactates, etc.

Note: Some of the compounds are not discussed in this chapter, but are described in Chapter 7, which is devoted to physical properties.
Source: Adapted from Gunstone, F.D., *The Chemistry of Oils and Fats*, Blackwell Publishing, Oxford, 2004.

TABLE 9.3 Materials, processes, and products of the oleochemical industry

Raw materials	Tall oil, lauric oils, palm oil, high- and low-erucic rapeseed oil, soybean oil, sunflower oil
Unit operations to produce basic oleochemicals	Splitting, distillation, fractionation, hydrogenation, methylation, hydrophilisation
Basic oleochemicals	Fatty acids, methyl esters, fatty alcohols, fatty amines, glycerol
Operations to produce downstream products from the basic oleochemicals	Amidation, dimerisation, epoxidation, ethoxylation, quaternisation, sulfation, sulfonation, saponification, transesterification
Oleochemical derivatives	Amides, dimer and trimer acids, epoxidised oils and esters, ethoxylates and propoxylates, sulfates, sulfonates, esters, soaps, salts
End-use markets	Building auxiliaries, candles, cleaning agents, cosmetics, detergents, flotation agents, food emulsifiers, inks, insecticides, leather treatment, lubricants, paints, pesticides, pharmaceuticals, plastics, soaps, textiles, tyres

Source: Adapted from Gunstone, F.D., *The Chemistry of Oils and Fats*, Blackwell Publishing, Oxford, 2004.

9.2.1 Fatty acids

Fat hydrolysis gives fatty acids and glycerol. The former are used in large quantities to make soaps and also as intermediates to produce methyl esters, amides, amines, and other important nitrogen-containing compounds, acid chlorides, anhydrides, ketene dimers, and peroxy acids and esters (Table 9.2).

As there is often confusion about the weight relationships between fats and fatty acids, it is worth noting that hydrolysis of glycerol trioleate (100 g) involves reaction with water (6.1 g) to produce oleic acid (95.7 g) and glycerol (10.4 g). The contribution of water is often overlooked and it is erroneously argued that because the reaction produces 10 g of glycerol there will only be 90 g of fatty acids. The theoretical yield of free acid is close to 96%.

The conversion of oils and fats to soaps (saponification) is carried out by a long-established process involving treatment with aqueous alkali at around 100ºC. Glycerol is obtained as a valuable by-product. The sodium and potassium salts are conventional soaps. Salts with other metals are used to promote polymerisation of drying oils, as components of greases and lubricants, and are incorporated into animal feeds for ruminants.

Fats can also be hydrolysed by water itself in a fat-splitting process to yield free acids. This is probably a

homogeneous reaction between fat and a small amount of water dissolved in the fat. The procedure is usually carried out in a continuous, high pressure (20 to 60 bar), uncatalysed, counter-current process at 250ºC, though lower temperatures are desirable for highly unsaturated oils. Under these vigorous conditions, both the fatty acids and the glycerol will be discoloured and may have to be distilled. Splitting capacity in Malaysia has risen from 0.68 million tonnes in 1996 to 1.77 million tonnes in 2004 (Lim, 2005). This is in addition to the production of 0.47 million tonnes of soap noodles. Further information is found in Malaysian Oil Palm Statistics (MPOB, 2005).

Toilet soaps are made from tallow (mainly in North and South America) or from vegetable oil (mainly palm oil) elsewhere in the world. The traditional process involves saponification (alkaline hydrolysis of vegetable oils or animal fats), but soaps are made increasingly by neutralisation of distilled fatty acids. There is a growing trade in soap noodles (sodium salts of fatty acids) that are converted elsewhere to coloured, scented, wrapped bars of toilet soap. This product is now in competition with liquid soaps (Table 9.7).

Hydrolysis promoted by lipases, such as those from *Rhizomucor miehei* and *Candida rugosa,* takes about 20 hours at 20ºC or 6 hours at 45ºC, but gives cleaner products with less waste than the fat splitting process. Despite this and the saving in energy costs, it is not yet widely used on an industrial scale.

9.2.2 Fatty esters

Esters can be made by esterification of acids by reaction with alcohols or from existing esters, including triacylglycerols, by reaction with alcohols (a process known as alcoholysis), acids (acidolysis), or other esters (interesterification).

Alcoholysis is more widely practised than acidolysis and includes the important reactions of esters (especially triacylglycerols) with methanol (methanolysis) or with glycerol (glycerolysis).

On an industrial scale ester production of methyl esters is most commonly undertaken by methanolysis of triacylglycerols (natural oils and fats) in the presence of an acidic, alkaline, or enzymatic catalyst. Large-scale methanolysis is used to make methyl esters for use as biofuel, as solvent, or as an intermediate in the production of alcohols. They can also be hydrolysed to acids.

Oils low in free acid can be converted directly to methyl esters with an alkaline catalyst. Glycerol is also produced in this reaction and is recovered as a second marketable product. In a continuous process for the conversion of vegetable oils to methyl esters, conversion is >98% using sodium hydroxide as the catalyst. Under optimum conditions the reaction requires 6 to 8 minutes and may even take place during passage through the reaction plant. Claims have been made for the use of calcium carbonate as an environmentally acceptable catalyst (Suppes et al., 2001).

Just as hydrolysis can occur without a catalyst at an elevated temperature, methanolysis is also possible without a catalyst at temperatures up to 200ºC, but this process is not used on an industrial scale.

Candida antarctica lipase has been used to promote the methylation of waste fatty acids. Conversions of around 95% are achieved at 30ºC in 24 hours and under appropriate conditions the lipase maintains it activity through 45 cycles. Enzymatic methanolysis has also been examined with a range of vegetable oils and waste edible oil using a fixed bed reactor.

Glycerolysis is another alcoholysis process employed on a commercial scale to convert triacylglycerols to mixtures of monoacylglycerols and diacylglycerols by reaction with glycerol in the presence of a basic catalyst when the following equilibrium is established:

$$\text{triacylglycerol} + \text{glycerol} \rightleftharpoons \text{monoacylglycerol} + \text{diacylglycerol}$$

The composition of the product mix depends on the amount of glycerol dissolved in the fat phase. It can also be modified by the use of appropriate solvents. Concentrates of monoacylglycerol (90 to 95%), produced by molecular distillation, are widely used as emulsifiers. A similar process has been described using enzymes as catalysts, but this has not been used on an industrial scale.

9.2.3 Fatty alcohols

Long-chain alcohols (RCH_2OH) with structures similar to the better-known acids (RCOOH) occur naturally in the free state and, more commonly, as esters. These last include wax esters made from long-chain alcohols and long-chain acids and occur in jojoba and other vegetable and animal waxes. Many insect pheromones are fatty alcohol acetates.

Long-chain alcohols are important oleochemicals produced on a commercial scale by hydrogenolysis of acids, methyl esters, or triacylglycerols. This is a catalytic process and, depending on the choice of catalyst, olefinic double bonds may also be reduced or be left unchanged. These processes are generally applied to natural mixtures and the products, therefore, are mixtures varying in chain length. C_8 to C_{14} alcohols are produced from lauric oils (coconut and palm kernel), C_{16} and C_{18} compounds from tallow, lard, palm oil, or palm stearin, and C_{22} alcohols from erucic acid-rich oils. Individual alcohols can be obtained by fractional distillation of the mixed products. Dodecanol and similar alcohols are also produced by the petrochemical industry through oligomerisation of ethene (ethylene). Commercial production of long-chain alcohols is now around 2 million tonnes annually of which two-thirds or more is fat-based. About 75% of these fatty

alcohols are used as alcohol sulfates, alcohol ethoxylates, or alcohol ethoxylate sulfates (see Section 9.3.3 to Section 9.3.5).

Lim (2005) reports that since 2001 new production capacity for C_{12} to C_{18} alcohols has been 0.78 million tones, while capacity of only 0.24 million tonnes has been closed. An increase in the capacity to produce alcohols of 135,000 tonnes in Malaysia and 60,000 tonnes in Indonesia was reported for 2005. Malaysian exports of fatty alcohols rose from 288,000 tonnes in 2003 to 328,000 tonnes in 2004 (MPOB, 2005).

Although commodity oils and fats are the starting point for most of these processes, the glycerol esters themselves are not generally used directly since, among other reasons, the valuable glycerol would be lost. More usually, hydrogenolysis is carried out on acids, methyl esters, or on wax esters made *in situ* from acids and alcohols. A wide range of minor products may also be formed during this reaction, including esters, aldehydes, alkanes, ethers, and acetals.

In the methyl ester route, acid-free esters are first made from the natural oils by methanolysis (with release and recovery of glycerol) and then subjected to hydrogenolysis using pure hydrogen (>99.9%) and a copper chromite catalyst, usually in a fixed bed reactor, at 250 to 300 bar and 210°C. The volatile mixture of hydrogen and methanol can be separated and each component recycled. The alcohol product is stripped of methanol and the long-chain alcohols are used as such or are fractionated by distillation into individual components. This procedure can be adapted to produce olefinic alcohols with a copper-zinc catalyst free of chromium. Nickel catalysts activated with chromium, iron, or preferably rhodium can also be used for reactions at 200 to 230°C and 200 bar.

Arguments have been presented for the acid route using some new technologies. This involves: (1) conversion of oil to acids and fractionation of these, some of which may be sold as acids; (2) preparation of methyl esters from acids using a resin bed as catalyst; and (3) reduction of esters to alcohols using a fixed bed catalyst (40 bar, 200 to 250°C, chromium-free catalyst). The combined procedures can be used flexibly to produce acids, methyl esters, and alcohols as required.

In the wax ester route, the starting materials are fatty acids (distilled or fractionated) and some pre-made, long-chain alcohols. At an appropriate temperature the acids and alcohols react without a catalyst to produce wax esters. These and pure hydrogen are then passed to the fixed-bed hydrogenation reactor charged with catalyst where hydrogenolysis takes place. Thereafter, hydrogen is separated from the alcohols. Some of these are returned to make more wax ester and the balance is distilled. This procedure has the advantage that it is not necessary to use or to recover methanol.

These commercial processes are limited by the rates of hydrogen transfer between the gas, liquid, and solid phases. Laboratory scale reactions, conducted under supercritical conditions with propane, are 5 to 10 times quicker than the conventional reaction. A copper-based catalyst free of chromium at 150 bar and 240 to 250°C is employed (van den Hark et al., 1999).

9.2.4 Fatty amines

Fatty amines, produced at a level of around 500 kt per annum, are the starting point for several types of nitrogen-containing compounds used as surfactants. Acids are first converted to nitriles — probably via amides — by reaction with ammonia at 280 to 360°C in the presence of alumina, thoria, titanium oxide, zinc oxide, manganese acetate, bauxite, or cobalt salts as catalysts.

$$RCOOH \rightarrow RCONH_2 \rightarrow RCN \rightarrow RCH_2NH_2 \rightarrow [RCH_2]_2NH$$

Hydrogenation of nitrile occurs with nickel or cobalt as catalysts. Double bonds may be reduced at the same time, but conditions are usually selected to minimise this. Some conversion of *cis* to *trans* isomers may also occur. The major product is usually the primary amine (RCH_2NH_2), but this may be accompanied by secondary ($[RCH_2]_2NH$) and tertiary amines ($[RCH_2]_3N$). The formation of secondary and tertiary amines can be promoted by adjusting the reaction conditions. Aldimine (RCH = NH), the first product in the conversion of nitriles to primary amines, reacts with more hydrogen to form primary amine, with preformed primary amine to form secondary amine, or with preformed secondary amine to give tertiary amine.

$$RC \equiv N \rightarrow RCH = NH \rightarrow RCH_2NH_2$$
reaction with hydrogen

$$RC \equiv N \rightarrow RCH = NH \rightarrow [RCH_2]_2NH$$
reaction with primary amine

$$RC \equiv N \rightarrow RCH = NH \rightarrow [RCH_2]_3N$$
reaction with secondary amine

Tertiary amines with two methyl groups (RCH_2NMe_2) are made from primary amines by reductive alkylation with formaldehyde (methanal), from *N,N*-dimethylalkyl amides by catalytic reduction, or from fatty alcohols by catalysed reaction with dimethylamine.

$$RCH_2NH_2 + 2CH_2O \rightarrow RCH_2NMe_2$$

$$RCONMe_2 \rightarrow RCH_2NMe_2$$

$$RCH_2OH + Me_2NH \rightarrow RCH_2NMe_2$$

Still other nitrogen-containing, surface-active compounds may be made from carboxylic acids, alcohols, and amines (see Section 9.3.7 and Table 9.9 and Table 9.10).

9.2.5 Glycerol

Oils and fats are mainly triacylglycerols and are generally used for dietary consumption in this form. However, in the oleochemical industry, oils and fats are used mainly to manufacture acids, soaps, methyl esters, alcohols, or nitrogen-containing derivatives and the production of these compounds will almost always involve the liberation of glycerol (1,2,3-propanetriol) at a level of around 10% of the oil or fat. This is a useful and valuable by-product and its economic value is an important part of the profitability of the oleochemical industry.

Glycerol is also a product of the petrochemical industry where it is made from propene via epichlorohydrin (1-chloro-2,3-epoxypropane). The increasing supply of glycerol from the oleochemical industry, the high price of propene, and the demand for epichlorohydrin for other purposes have together made the petrochemical supply route less important. It is now about 5% or less of total supply compared with 25% 20 years ago (Gunstone and Heming, 2004).

Glycerol is available in several grades varying in purity and the requirements of the industries to which it is sold. Refined material is at least 86.5% pure and generally greater than 99.5%. Its value lies in its physical properties: it is hygroscopic, colourless, odourless, viscous, sweet-tasting, low boiling, nontoxic, emollient, a good solvent, and water-soluble. It is also easily biodegradable. Its major uses include oral care products, food and food emulsifiers, tobacco products, polyurethanes, prescription drugs, over-the-counter medicines, and cosmetics. Attempts are being made to develop new uses by conversion to other valuable compounds, such as glycidol (2,3-epoxypropanol), glycerol carbonate, and polyglycerols and their esters (Barrault et al., 2005, and Stepan website). In some of its uses, glycerol (produced at an annual level of around 1 million tonnes) competes with other polyols, such as pentaerythitol and trimethylolpropane (together 0.4 million tonnes), sorbitol (1.1 million tonnes.), propylene glycol (1.5 million tonnes), and ethylene glycol (7.5 million tonnes). The figures in parentheses represent annual production levels in 2003.

In 2003, the annual production of glycerol (930 kt) came from countries with significant oleochemical industries including the U.S., Europe, Japan, and Southeast Asia. Significant importers were the U.S. (37% of its glycerol requirement) and Japan (50% of its glycerol requirement), while Southeast Asia was the major exporting region. Four ASEAN (Association of Southeast Asian Nations) countries (Malaysia, Indonesia, Philippines, Thailand, and Singapore) alone exported around 164,000 tonnes of glycerol in 2003. Table 9.4 clearly shows that ASEAN countries are now important producers of glycerol and have become the dominant exporter of this commodity.

Sources of glycerol by oleochemical products are detailed in Table 9.5. Between 1999 and 2008, glycerol production is expected to rise 38% (from 804 to 1110 kt). Changes in the supply levels from various oleochemical processes over this 10-year period are soaps (–58kt), fatty acids (+88 kt), biodiesel (+293 kt), fatty alcohols (+32 kt), and petrochemical glycerol (–50 kt). These figures demonstrate the growing importance of biodiesel production and the continuing demand for fatty acids and fatty alcohols as sources of glycerol. The change through increased biodiesel production is mainly in Europe, but is expected to become more apparent in North America and elsewhere. The market for fatty acids continues to increase and new plants are being established in China and other developing countries. These will add to local supplies of glycerol and affect import requirements.

In 2003, 41% of the European glycerol supply came from biodiesel production and that share is expected to increase. Following changes in taxation rules in Germany for a 95:5 blend of diesel with biodiesel, usage of biodiesel is expected to increase rapidly leading eventually to the production of 140 kt of glycerol in Germany, in addition to part of the 65 kt produced in 2003 for the existing pure biodiesel market. The biodiesel market in the rest of Europe will also move ahead strongly based mainly on locally produced rapeseed oil. European biodiesel capacity in Europe in 2004 was 2.2 million tonnes. New projects for large biodiesel plants continue to be announced

TABLE 9.4 Production, consumption, exports, and imports of glycerol (kilotonnes) in 2003

	Production	Consumption	Exports	Imports	Imports From:
World	930[a]	936	251	248	
U.S.	142	201	24	75	SE Asia, Europe, S. America
Europe	315	325	25	35	SE Asia
China	20	65	2	45	Malaysia and Indonesia
Japan	45	85	2	43	SE Asia
ASEAN[b]	197	33	164	–	
Rest of world	211	227	34	50	

[a] Details of sources by oleochemical products are given in Table 9.5.

[b] Malaysia, Indonesia, Philippines, Thailand, and Singapore.

Source: Gunstone, F.D. and Heming, M.P.D., Glycerol – an important product of the oleochemical industry, *Lipid Technol.*, 16, 177–179, 2004.

TABLE 9.5 Production of glycerol (kilotonnes) in 1999, 2003, 2004, and forecast for 2008 by the oleochemical product of which it is a by-product

	1999	2003	2004	2008
Total	**804**	**930**	**970**	**1110**
Soaps	198	180	170	140
Fatty acids	322	350	365	410
Biodiesel	57	160	210	350
Fatty alcohol	108	110	120	140
Synthetic	75	80	50	25
Other	44	50	55	45

Source: Gunstone, F.D. and Heming, M.P.D., Glycerol – an important product of the oleochemical industry, *Lipid Technol.*, 16, 177–179, 2004.

although doubts have been expressed about the economic viability of some of these programmes. Nevertheless, it is quite possible that European biodiesel capacity will reach 3 million tonnes by the end of 2005, with the largest capacities being in Germany (1.1 million tonnes/year), Italy (0.6 million tonnes/year) and France (0.4 million tonnes/year) (see Section 9.6).

References

Barrault, et al., Polyglycerols and their esters as an additional use of glycerol, *Lipid Technol.*, **17**, 131–135, 2005.

Dumelin, E.E., Biodiesel — a blessing in disguise? *Eur. J. Lipid Sci. Technol.,* **107**, 63–64, 2005.

Gunstone, F.D., *The Chemistry of Oils and Fats*, Blackwell Publishing, Oxford, 2004, Chaps. 8, 11.

Gunstone, F.D. and Heming, M.P.D., Glycerol — an important product of the oleochemical industry, *Lipid Technol.*, **16**, 177–179, 2004.

Lim, S., Oleochemicals — biodiesel and glycerine overshadow market, *Oils Fats Int.,* **21** (1) 37–39, 2005.

MPOB (Malaysian Palm Oil Board), *2004 Malaysian Oil Palm Statistics,* 2005.

Stepan (Stephan Company): www.stepan.com

Suppes, G.J., et al., Calcium carbonate catalysed alcoholysis of fats and oils, *J. Am. Oil Chem. Soc.,* **78**, 139–145, 2001.

Van den Hark, S., et al., Hydrogenation of fatty acid methyl esters to fatty alcohols at supercritical conditions, *J. Am. Oil Chem. Soc.,* **76**, 1363–1370, 1999.

9.3 Surfactants

9.3.1 Introduction

Surfactants are surface-active molecules as a consequence of their amphiphilic nature. This means that one part of a surfactant molecule (the alkyl chain) is lipophilic (hydrophobic) and the other part (the polar head group) is hydrophilic (lipophobic). The balance between these forces is an important property of a surfactant molecule and is defined as the hydrophilic lipophilic balance (HLB) by the equation:

HLB = 20 (molecular weight of the hydrophilic portion/ molecular weight of the whole molecule)

Typically emulsifiers have HLB values of 5 to 6, wetting agents of 7 to 9, and detergents of 13 to 15.

Surfactants are produced both by the petrochemical and oleochemical industries, though only the latter will be considered in this section. This is most obvious in the production of long-chain alcohols. There are environmental and economic reasons why lipid-based molecules find favour in a time of high-priced oil and gas.

Amphiphilic molecules can exist comfortably at an oil–water interface and reduce the surface tension at such interfaces. This property is fundamental in all living systems and in many foods and other manmade systems. Depending on the HLB, appropriate amphiphilic molecules influence a range of important surfactant properties, such as emulsification, deemulsification, wetting, foaming, defoaming, water-repelling, dispersing, solubilising, detergency, sanitising, lubricity, and emolliency. The simplest and oldest examples are soaps, such as sodium palmitate in which the palmitic acid chain is lipophilic and the carboxylate group is hydrophilic.

The lipophilic alkyl chain varies mainly in chain length with C_{12} and C_{14} chains from the lauric oils; C_{16} and C_{18} chains from tallow, palm oil, or palm stearin; and the C_{20} and C_{22} chains from fish oils or high-erucic rapeseed oil. The alkyl chain may also have some unsaturation and may be branched. The polar head group shows greater variations through carboxylates, sulfates and sulfonates, phosphates, partial esters of polyhydric alcohols, such as glycerol and carbohydrates, polyoxyethlene derivatives of alcohols or amines, derivatives of amino acids, and many other nitrogen-containing molecules.

There are four major groups of surfactants – anionic, nonionic, cationic, and zwitterionic (amphoteric) – with these terms describing the nature of the head group. Of these, anionics are used in greatest amount, but nonionics are increasing faster than any of the other groups. Some figures of usage in 2000 are given in Table 9.6. For automatic dishwashers two, three, or four surfactants may be combined in a single washing tablet.

Many complex molecules have been designed and synthesised as superior surfactants. Typical of these is the amphoteric surfactant shown below with four functional groups (amino, carboxy, hydroxy, and ether groups) (Hidaka et al., 2003).

$$C_{12}H_{25}OCH_2CH(OH)CH_2N(CH_2CH_2OH)\ CH_2CH_2COONa$$

A detergent is a formulation of many components (Hargreaves, 2003) in which the surfactant is responsible for washing and cleansing properties. Detergents enter widely into daily life and play an important part in keeping the human environment clean and wholesome. Their many uses include: cleaning agents for floors, surfaces, laundry, dishes, and personal care products. They are frequently used in pharmaceuticals and as lubricants, and are employed in industries devoted to food, agriculture, metal-working, textiles, and building. Appel (2000) has summarised the

TABLE 9.6 Consumption of surfactants (million tonnes) in 2000 by category and usage in three countries/regions of the world (excluding 7 million tonnes of soaps)

	Western Europe	North America	Japan
Total	2.61	3.34	1.19
By category			
Anionic	1.32	1.95	0.49
Nonionic	0.97	1.04	0.56
Cationic	0.25	0.27	0.10
Amphoteric	0.07	0.09	0.04
By usage			
Household products	1.41	1.32	0.32
Cosmetics and toiletries	0.12	0.19	0.13
Cleaning products	0.21	0.28	0.10
Textiles	0.16	0.31	0.12
Mining and petroleum	0.30	0.37	0.11
Plastics and paints	0.16	0.60	0.06
Agrochemicals	0.08	0.12	0.04
Other	0.15	0.15	0.31

Note: Other information is available in references: Anon., 2000 2002a,b, 2003.
Source: Adapted from Gunstone, F.D., *The Chemistry of Oils and Fats*, Blackwell Publishing, Oxford, 2004.

modern methods of detergent manufacture and Berna et al. (1998) have reviewed laundry products still used in bar form for manual washing. Rosen and Dahanayake (2000) have written a book on the industrial utilization of surfactants and Hargreaves (2003) has authored a simple but useful work on formulation containing a large number of typical recipes some of which are cited in Table 9.7.

There has been a general consolidation of the surfactants industry both of suppliers and retailers (Anon., 2001).

Many natural products have surfactant properties. Hill (2001) has reviewed the use of oils and fats as oleochemical raw materials. Dembitsky (2004, 2005a-e) has demonstrated the diversity of these compounds and has reported their chemical structures and biological activities. Many microorganisms also produce biosurfactants (Solaiman, 2005). Urata and Takaishi (1999, 2002) have discussed a number of synthetic routes to novel compounds capable of self-assembly.

Analytical methods for the examination of surfactants have been reported by Thin Sue Tang (2001), Morelli and Szajer (2000, 2001), and Waldhoff and Spilker (2005). A useful website is The Surfactants Virtual Library (see reference list).

TABLE 9.7 Selected typical formulations for a range of products indicating the surfactants present

Product	Formulation	%
Shampoo for dry hair	40% triethanolamine lauryl sulfate	8.0
	27% ammonium lauryl sulfate	6.0
	70% sodium lauryl ether sulfate	4.0
	Coco amido propyl betaine	7.0
	Coconut diethanolamide and citric acid, salt, perfume, colour, preservative, and water	2.0
Foam bath	27% sodium lauryl ether sulfate	60.0
	30% lauryl betaine and salt, citric acid, perfume, colour, preservative, and water	5.0
Shower gel	30% sodium lauryl ether sulfate	40.0
	Coconut diethanolamide	2.0
	30% alkylamido propyl betaine	5.0
	Cocoamine oxide and salt, lactic acid, perfume, colour, preservative, and water	2.0
Liquid soap	27% sodium lauryl ether sulfate	20.0
	Monoethanolamine lauryl sulfate	10.0
	30% lauryl betaine and salt, citric acid, perfume, colour, preservative, and water	7.0
Toothpaste	Glycerol	25.0
	Sodium lauryl sulfate and sodium carbomethoxycellulose, sodium monofluorophosphate, dicalcium phosphate dihydrate, flavour, preservative, and water	1.5
Moisturising cream	Caprylic/capric triacylglycerols	13.0
	Octyl cocoate	3.0
	Cetyl esters	3.0
	Cetyl/stearyl alcohol	3.0
	Polysorbate 60	3.0
	Sorbitan stearate	2.0
	Glycerol and hydrolysed vegetable protein, perfume, colour, preservative, and water	3.0
Dish washing liquids (20% active)	Sodium dodecylbenzene sulfonate	23.3
	Coconut diethanolamide	2.0
	27% sodium lauryl ether sulfonate and salt, perfume, colour, preservative, and water	13.3
Carpet shampoo	28% sodium lauryl ether sulfonate	35.7
	Coconut diethanolamide	3.0
	Isopropanol and perfume, colour, preservative, and water	10.0
Screenwash	30% sodium lauroyl sarcosinate (1.0%)	1.0
	Isopropanol (25.0%), colour, and water	25.0

Source: Adapted from Hargreaves, T., *Chemical Formulation*, RSC, Cambridge, U.K., 2003.

9.3.2 Anionic surfactants from carboxylic acids

As the name indicates, anionic surfactants have a negatively charged species and a counterion that is usually a metal, but may be a type of ammonium group. The anionics are used in greater volume than any other class of surfactants. Soap belongs to this category and is still the surfactant used in largest amount. In many countries, hard (tablet) soap is being replaced by a liquid soap that is not a carboxylate salt (see Table 9.7). Soap has the disadvantage that it can only be used at pH8 and above and that it forms an insoluble scum with the calcium salts present in hard water. Alternative and superior surfactants, therefore, have been developed. These are generally sulfates or sulfonates in place of carboxylates. In all of these, the alkyl chain is the most expensive component in the surfactant.

Carboxylates	RCOOH
Fatty alcohol sulfates	$ROSO_3H$
Fatty alcohol ether sulfates	$R(OCH_2CH_2)_nOSO_3H$
α-Sulfonated esters	$RCH(SO_3H)COOCH_3$

Sodium and potassium salts of fatty acids (traditional soaps) are still made by saponification of appropriate fats and also increasingly by neutralisation of carboxylic acids resulting from splitting (see Section 9.2.1).

Cohen and Trujillo (1998) have reported the synthesis, characterization by GC-MS (gas chromatography-mass spectrometry), and by infrared spectroscopy, surface tension, and specific conductivity of methyl ester sufonates. The production of methyl ester sulfonates in the U.S. has been reported (Watkins, 2001). Scheibel (2004) has discussed changes in anionic surfactant technology in the laundry detergent industry.

9.3.3 Anionic surfactants from alcohols

Anionic surfactants of various kinds can be made from fatty alcohols or fatty alcohol ethoxylates (see Section 9.2.3 and Section 9.3.4) mainly as sulfates through reaction with sulfur trioxide or chlorosulfonic acid and used as sodium, ammonium, or monoethanolamine ($HOCH_2CH_2NH_2$) salts. The long-chain alcohols may also be used as phosphates (phosphorus pentoxide, Tracy and Reierson, 2002), sulfosuccinates (maleic anhydride and sodium sulfite), ethoxy carboxylates (sodium chloroacetate), or carbonate ethoxylates (dimethyl carbonate) by reaction with the reagents indicated in parentheses (Table 9.8).

These are active components in detergents used in personal care products and for washing clothes and hard surfaces (floors, walls, dishes). Surfactant, in the range of 5 to 20%, is accompanied by other materials, such as phosphate, zeolite, bleaching agent, optical brightener, fragrance, and water. The sarcosinates, taurates, and isethionates (Table 9.8) are long-chain amides of sarcosine and taurine, respectively, or are esters of isethionic acid. α-Sulfonate esters are made from methyl esters of saturated acids by reaction with sulfur trioxide. The product, after neutralisation, is a mixture of monoester salts (from $RCH(SO_3H)COOCH_3$) and di-sodium salts (from $RCH(SO_3H)COOH$) usually in a ratio of 80:20.

Further details are given in chapters written by Porter (1997) and by Roberts (2001).

9.3.4 Nonionic surfactants

Nonionic surfactants contain the usual type of lipophilic chain from a petrochemical or oleochemical source. The head group is not charged, but is polar through the presence of an appropriate collection of hydroxy, amino, or ether groups. The last come from ethylene oxide or propylene oxide products (see Section 9.3.5) and the former from glycerol, polyglycerol, low molecular weight carbohydrates, or amino acids or other amines.

Typical structures include polyethylene oxide derivative of the fatty alcohol ROH (alcohol ethoxylate AEO), such as $R(OCH_2CH_2)_nOH$ and bis-polyethylene oxide derivatives of the fatty amine RNH_2, such as

$$HO(CH_2CH_2O)_n\,NR(OCH_2CH_2)_mOH$$

Ethylene oxide is itself a product of the petrochemical industry and a hazardous chemical with undesirable environmental properties. It is for this reason that there is growing interest in the acyl and alkyl derivatives of glucose and other carbohydrates where all the reactants are

TABLE 9.8 Anionic surfactants produced from fatty alcohols and their ethoxylates

Name	Structure[a]	Typical Uses
Alcohol sulfates	$ROSO_3H$	Shampoo, toothpaste
Ethoxy sulfates	$AEOSO_3H$	Shampoo, bubble bath
Monoacylglycerol sulfates	$RCOOCH_2CH(OH)CH_2OSO_3H$	
Ethoxy phosphates	$AEOPO_3H_2$ or $(AEO)_2PO_2H$	Electroplating
Sulfosuccinates	$AEOCOCH_2CH(OSO_3Na)COONa$	Carpet cleaner, oil spill dispersants
Ethoxycarboxylates	$AEOCH_2COONa$	
Carbonate ethoxylates	$ROCOO(CH_2CH_2O)_nH$	
Sarcosinates	$RCON(CH_3)CH_2COOH$	Corrosion inhibitor
Taurates	$RCONHCH_2CH_2SO_3H$	
Isethionates	$RCOOCH_2CH_2SO_3H$	Toilet bars

[a] RO represents a fatty alcohol, RCOO an acyl group and AEO an alcohol ethoxylate $R(OCH_2CH_2)_nO$.

natural products coming from renewable resources (Section 9.3.7). Bognolo (1997) has written a full account of nonionic surfactants.

9.3.5 Ethoxylation and propoxylation of alcohols and esters

A substantial portion of the medium and long-chain alcohols are used only after conversion to ethoxylates or propoxylates. Ethoxylation of long-chain alcohols with ethylene oxide occurs at 135°C under pressure in 30 minutes in a reaction usually catalysed by ~ 0.2% of NaOH or KOH. The product is hydrophilic by virtue of one hydroxyl group and many ether links and may be a mixture of compounds with up to 20 ethylene oxide (EO) units (Figure 9.1). Since important surfactant properties vary with the number and range of EO units, there is a drive to produce materials with different narrow ranges of EO units.

For example, when dodecanol is reacted with ethylene oxide (50% wt ≡ 4.4 mol) the product is a mixture of compounds with up to 20 or more EO units and no individual compound greater than 10%. With proprietary catalysts, such as $ZrSO_4(OR)_2$ or similar aluminium compounds, the products contain only 0 to 10 EO units with those having 4 to 6 EO units each around 20% and those with 3 and 7 units ~ 10%. These narrow range ethoxylates have good stability and skin mildness in liquid dish-washing products (Di Serio et al., 1998).

The ethoxylation of esters, rather than alcohols, is an interesting development in this field since products with improved surfactant properties can be obtained from esters that are less expensive starting materials than the alcohols. Using a methyl ester and an appropriate catalyst, such as a composite aluminum and calcium metal oxide at 180°C and 3 bar the product has a narrow range of molecular weights with mainly 5 to 10 ethylene oxide units (see Figure 9.1). While alcohol ethoxylates can assume a linear arrangement ester ethoxylates are necessarily bent (boomerang shape) because of the trigonal ester carbon atom. The ester products have outstanding dermatological properties (Cox et al., 1998; Hreczuch et al., 2001).

The reaction is not confined to methyl esters and has been applied to compounds with two or more ester functions. Interesting products result from triacylglycerols including natural mixtures, such as the lauric oils (coconut and palmkernel) and tallow. Reaction can occur at all three ester functions to give products with the structure shown in Figure 9.1 Subsequent partial hydrolysis gives a mixture of ethoxylated triacylglycerols (unreacted material), fatty acid soaps (RCOONa), and products in which 1, 2, or 3 acyl groups have been removed. The composition of the mixture depends on the degree of hydrolysis. Even without further separation this mixture has good surfactant properties (Cox and Weerasooriya, 2000).

Another development is the replacement of ethylene oxide, wholly or in part, by propylene oxide in the reaction both with alcohols and esters. The repeating unit ($-CH_2CH_2O-$) is then replaced by ($-CHMeCH_2O-$) or there is a mixture of both units in the poly-ether chain. The resulting branched-methyl compounds have interesting modified surfactant properties, important among which is their greater ability to reduce foaming when compared with the ethylene oxide derivatives. This reaction requires a calcium aluminum complex as catalyst (Cox et al., 1998; Weerasooriya, 1999). Further information is given by Bognolo (1997) and Gunstone (2001).

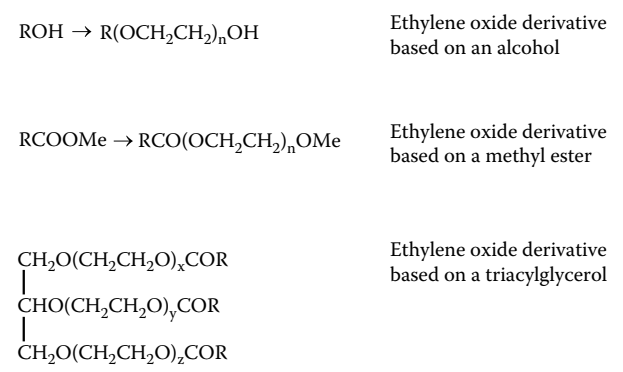


FIGURE 9.1 Ethylene oxide derivatives of alcohols, methyl esters, and triacylglycerols.

9.3.6 Alkyl polyglycosides

The term alkyl polyglycoside is the name given to technical products made from starch (or other source of glucose) and a fatty alcohol. These are sugar ethers in contrast to the sugar esters used in olestra. The alcohol is usually a mixture of $C_{8/10}$, $C_{12/14}$, or $C_{16/18}$ alcohols derived from appropriate fatty acid sources. All the substrates are renewable resources. The reaction between starch and alcohol is usually catalysed by acids, such as sulfuric or 4-toluenesulfonic and is accompanied by extensive depolymerisation of the carbohydrate polymer so that the product is mainly, but not entirely, an alkyl glucoside. In the two-step process (see equation below) butanol is used first and in the second step the fatty alcohol mixture (ROH) of desired chain length. The product is a mixture of compounds with R groups differing in chain length and values of *n* lying between 1 and 5. The average value of *n* (degree of polymerisation) is usually 1.3 to 1.7. Products with a value of 1.3 will contain molecules with one (~60%), two (~20%), three (~10%), and four and five glucose units. Products made from alcohols having 8 to 14 carbon atoms are water-soluble and are used as surfactants, those with 16 and 18 carbon atoms are not water-soluble, but are used as emulsifying agents and in cosmetic preparations (Hill et al., 1997).

$$\text{starch} + \text{BuOH} \rightarrow [\text{glucose}]_n\text{OBu}$$

$$[\text{glucose}]_n\text{OBu} + \text{ROH} \rightarrow [\text{glucose}]_n\text{OR}$$

Piispanen et al. (2004) have described the structures and structure/property relationships of surfactants derived from natural products and Dembitsky (2004, 2005a-d) has reported the structure and biological activity of a large number of natural glycoside surfactants.

The acyl esters of sugars are not yet commercial surfactants, but there is considerable interest in the preparation of acylated derivatives of fructose (Jung et al., 1998), glucose, or other monosaccharides, and of sucrose. These can be prepared chemically or, more specifically, with enzymatic catalysts and studies of their synthesis, hydrolysis, biodegradation, and some surfactant properties have been reported (Allen and Tau, 1999; Baker et al., 2000a,c; Polat et al., 2001).

9.3.7 Cationic surfactants

Cationic surfactants are nitrogen containing-compounds. They show high substantivity (i.e., strong adherence) to natural surfaces and find extensive use in fabric softening, hair conditioning, corrosion inhibition, mineral flotation, and as bactericides (Karsa, 2001). Levinson (1999) has reviewed rinse-added fabric softener technology.

Alcohols and amines add to acrylonitrile and, after catalytic hydrogenation, furnish ether amines (often written as one word) and diamines as shown in the equations below (Table 9.9 and Table 9.10). The diamine can react again with acrylonitrile to give a triamine $RNH(CH_2)_3NH(CH_2)_3NH2$.

$$\text{ROH} + CH_2{=}CHCN \rightarrow RO(CH_2)_2CN \rightarrow RO(CH_2)_3NH_2 \text{ (ether amine)}$$

$$RNH_2 + CH_2{=}CHCN \rightarrow RNH(CH_2)_2CN \rightarrow RNH(CH_2)_3NH_2 \text{ (diamine)}$$

Ether amines and diamines react with ethylene oxide to give ethyloxylated products (Table 9.8 and Table 9.9) as in the following equations:

$$RO(CH_2)_3NH_2 \rightarrow RO(CH_2)_3NH(CH_2CH_2O)_nH$$
(ethoxylated ether amine)

$$RNH(CH_2)_3NH_2 \rightarrow RNH(CH_2)_3NH(CH_2CH_2O)_nH$$
(ethoxylated diamine)

$$RNH_2 \rightarrow H(OCH_2CH_2)_nN(R)(CH_2CH_2O)_mH$$
(ethoxylated amine)

$$RCONH(CH_2)_2NH(CH_2)_2NHCOR$$
(diamidoamine or diacylated triamine)

Diamidoamines (or diacylated amines) formed from carboxylic acids and diethylene triamine ($H_2N(CH_2)_2NH(CH_2)_2NH_2$) have the structure shown and readily cyclise to imidazolines (Figure 9.2).

Further information is provided by James (1997) and by Franklin et al. (2001).

TABLE 9.9 Cationic surfactants made from fatty amines or fatty alcohols

Product Name	Product Structure	Starting Material	Reactant
Ether amine	$RO(CH_2)_3NH_2$ [a]	ROH	$CH_2{=}CHCN$
Diamine	$RNH(CH_2)_3NH_2$ [a]	RNH_2	$CH_2{=}CHCN$
Ethoxylated ether amine	$RO(CH_2)_3NH(CH_2CH_2O)_nH$	$RO(CH_2)_3NH_2$	Ethylene oxide
Ethoxylated diamine	$RNH(CH_2)_3NH(CH_2CH_2O)_nH$	$RNH(CH_2)_3NH_2$	Ethylene oxide
Ethoxylated amine	$H(OCH_2CH_2)_nN(R)(CH_2CH_2O)_mH$	RNH_2	Ethylene oxide
Diamido amine[b]	$RCONH(CH_2)_2NH(CH_2)_2NHCOR$	$H_2N(CH_2)_2NH(CH_2)_2NH_2$	RCOOH

[a] Product after catalytic hydrogenation of an intermediate nitrile.
[b] This is the name given to a diacylated triamine. The products are readily cyclised to imidazolones (Figure 9.2).
Note: R represents an alkyl chain.

TABLE 9.10 Cationic surfactants made from fatty amines

Products	Structure	Reactants
Amines	RNH_2, R_2NH, R_3N	Nitriles – hydrogenation
Quaternary salts (quats)	$[R_2NMe_2]^+ X^-$	Tertiary amines and RX
Amine oxides	$[RNMe_2]^+ O^-$	Tertiary amines and H_2O_2
Amido amines	$RCONH(CH_2)_3NMe_2$	Polyamines and RCOOH
Imidazolines	Figure 9.2	Polyamines and RCOOH
Ester amines	$RCOOCH_2CH_2NMe_2$	Ethanolamines and RCOOH
Ether amines	$RO(CH_2)_3NH_2$	Fatty alcohols and acrylonitrile

Source: Adapted from Gunstone, F.D., *The Chemistry of Oils and Fats*, Blackwell Publishing, Oxford, 2004.

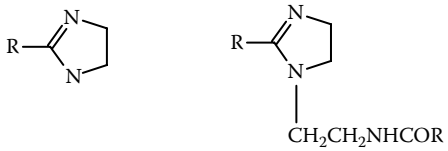


FIGURE 9.2 Imidazolines from RCOOH and diamine ($H_2N(CH_2)_2NH_2$) or triamine ($H_2N(CH_2)_2NH(CH_2)_2NH_2$).

9.3.8 Gemini surfactants and cleavable surfactants

Most surfactants contain one lipophilic chain and one hydrophobic head group. Gemini or dimeric surfactants contain two of each of these linked together by a short aliphatic group or through an aromatic ring. A book devoted to this subject has been edited by Zana and Xia (2003). One example formulated below shows two quaternary groups linked through a tetramethylene spacer (Rosen and Tracy, 1998). Similar compounds with an aromatic spacer have been prepared and assessed for protection of steel fabrics against 2M hydrochloric acid. (Negm and Mohamed, 2004).

$$R(Me)_2N^+(CH_2)_4N^+(Me)_2\ R$$

$$R_3N^+CH_2COOC_6H_4OCOCH_2N^+R_3$$

Unlike conventional surfactants, gemini consist of two molecules of monomeric surfactant linked through a flexible spacer. Such molecules are capable of wide variations in terms of each of their three components – lipophilic chain, head group, and spacer — and frequently show remarkable properties. Gemini surfactants generally display unusual patterns of self-assembly and some intriguing physicochemical properties. For example, ionic Gemini surfactants have critical micelle concentrations two orders of magnitude lower than their monomeric alkylsulfonate analogues and are very efficient at reducing surface tension both on their own and in combination with conventional surfactants. Gemini surfactants have much lower Krafft points and higher solubility in water compared to their monomeric counterpoints. Valivety et al. (1998) have described the synthesis of amino acid based gemini surfactants. One example is the compound formulated below and based on myristic acid (2 mols), serine (2 mols) and1,10-dihydroxydecane (1 mol).

$$C_{13}H_{27}COOCH_2CH(NH_2)COO(CH_2)_{10}OCOCH(NH_2) - CH_2OCOC_{13}H_{27}$$

decanediyl-1,10-bis-(3-*O*-myristoyl-L-serine)

Another group of surfactants have a weak bond built into the molecule. These are interesting because this feature leads to improved biodegradabilty and opens up the possibility of using these compounds for drug delivery. The weak bonds can be broken when required with the help of enzymes, by reactions occurring at sewage plants, or by chemical or physical processes involving acid, alkali, ozone, heat, or ultraviolet light. These compounds are generally acetals/ketals or ortho esters (Hellberg, 2003).

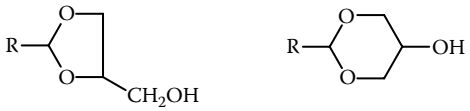


FIGURE 9.3 Acetals from RCHO and glycerol.

Cyclic acetals/ketals result when aldehydes or ketones react with polyhydric alcohols, such as glycerol (Figure 9.3), pentaerythryitol, or glucose. The products are 1,3-dioxolanes (5-membered hetero ring) or 1,3-dioxanes (6-membered hetero ring). Any unreacted hydroxyl groups can be further functionalised. These compounds are made under anhydrous acidic conditions and are readily hydrolysed under aqueous acidic conditions (Hellberg et al., 2000a).

Ortho-esters are made from ethyl orthoformate [$HC(OEt)_3$], alcohols, and monomethyl polyethylene glycol ($HO(C_2H_4O)_nMe$) in the presence of aluminum chloride. The product is a mixture of many compounds having the structures shown below in which x, y, and z have values 0-3 and x + y + z = 3. The products formed in largest amount have values of x, y, and z of 1,1,1 or 0,2,1, or 0,1,2. Such product mixtures are used for temporary emulsions, hard surface cleaners, textile treatment processing, etc. They are hydrolysed under mild acid or alkaline conditions and have good biodegradability (Hellberg et al., 2000b).

$$\begin{array}{c} [OR]_y \\ | \\ H-C-[O(C_2H_4O)_nMe]_z \\ | \\ [OEt]_x \end{array}$$

Ono et al. (2004, 2005) have described the preparation of cleavable surfactants from methyl pyruvate and from diethyl tartrate as shown below.

$$CH_3COCOOMe \rightarrow CH_3CH(OR)_2COONaR$$
$$= C_8H_{17},\ C_{10}H_{21},\ \text{or}\ C_{12}H_{23}$$

$$[EtO_2CCH(OH)]_2 \rightarrow [RO_2CCH(OSO_3Na)]_2R$$
$$= C_8H_{17},\ C_{10}H_{21},\ \text{or}\ C_{12}H_{23}$$

Quaternary ammonium compounds, much used as rinse-aids in the past, have been largely replaced by ester quats. Typical structures of these two categories of compounds are shown:

quat: $R_2N^+Me_2\ X^-$

ester quat: $(RCOOCH_2CH_2)_2N^+Me_2\ X^-$

The ester quats are stable to acids, but are easily hydrolysed by alkali to soap and the compound ($(HOCH_2CH_2)_2\ N^+Me_2X^-$). They show better environmental characteristics than the quats themselves (Hellberg, 2002).

Alkyl ethoxylates ($R(OC_2CH_2)_nOH$) are viscous oils that are not always easy to handle, but they react with carbon dioxide to form carbonates ($R(OCH_2CH_2)_n\ OCO_2Na$). These are solid and are easily incorporated into granular detergents. In an alkaline solution, the ethoxylates are quickly regenerated from the carbonates.

References

Allen, D.K. and Tao, B.Y., Carbohydrate-alkyl ester derivatives as biosurfactants, *J. Surfact. Deterg.*, **2**, 383–390, 1999.

Anon., Publications and reports, *J. Surfact. Deterg.,* **3**, 139–141, 2000.

Anon., Consolidation in the surfactants industry, *Inform,* **12**, 872–882, 2001.

Anon., Publications and reports, *J. Surfact. Deterg.,* **5**, 426, 2002a.

Anon., LAS decline, *Inform,* **13**, 704–705, 2002b.

Anon., Publications and reports, *J. Surfact. Deterg.,* **6**, 396, 2003.

Appel, P.W., Modern methods of detergent manufacture, *J. Surfact. Deterg.*, **3**, 395–405, 2000.

Baker, I.J.A., et al., Sugar fatty acid ester surfactants: structure and ultimate aerobic biodegradability, *J. Surfact. Deterg.,* **3**, 1–11, 2000a.

Baker, I.J.A., et al., Sugar fatty acid ester surfactants: biodegradation pathways, *J. Surfact. Deterg.,* **3**, 13–27, 2000b.

Baker, I.J.A., et al., Sugar fatty acid ester surfactants: base-catalysed hydrolysis, *J. Surfact. Deterg.,* **3**, 29–32, 2000c.

Berna, J.L., et al., Laundry products in bar form, *J. Surfact. Deterg.*, **1**, 263–271, 1998.

Bognolo, G., Nonionic surfactants, in *Lipid Technologies and Applications,* Gunstone, F.D., Padley, F.B., Eds., Marcel Dekker, New York, 1997, Chap 25.

Cohen, L. and Trujillo, F., Synthesis, characterisation, and surface properties of sulfoxylated methyl esters, *J. Surfact. Deterg.,* **1**, 353341, 1998.

Cox, M.F. and Weerasooriya, U., Impact of molecular structures on the performance of methyl ester ethoxylates, *J. Surfact. Deterg.*. **1**, 11–22, 1998.

Cox, M.F. and Weerasooriya, U., Enhanced propoxylation of alcohols and alcohol ethoxylates, *J. Surfact. Deterg.*. **2**, 59–68, 1999.

Cox, M.F. and Weerasooriya, U., Partially saponified triglyceride ethoxylates, *J. Surfact. Deterg.*. **3**, 213, 2000.

Cox, M.F. et al. Methyl ester propoxylates, *J. Surfact. Deterg.*. **1**, 167–175, 1998.

Dembitsky, V.M., Astonishing diversity of natural surfactants: 1. Glycosides of fatty acids and alcohols, *Lipids*, **39**, 933–953, 2004.

Dembitsky, V.M., Astonishing diversity of natural surfactants: 2. Polyester glycosidic ionophores and macrocyclic glycosides, *Lipids*, **40**, 219–248, 2005a.

Dembitsky, V.M., Astonishing diversity of natural surfactants: 3. Carotenoid glycosides and isoprenoid glycolipids, *Lipids*, **40**, 535–557, 2005b.

Dembitsky, V.M., Astonishing diversity of natural surfactants: 4. Fatty acid amide glycosides, their analogues and derivatives, *Lipids*, **40**, 641–660, 2005c.

Dembitsky, V.M., Astonishing diversity of natural surfactants: 5. Biologically active glycosides of aromatic metabolites, *Lipids*, **40**, 869–900, 2005d.

Dembitsky, V.M., Astonishing diversity of natural surfactants: 6. Biologically active marine and terrestrial alkaloid glycosides, *Lipids*, **40**, 1081–1105, 2005e.

Di Serio, M., et al., Narrow-range ethoxylation of fatty alcohols promoted by a zirconium alkoxide sulfate catalyst, *J. Surfact. Deterg.,* **1**, 83–91, 1998.

Franklin, R., et al., Cationic and amine-based surfactants, in *Oleochemical Manufacture and Applications,* Gunstone, F.D., Hamilton, R.J., Eds., Sheffield Academic Press, Sheffield, U.K., 2001, Chap 2.

Gunstone, F.D., Chemical reactions of fatty acids with special reference to the carboxyl group, *Eur. J. Lipid Sci. Technol.,* **103**, 307–314, 2001.

Gunstone, F.D., *The Chemistry of Oils and Fats*, Blackwell Publishing, Oxford, 2004, Chaps. 8, 11.

Gunstone, F.D. and Heming, M.D., Glycerol – an important product of the oleochemical industry, *Lipid Technol.*, **16**, 177–179, 2004.

Hargreaves, T., *Chemical Formulation*, RSC, Cambridge, U.K., 2003.

Hellberg, P-E., Cleavable surfactants — giving an unstable advantage, *Lipid Technol.*, **15**, 101–105, 2003.

Hellberg, P-E., Ortho ester-based cleavable cationic surfactants, *J. Surfact. Deterg.*, **5**, 217–227, 2002.

Hellberg, P-E., et al., Cleavable surfactants, *J. Surfact. Deterg.*, **3**, 81–91, 2000a.

Hellberg, P-E., et al., Nonionic cleavable ortho ester surfactants, *J. Surfact. Deterg.*, **3**, 369–379, 2000b.

Hidaka, H., et al., Preparation and properties of new tetrafunctional amphoteric surfactants bearing amino, carboxyl, and hydroxyl groups and an ether bond, *J. Surfact. Deterg.,* **6**, 131136, 2003.

Hill, K., et al., *Alkylpolyglycosides: Technology, Properties, and Applications*, VCH, Weinheim, Germany, 1997.

Hill, K., Fats and oils as oleochemical raw materials, *J. Oleo Sci.,* **59**, 433–444, 2001.

Hreczuch, W., et al., Direct ethoxylation of longer-chain aliphatic ester, *J. Surfact. Deterg.,* **4**, 167–173, 2001.

James, A.D., Cationic surfactants, in *Lipid Technologies and Applications,* Gunstone, F.D., Padley, F.B., Eds., Marcel Dekker, New York, 1997, Chap 24.

Jung, S., et al., Structure and surface-active property determinations of fructose mono-oleates, *J. Surfact. Deterg.*, **1**, 53–57, 1998.

Karsa, D. R., Quaternary ammonium surfactants, *Lipid Technol.*, **7**, 81–86, 2001.

Levinson, M.I., Rinse-added fabric softener technology at the close of the twentieth century, *J. Surfact. Deterg.,* **2**, 223–235, 1999.

Morelli, J.J. and Szajer, G., Analysis of Surfactants: Part I, *J. Surfact. Deterg.,* **3**, 539–552, 2000.

Morelli, J.J. and Szajer, G., Analysis of surfactants: Part II, *J. Surfact. Deterg.,* **4**, 75–83, 2001.

Negm, N.A. and Mohamed, A.S., Surface and thermodynamic properties of diquaternary bola-form amphphiles containing an aromatic spacer, *J. Surfact. Deterg.*, **7**, 23–30. 2004.

Ono, D., et al., Synthesis and properties of soap types of double-chain cleavable surfactants derived from pyruvate, *J. Oleo Sci.,* **53**, 89–95, 2004.
Ono, D., et al., Preparation and properties of bis(sodium sulfate) types of cleavable surfactants derived from diethyl tartrate, *J. Oleo Sci.,* **54**, 51–57, 2005.
Piispanen, P.S., et al., Surface properties of surfactants derived from natural products. Syntheses and structure/property relationships – Part 1, solubility and emulsification, Part 2, foaming, dispersion and wetting, *J. Surfact. Deterg.,* **7**, 147–159, 161–167, 2004.
Polat, T. and Linhardt, R.J., Syntheses and applications of sucrose-based esters, *J. Surfact. Deterg.*, **4**, 415, 2001.
Porter, M.R., Anionic detergents, in *Lipid Technologies and Applications,* Gunstone, F.D., Padley, F.B., Eds., Marcel Dekker, New York, 1997, Chap 23.
Roberts, D.W., Manufacture of anionic surfactants, in *Oleochemical Manufacture and Applications,* Gunstone, F.D., Hamilton, R.J., Eds., Sheffield Academic Press, Sheffield, U.K., 2001, Chap 3.
Rosen, M.J. and Tracy, D.J., Gemini surfactants, *J. Surfact. Deterg.*, **1**, 547–554, 1998.
Rosen, M.J. and Dahanayake, M., *Industrial Utilisation of Surfactants, Principles and Practice*, AOCS Press, Champagne, IL, 2000.
Schiebel, J.J., The evolution of anionic surfactant technology to meet the requirements of the laundry detergent industry, *J. Surfact. Deterg.*, **7**, 319–328, 2004.
Solaiman, D.K.Y., Applications of microbial biosurfactants, *Inform*, **126**, 408–410, 2005.
The Surfactants Virtual Library: www.surfactants.net
Thin Sue Tang, Analysis of oleochemicals, in *Oleochemical Manufacture and Applications,* Gunstone, F.D., Hamilton, R.J., Eds., Sheffield Academic Press, Sheffield, U.K., 2001, Chap 8.
Tracy, D.J. and Reierson, R.L., Commercial synthesis of monoalkyl phosphates, *J. Surfact. Deterg.*, **5**, 169–172, 2002.
Urata, K. and Takaishi, N., Newer synthetic approaches to surfactants, lipids, and related compounds based on C-3 building blocks: Recent advances related to fatty chemistry, *J. Surfact. Deterg.*, **2**, 91–103, 1999.
Urata, K. and Takaishi, N., Self-assembly of compounds based on a glycerin skeleton as a C-3 building block, *J. Surfact. Deterg.*, **5**, 287294, 2002.
Valivety, R., et al., Application of enzymes to the synthesis of amino acid-based bola and gemini surfactants, *J. Surfact. Deterg.*, **1**, 177–185, 1998.
Waldhoff, H. and Spilker, R., Eds., *Handbook of Detergents, Part C: Analysis,* vol. 123, Marcel Dekker, New York, 2005.
Watkins, C., Methyl ester sulfonates, *Inform*, 12, 11531159. 2001.
Weerasooriya, U., Ester alkoxylation technology, *J. Surfact. Deterg.*, **2**, 373–381, 1999.
Zana, R. and Xia, J., Eds., *Gemini Surfactants: Synthesis, Interfacial and Solution-Phase Behaviour and Applications,* Marcel Dekker, New York, 2003.

9.4 Cosmetics and personal care products

9.4.1 Introduction

This review covers two aspects of using lipids in cosmetic and skin-care formulations. The physicochemical properties of lipids make them suitable for controlling product consistency and to favourably influence the skin feel of the preparation. Knowledge about polymorphism and crystallisation kinetics is essential to optimise formulations based on lipids. The second aspect of lipids in cosmetic formulations being covered is the ability of essential fatty acids, tocopherols, and phytosterols to improve skin health. Lipids are important building blocks of the cell membranes; they act as antioxidants and anti-inflammatory agents and can have a fundamental effect on collagen production and break down in the skin. All of this illustrates the versatility of lipids as formulating tools in skin care and cosmetics.

Lipids are important multifunctional ingredients commonly used in cosmetic and personal care products. They may function as emollients, moisturisers, emulsifiers, solubilisers, dispersing agents, texturisers and skin-feel improvers. Some lipids show interesting bioactivity (anti-inflammatory and antioxidative) and influence protein synthesis and degradation in the skin. They find uses in skin-care emulsions, ointments, and balms as well as colour cosmetics and personal care products, such as shower gels, shampoos, and hair conditioners.

The definition of lipids in cosmetic applications is variable and in this review the emphasis will be on acylglycerols, tocopherols, and phytosterols. The use of lipids and lipid-derived materials as texturisers will first be described, followed by examples of their bioactivity and interactions with the skin.

9.4.2 Texture control using solid and semisolid lipids

A cosmetic skin-care formulation is a complex mixture of ingredients with varying functionality. A majority of such products are stabilised emulsions of an emollient in water with added bioactive materials for delivering real or apparent benefits to the well-being of the skin. The formulation needs to deliver both water-soluble actives and oil-soluble ingredients to the stratum corneum and preferentially control the penetration of these substances to the epidermis and dermis. The formulation regularly has to have a shelf life of more than 24 months, putting a lot of requirements on the emulsifying and stabilising system. Finally, the formulation also needs to be aesthetically and sensorially acceptable in order to fulfill the demands of the consumer.

The main component in the oil phase of skin-care emulsions is the emollient. It comprises one or more oils of differing composition and chemical structure. Many lipid-based and lipid-derived materials have been suggested for use as emollients, including esters of long-chain fatty acids with both short- and long-chain alcohols, natural and synthetic triacylglycerols, and naturally occurring hydrocarbons, such as squalene and squalane. The primary function of these emollients is to lubricate the skin, decrease the water permeability, and to act as a carrier of

the actives in the formulation. A secondary function of increasing importance is to influence the formulation texture and the sensory aspects when applying the emulsion to the skin.

Important considerations when selecting emollients are their effects on emulsion structure and how that effect is translated to sensory properties on application (Wiechers et al., 2004). Physicochemical properties, such as viscosity and polarity, are strongly related to the ability of an emollient to spread onto and penetrate into the skin. By combining emollients with different characteristics in terms of spreading ability and viscosity, optimal performance on different timescales can be obtained. The ability of an emollient to interact with surfactants in liquid crystalline phases also plays a significant role in the performance (Wiechers, 2003).

The microstructure and the resulting texture of cosmetic emulsions can be strongly influenced using semisolid and solid crystallising materials in the oil phase. By a careful selection of semisolid fats and waxes, the consistency of a cosmetic cream or lotion can be fine tuned to obtain optimal sensory properties and good product stability. In this context, the crystallisation behaviour of the ingredients used is strongly influencing the final result. There are three physicochemical concepts that are useful in the design of an emollient for cosmetic creams: the solids content of the oil phase, the polymorphic behaviour of the solids, and, finally, the kinetics of crystallisation.

9.4.3 Solid phase content

First of all, the solid phase content is directly correlated with the consistency and sensory properties of the product. In general terms, the more solids at a given temperature, the harder and more brittle the emulsion will appear. It is important to optimise the solid phase content for the different temperature ranges that the product will encounter. For example, the solid phase content at body or skin temperature (34 to 37°C) determines the sensory properties and the spreadability of the product. The solids content at 40 to 45°C will have an influence on the high temperature storage stability of the formulation. Finally, the solids content at 20 70 25°C will determine the texture and consistency of the product at room temperature. Characteristic ranges for skin-care creams are less than 50% solids at room temperature, less than 30% solids at body temperature, and 5 to 15% solids at 40°C (Figure 9.4). With these solids ranges, a product will have good stability during storage, be nicely spreadable on the skin, and have good high temperature resistance.

9.4.4 Polymorphism

The polymorphic behaviour of the solids used in cosmetic formulations is generally not well known by the formulators. Most simple waxes (wax esters and hydrocarbon-based waxes) crystallise in orthorhombic crystals similar to the beta-prime polymorph of triacylglycerols (Small, 1984). Semisolid and solid triacylglycerol based ingredients display a more complex crystallisation behaviour including the alpha-, beta-prime and beta forms. Many hydrogenated vegetable fats, such as hydrogenated soybean oil and hydrogenated palm oil, as well as the lauric oils, coconut oil, and palm kernel oil are generally stable in the beta-prime form. Many of the exotic "butters" used in cosmetic applications — cocoa butter, shea butter,

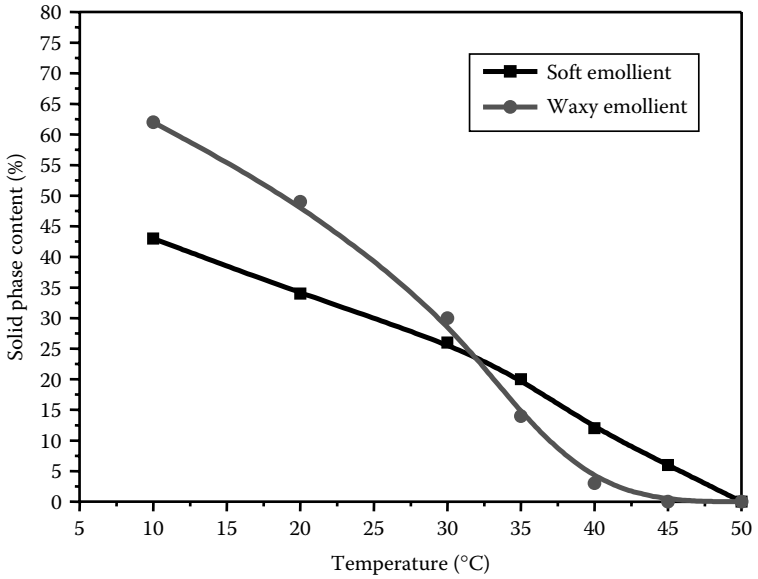


FIGURE 9.4 Solid phase content as a function of temperature for two typical emollient blends: (a) an emollient with soft, plastic consistency at room temperature and good stability at elevated temperatures, and (b) an emollient with hard, waxy texture at room temperature and almost complete melting at body temperature. Solid phase content in the mixtures was determined by low resolution pulsed NMR after tempering at 26°C for 40 hours.

shorea butter and so on — crystallise in the triple-chain packed beta form. These butters normally require a good control of the crystallisation conditions ("tempering") when used in higher concentrations if problems with the storage stability are to be avoided. When mixing ingredients from different groups, the resulting complex polymorphic behaviour can strongly influence the stability and sensory properties of the formulation. The best results in both aspects are obtained if the polymorphic behaviour of the system is matched; all ingredients should be either in the beta prime or in the beta modification. For further discussion of polymorphism, see Section 7.2.4.

9.4.5 Crystallisation kinetics

The response of a formulation to processing, especially cooling conditions, is dependent on the inherent crystallisation behaviour of the mixture of solids used in the product. Cooling conditions available in cosmetic product manufacturing frequently are not designed for rapid cooling, a fact the formulator needs to consider when selecting ingredients. Control of cooling conditions is also relevant when considering scale-up effects and product stability during storage. The most obvious influence of cooling conditions is on product consistency. In most systems, rapid cooling results in a massive burst of crystal nuclei that will have a limited potential of growth, resulting in small crystals and a harder consistency (Figure 9.5). The surface of the product is smooth and glossy if the crystals are small. Slow cooling results in fewer crystal nuclei that will grow to large sizes with time. Such systems are usually softer in consistency and spread more easily. However, uncontrolled growth can also lead to a grainy product with obvious "bloom" on the surfaces.

9.4.6 Combined effects

The combination of polymorphic behaviour and response on cooling will determine the morphology of the crystals produced. Depending on the shape and size distribution of the crystals, the consistency can be softer or harder. Needle-like crystals, characteristic of the beta-prime polymorphs of fats and wax esters, will give a higher degree of elasticity compared to the more regularly shaped crystals associated with the beta polymorphs. Shear during cooling will also influence the shape and the size distributions of the particles. Rapid cooling can sometimes lock the particles in an unstable polymorph that will slowly transform on storage, resulting in undesired consistency changes.

Both polymorphism and the solid phase content are dependent on the liquid phase used in the formulation. The solubility of the fats and waxes used will be different in different emollients and unexpected effects on product consistency and stability can be seen when the emollient composition is changed. In emulsions, the emulsion droplet sizes can also influence both solid phase content as well as polymorphic forms. If the emulsion droplets are small enough, a super-cooling of the oil phase can sometimes be observed with unstable crystal forms such as alpha being more stable.

In anhydrous formulations, such as balms and ointments, as well as colour cosmetics (lipsticks, pencils, mascaras and foundations), the compatibility between the solid phases becomes very important for product performance (Matsuda and Yamaguchi, 2001). Co-crystallising solids that cover a wide melting point range can give very stable formulations with excellent consistency, stability, and skin feel.

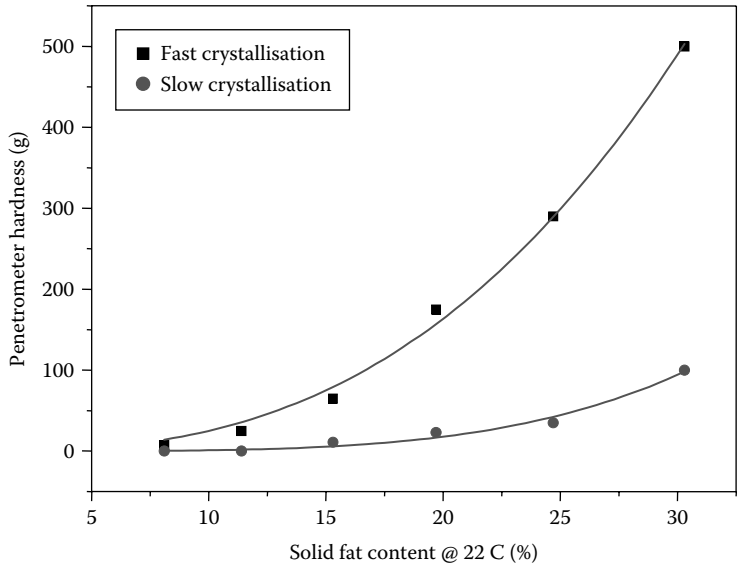


FIGURE 9.5 Hardness of emollient mixtures crystallised at different conditions: (a) rapidly cooled from melt to 20°C, cooling rate 10°C/minute, and (b) slowly cooled from melt to 20°C, cooling rate 0.3°C/minute. Solid phase content in the mixtures was determined by low resolution pulsed NMR at 20°C after tempering for 2 hours.

9.4.7 Bioactive lipids in skin-care applications

The skin is the largest organ of the human body, covering about 1.5 to 2 m^2, presenting a total thickness of less than 2 mm in most locations and weighing about 4 kg in adults. It is the only organ completely exposed to the environment, making the major role of the skin that of a barrier against air, microorganisms, and environmental pollutants. The outermost layer, stratum corneum or the horny layer, consists of cells named corneocytes embedded in a lipid matrix. The lipids form an intercellular lamellar sheet between the cornified cells and constitute the primary barrier of the skin. The structure and function of the stratum corneum is extensively studied and several models for the interaction between the epidermal lipids and the corneocytes have been proposed, including the "brick-and-mortar" of Michaels (Michaels, Chandrasekaran, and Shaw, 1975), the "domain mosaic model" of Forslind (Forslind, 1994), and more recently the "single gel phase" model of Norlén (Norlén, 2001). A schematic cross section through human skin is shown in Figure 9.6. The outermost part of the epidermis is normally considered as the main target for cosmetic and personal care products.

For optimal function, the skin requires a selection of different lipids. There are two main types of skin lipids; on the skin surface, there is the sebum, generated in the sebaceous glands and dominated by triacylglycerols, wax esters, and squalene. The epidermal lipids, which are generated in the epidermal cells, are essential for maintaining the integrity of the skin barrier by preventing the penetration of impurities, chemicals, microorganisms, and water, while also protecting against undesirable water loss through the skin. The main components of the epidermal lipids are ceramides (15 to 41%), free fatty acids (7 to 23%), cholesterol (13 to 34%), cholesterol esters (4 to 17%), cholesterol sulfate (1.5 to 4.5%), and triacylgly-cerols (5 to 25%) (Engblom, 1996).

9.4.8 Lipids in skin care

Lipids are important ingredients in all skin-care categories and of special importance for dry and sensitive skin, and for antiaging and protecting skin-care formulations. Apart from acting as emollients, many lipids also function as delivery agents for various bioactive materials in the formulations.

Dry-skin conditions have become a widespread problem in many parts of the world mainly due to life-style changes, including altered dietary patterns, changes in workplace conditions, and a comparatively older population. Dry skin is characterised by a reduced content of water and an altered lipid composition in the stratum corneum. A defective skin barrier results in increased water evaporation and an increased sensitivity to the environment. Thus, an ideal skin-care formulation should contain ingredients that improve barrier function and repair as well as supplement the natural epidermal lipids (Loden and Maibach, 2005; Park, 2001).

9.4.9 Essential fatty acids

Essential fatty acids are those polyunsaturated fatty acids (PUFA) that are necessary for good health, but cannot be synthesized in the body. Dry and atopic skin shows a decrease in linoleic acid content, the important precursor of ceramides essential for the barrier function of the skin (Horrobin, 2000). Both topical application and dietary intake of essential fatty acids have been shown to restore dry skin conditions as well as having therapeutic effects on skin disorders, such as atopic dermatitis, psoriasis, and

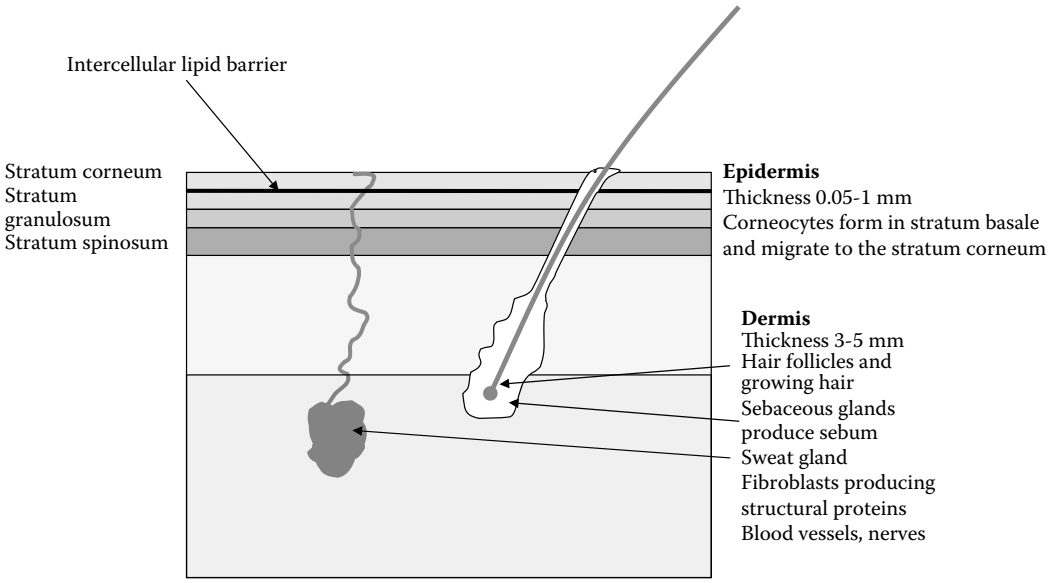


FIGURE 9.6 Schematic representation of skin structure.

acne (Conti, 1996; Spector, 1999). Common vegetable oils with a high content of essential fatty acids, such as linoleic acid, that can be used as emollients in skin-care products, are products derived from rapeseed, sunflower, corn, soybean, arachis (groundnut, peanut), and cottonseed.

Another polyunsaturated fatty acid – γ-linolenic acid – has also shown potential for treating dry skin conditions caused by atopic dermatitis. γ-Linolenic acid metabolism leads to antiinflammatory prostaglandins that can ameliorate the effects of inflammatory skin disorders (Horrobin, 2000; Ziboh, Miller, and Cho, 2000). Oils derived from evening primrose, borage, blackcurrant, and echium are known for their high content of γ-linolenic and stearidonic fatty acids (Section 2.3.1.5).

Due to the high sensitivity of polyunsaturated fatty acids with respect to oxidation, the addition of an optimised antioxidant system during oil processing is recommended. The added antioxidant will protect the oil during storage, but also contribute to the protection of the formulation.

9.4.10 Tocopherols

The human skin is rich in lipids, proteins and DNA, all of which are extremely sensitive to oxidation and a proper protection against oxidation is necessary for the health of the skin (Kohen, 1999; Rengarajan, 1999). Beside the obvious oxidative damage to the skin constituents, free radicals and reactive oxygen species can initiate inflammatory reactions and activate matrix metalloproteinases (Brenneisen, Sies, and Scharffetter-Kochanek, 2002). Free radicals are closely linked with aging and oxidative stress in the skin, being associated not only with decreased cell viability and DNA damage, but as a significant agent in the skin-aging process when the skin loses its elasticity and regenerative power. It, therefore, is essential to protect the skin against the effects of UV radiation and oxygen-derived free radicals and many formulation strategies to achieve this have been developed during the past decades.

The lipophilic tocopherols are essential for the stabilisation of biological membranes, especially those containing large amounts of polyunsaturated fatty acids. α-Tocopherol is particularly effective in protecting against oxidative damage of cellular membranes and biomolecules, such as lipids, proteins, and nucleic acids (Kohen 1999, Nachbar and Korting, 1995; Thiele et al., 2001). The tocopherols act as antioxidants primarily by a free radical scavenging mechanism. It has been shown to be of obvious physiological advantage to deliver tocopherols topically, which can ameliorate the early phase of an oxidative stress response. For example, topical application of vitamin E is shown to reduce the appearance of fine facial lines and wrinkles as well as increasing stratum corneum hydration and enhancing its water-binding capacity (Gehring, Fluhr, and Gloor, 1998). Synergistic effects between tocopherols and other antioxidants, such as flavonoids and ascorbic acid, are shown to offer significant antioxidant and membrane stabilising properties in human skin (Kitazawa et al., 1997; Shindo et al., 1994). In addition, α-tocopherol has been demonstrated *in vitro* to reduce the age-dependent increase of collagenase activity, potentially delaying the progression of skin aging (Ricciarelli et al., 1999).

The antiinflammatory action of γ-tocopherol has been demonstrated in dietary studies (Jiang et al., 2001; Jiang et al., 2000;, Jiang and Ames, 2003). The combination of α- and γ-tocopherol found in many seed oils is interesting for the dual activity these oils can have when used as emollients in topical applications. These nontoxic and biologically active substances are present at varying quantities in vegetable oils. Typical compositions for some common oils are given in Chapter 2.

9.4.11 Sterols and triterpene alcohols

Sterols (desmethylsterols), triterpene alcohols (4,4-dimethylsterols) and their derivatives such as hormone and vitamin precursors impart various important biological functions within the body. Desmethylsterols are commonly found in vegetable oils in concentrations ranging from 0.1 to 1% (Section 2.2.19; Table 2.47). The predominant sterol in seed oils is normally beta-sitosterol, but other sterols can also be found in significant amounts in specific plants. Triterpene alcohols are normally present in much lower concentrations in seed oils.

Uniquely, high concentrations of triterpene alcohols are found in shea butter, the fat extracted from the kernels of the shea tree (Vitellaria paradoxa or Butyrospermum parkii) (Peers, 1977), growing in the arid regions of sub-Saharan Africa.

9.4.12 Antiinflammatory effects of sterols and tocopherols

Phytosterols from rapeseed oil have been shown to impart antiinflammatory and healing effects on surfactant damaged skin (Loden and Andersson, 1996). The benefits using the combination of tocopherols and phytosterols in an emollient were demonstrated in a clinical study using rapeseed oil fractions with different levels of sterols and tocopherols. It was shown that a pretreatment with rapeseed oil fractions reduced the transepidermal water loss (TEWL) and erythema in skin treated with sodium lauryl sulfate solutions. The reference materials were petrolatum, an inert occlusive emollient, borage oil, which is rich in γ-linolenic acid, and a hydrocortisone cream, a known antiinflammatory preparation. The fractionated rapeseed oil showed activity comparable to the hydrocortisone cream, while no effects were observed with petrolatum and borage oil. Phytosterols are also known for having a structural role by interacting with the lameIlar lipid layers, strengthening the lipid barrier, and improving dry skin conditions, squamation, and erythema (Chlebarov, 1989).

9.4.13 Bioactivity of triterpene alcohols

There have been many bioactivity studies conducted with various types of triterpene alcohols. The studies performed with these alcohols indicate that there are at least two areas where interesting bioactive effects can be expected. The antiinflammatory effects of phytosterols, including the triterpene alcohols, are well demonstrated for both the free alcohols and their esters. The second effect is associated with the synthesis and degradation of the structural proteins collagen and elastin.

Many individual triterpene alcohols and their natural mixtures have been investigated for their antiinflammatory properties. Several studies show that the two amyrins as well as lupeol and butyrospermol are antiinflammatory in different types of inflammation models. For example, Akihisa et al. (1997) presented data on the antiinflammatory effect of a large number of triterpene alcohols found in Theaceae oils (Camellia and Sasanqua), including butyrospermol, lupeol, alpha- and beta-amyrin, as well as taraxasterol, psitaraxasterol, and 24-methylenedammarenol (all of them also found in high concentrations in shea butter). All of these triterpene alcohols (in the form of free alcohols) showed inhibitory activities in the same concentration range as the control substance indomethacin, when tested in an inflammation model in mice. The mechanism for the inflammatory action of lupeol and its esters was investigated by Fernandez et al. (2001). The antiinflammatory activity of lupeol was studied in models demonstrating effects on two different inflammatory pathways. Topically applied lupeol had a significant antiinflammatory effect in the TPA model (cyclooxygenase pathway), while the effect was less pronounced in the arachidonic acid-induced oedema (lipoxygenase pathway), only the highest concentration tested gave any significant effect. It was concluded that lupeol is an inhibitor of certain proinflammatory mediators, such as prostaglandin E2 (a cyclooxygenase metabolite) and cytokines, but not leukotrienes (lipoxygenase metabolites).

Some of the triterpene alcohols found in shea butter are also inhibitors of protein degrading enzymes, proteases. Different types of proteases are active in the skin, degrading collagen and elastin, two of the major structural proteins contributing to the toughness and strength of the skin. The production of collagen and elastin decreases with increasing age, resulting in thinner and less elastic skin. The effects of this natural aging process can be alleviated by stimulating collagen and elastin synthesis or by inhibiting the activity of the collagenases and elastases. Proteases are also implicated in the breakdown of connective tissue in rheumatoid arthritis and the triterpene alcohols and their derivatives have been investigated as alternatives to conventional pharmaceutical products, such as hydrocortisone and indomethacin.

Several studies have been conducted to evaluate the inhibitory effect of triterpene alcohols on different types of proteases. For example, Hodges et al. (2003) showed that lupeol and its palmitate and linoleate were inhibitors of trypsin activity, while no effect on porcine pancreatic elastase was observed. Metalloproteases (e.g., collagenase) and serine proteases (trypsin, chymotrypsin, porcine pancreatic elastase, human leukocyte elastase) are inhibited *in vitro* by various types of triterpenes, including lupeol and its esters. Rajic et al. (2000) showed that esterification increases the degree of inhibition of trypsin and chymotrypsin. Lupeol palmitate, lupeol linoleate, and alpha-amyrin linoleate were potent trypsin inhibitors, while free lupeol and alpha-amyrin were less efficient. Chymotrypsin was inhibited by lupeol, the other tested compounds being weaker inhibitors. These examples show that there is a potentially useful effect of the triterpene alcohols from shea butter to prevent aging effects on the skin by inhibiting the degrading activity of proteases. Triterpene derivatives are also known for stimulating collagenesis of skin fibroblasts (Laugel, 1998), indicating a second mechanism for delaying skin aging and changes due to lowered content of the structural proteins.

9.4.14 Products enriched with minor lipids

Normally the natural levels of triterpene alcohols, sterols, and tocopherols are too low for having extensive effect on topical application. Vegetable oils with naturally high contents of sterols include wheat germ oil, rapeseed oil, and soybean oil. Shea butter, avocado oil, rice bran oil, and olive oil also contribute to high levels of triterpene alcohols (4,4-dimethylsterols). The naturally occurring levels of these minor components can be increased by different processing methods including saponification followed by extraction and distillation (Clark, 1996). A nondestructive way of isolating these components is based on low-temperature solvent fractionation (Alander et al., 2005; Mellerup, Bach, and Enkelund, 2002). These concentrated fractions can be used as part of the emollient system in a cosmetic formulation to deliver the bioactive minor components to the skin.

References

Akihisa, T. et al. 1997, Triterpene alcohols from camellia and sasanqua oils and their anti- inflammatory effects, *Chem. Pharm. Bull. (Tokyo)*, **45**, 2016–2023.

Alander, J. et al. 2005, *Fractionation process*, EP 1084215, Karlshamns AB, (patent).

Brenneisen, P. et al. 2002, Ultraviolet-B irradiation and matrix metalloproteinases: from induction via signaling to initial events, *Ann. N. Y. Acad. Sci*, **973**, 31–43.

Chlebarov, S., 1989, *Notabene Medici*, vol. 2 and 3.

Clark, J.P. 1996, Tocopherols and sterols from soybeans, *Lipid Technol.*, **8**, 111–114.

Conti, A., 1996, Seasonal influences on stratum corneum ceramide I fatty acids and the influence of topical essential fatty acids, *Int. J. Cosmet. Sci.*, **18**, 1–12.

Engblom, J., 1996, *On the phase behaviour of lipids with respect to skin barrier function,* Department of Food Technology, Lund University, Lund, Sweden.

Fernandez, M.A. et al. 2001, New insights into the mechanism of action of the anti-inflammatory triterpene lupeol, *J. Pharm. Pharmacol.*, **53**, 1533–1539.
Forslind, B., 1994, A domain mosaic model of the skin barrier, *Acta Derm. Venereol.*, **74**, 1–6.
Gehring, W. et al. 1998, Influence of vitamin E acetate on stratum corneum hydration, *Arzneimittelforschung.*, **48**, 772–775.
Hodges, L.D. et al. 2003, Antiprotease effect of anti-inflammatory lupeol esters, *Mol. Cell Biochem*, **252**, 97–101.
Horrobin, D.F., 2000, Essential fatty acid metabolism and its modification in atopic eczema, *Am. J. Clin. Nutr.*, **71**, 1 Suppl, 367S–372S.
Jiang, Q. and Ames, B.N., 2003, Gamma-tocopherol, but not alpha-tocopherol, decreases proinflammatory eicosanoids and inflammation damage in rats, *FASEB J.*, **17**, 816–822.
Jiang, Q. et al. 2001, Gamma-tocopherol, the major form of vitamin E in the U.S. diet, deserves more attention, *Am. J. Clin. Nutr.*, **74**, 714–722.
Jiang, Q. et al. 2000, Gamma-tocopherol and its major metabolite, in contrast to alpha-tocopherol, inhibit cyclooxygenase activity in macrophages and epithelial cells, *Proc. Natl. Acad. Sci. U.S.A.*, **97**, 11494–11499.
Kitazawa, M. et al. 1997, Interactions between vitamin E homologues and ascorbate free radicals in murine skin homogenates irradiated with ultraviolet light, *Photochem. Photobio.*, **65**, 355–365.
Kohen, R., 1999, Skin antioxidants: their role in aging and in oxidative stress—new approaches for their evaluation, *Biomed. Pharmacother.*, **53**, 181–192.
Laugel, C., 1998, Incorporation of triterpenic derivatives within an o/w/o multiple emulsion: structure and release studies, *Int. J. Cosmet. Sci.*, **20**, 183–191.
Loden, M. and Andersson, A.C., 1996, Effect of topically applied lipids on surfactant-irritated skin, *Br. J. Dermatol.*, **134**, 215–220.
Loden, M. and Maibach, H.I., 2005, *Dry Skin and Moisturizers: Chemistry and Function*, 2nd ed., CRC Press, Boca Raton, FL.
Matsuda, H. and Yamaguchi, M., 2001, Separation and crystallization of oleaginous constituents in cosmetics —sweating and blooming, in *Crystallization Processes in Fats and Lipid Systems*, Garti, N. and Sato, K., Eds, Marcel Dekker, New York, pp. 485–503.
Mellerup, J. et al. 2002, A process for preparing vegetable oil fractions rich in non-tocolic, high-melting, unsaponifiable matter, WO0250221, Aarhus Oliefabrik A/S, (patent).
Michaels, A.S. et al. 1975, Drug permeation through human skin; theory and *in vitro* experimental measurement, *AIChE J.*, **21**, 985–996.
Nachbar, F. and Korting, H.C., 1995, The role of vitamin E in normal and damaged skin, *J. Mol. Med.*, **73**, 7–17.
Norlen, L., 2001, Skin barrier structure and function: the single gel phase model, *J. Invest. Dermatol.*, **117**, 830836.
Park, W.S., 2001, Improvement of skin barrier function using lipid mixtures, *SÖFW-J.*, **127**, 9.
Peers, K.E., 1977, The non-glyceride saponifiables of shea butter, *J. Sci. Food Agric.*, **28**, 1000–1009.
Rajic, A. et al. 2000, Inhibition of serine proteases by anti-inflammatory triterpenoids, *Planta Medica*, **66**, 206–210.
Rengarajan, H., 1999, Skin delivery of Vitamin E, *J. Cosmet. Sci.*, **50**, 249–279.
Ricciarelli, R. et al. 1999, Age-dependent increase of collagenase expression can be reduced by alpha-tocopherol via protein kinase C inhibition, *Free Radic. Biol. Med.*, **27**, 729–737.
Shindo, Y. et al. 1994, Enzymic and non-enzymic antioxidants in epidermis and dermis of human skin, *J. Invest. Dermatol.*, **102**, 122–124.
Small, D.M., 1984, Lateral chain packing in lipids and membranes, *J. Lipid Res.*, **25**, 1490–1500.
Spector, A.A., 1999, Essentiality of fatty acids, *Lipids*, **34**, S1–S3.
Thiele, J.J. et al. 2001, The antioxidant network of the stratum corneum, *Curr. Probl. Dermatol.*, **29**, 26–42.
Wiechers, J.W., 2003, The EEC concept: how emollients and emulsifiers work together to create more efficacious cosmetic products, *SÖFW-J.*, **129**, 2225.
Wiechers, J.W. et al. 2004, Formulating for efficacy, *Int. J. Cosm. Sci.*, **26**, 173–182.
Ziboh, V.A. et al. 2000, Metabolism of polyunsaturated fatty acids by skin epidermal enzymes: generation of antiinflammatory and antiproliferative metabolites, *Am. J. Clin. Nutr.*, **71**, 361S–366S.

9.5 Lubricants

9.5.1 Introduction

Lubrication is the use of a material to improve the smoothness of movement of one surface over another. The material used to achieve this is called a lubricant. These are usually liquids or semiliquids, but may be solids or gases or any combination of solids, liquids, and gases.

Smoothness of movement is improved by reducing friction. However, this is not always the case, and there may be situations in which it is more important to maintain steady friction than to obtain the lowest possible friction.

In addition to simply lubricating the metal parts that come in contact, lubricants are expected to reduce or control friction between metal parts to save energy, reduce wear or prevent weld of metal surfaces, clean metal surfaces of dirt or sludge to prevent scratching or scoring, clean metal surfaces of water and acids to prevent corrosion, and often to prevent overheating.

Annual consumption of oil-based lubricants in the U.S. is close to 10 million metric tons and valued at more than $8 billion (USD). The U.S. usage accounts for 27% of the world lubricant consumption (37.5 million metric tons). With a share of almost a third of global lubricant consumption, Asia-Pacific remains the leading lubricant region, followed by Europe and North America. More than 70% of total lubricant volume is used in motor oils for automotive engines and approximately 10% in hydraulic fluids. Other application areas, mostly industrial lubricants, are less significant.

Lubricants are usually divided into four basic classes.

9.5.1.1 Liquid lubricants or oils

Liquid lubricants cover mineral oils, fatty oils, synthetics, emulsions, or even process fluids. Mineral oils are most often used as the base stock in lubricant formulation. Synthetic oils (synthetic esters, phosphate esters, silicones, and fluorocarbons) are used for lubricants, which are

expected to operate in extreme conditions, i.e., high performance aircraft, missiles, and in space. Vegetable oils are used in formulating lubricants intended for the food and pharmaceutical industries, but even in these applications their use is quite limited.

The advantages and disadvantages of oils stem from their ability to flow easily. Thus, on the credit side, it is very easy to pour them from a container, to feed them into a bearing by dripping, splashing or pumping, and to drain them out of a machine when no longer fit for use. Other advantages are the cooling of a bearing by carrying away heat, and cleaning it by removing debris.

9.5.1.2 Greases

Greases are more than very viscous lubricating oils. Technically they are oils, which contain a thickening agent to make them semisolid. Thus, grease consists of oil constrained by microscopic thickener fibres to produce a stable and colloidal structure or gel.

Greases contain three basic active ingredients: a base oil, additives, and thickener. The base oil may be mineral, synthetic, or vegetable oil. For thickeners, metal soaps and clays are mostly used apart from some nonsoap thickeners, which are inorganic (silica and bentonite clays) or organic (polyurea) materials. Metal soaps are prepared by heating fats or oils in the presence of an alkali, e.g., NaOH. Fats and oils can be animal or vegetable origin, and are produced from cattle, fish, castor bean, coconut, cottonseed, etc. The reaction products are soap, glycerol, and water. Soaps are very important in the production of greases. The most commonly used soap-type greases are calcium, lithium, aluminum, and sodium. In most cases the oil plays the most important role in determining the grease performance, but in some instances the additives and the thickener can be critical. Very often additives, which are similar to those in lubricating oils, are used.

The behavior of greases is very similar to that of oils, but the former are used where the advantages of easy flow are outweighed by the disadvantages. Greases do not easily leak out of a machine or container, do not migrate away, and will form an effective seal against contaminants.

9.5.1.3 Solids or dry lubricants

The lubricants used in solid form may be bulky solids, paint-like coatings, or loose powders interposed between two surfaces in moving contact. Depending upon the nature of the two surfaces, a wide variety of solid materials can reduce friction and prevent seizure. For example, dust, sand, or gravel on the surface of a road can cause vehicles to skid because they decrease friction between tires and the road surface. The majority of solid lubricant applications are met by only three materials: graphite, molybdenum disulfide, and PTFE (polytetrafluoroethylene).

The advantages and disadvantages of solid lubricants are rather like the extremes for greases, where the lubricant will not flow at all. Similarly, the advantages and disadvantages of gas lubricants are like the extremes of oils, where the flow properties are almost too good.

9.5.1.4 Gases

The gas used in gas bearings is generally air, but any gas can be used so long as it does not attack the bearings or decompose.

9.5.2 Lubricant base oils

Lubricants are made from a base oil (80 to 100%) and suitable additive package. The additives are used to enhance the most important properties for each specific application. Most base oils originate from petroleum, including many synthetic esters and poly-alpha-olefins. Less than 2% of the base oils are the product of oleochemical and related industries. The primary area of their application has been as hydraulic fluids. The various base oils used in lubricant formulations are described below.

9.5.2.1 Mineral/petroleum oils

Since mineral oils are the most widely used lubricating oils, they are often the standard with which other oils are compared. Mineral oils are generally oils obtained from petroleum, although they may come from similar sources, such as oil shales and tar stands. The mineral oils used for lubrication were originally distilled fractions with suitable viscosity for lubrication. But now they are obtained through various steps of refining and extraction before being blended with specialty chemicals, called additives, to enhance existing performance characteristics. Mineral oils are mainly hydrocarbons of three basic types: paraffins, naphthenes, and compounds containing aromatic systems. Finally, there is usually a small proportion (~2%), containing aromatic ring systems. In addition to these hydrocarbons, there may be small quantities of compounds containing other elements, such as oxygen, sulfur, phosphorus, or nitrogen. Mineral base oils used for lubricants are generally molecules with 20 to 50 carbon atoms.

Mineral base oils continue to be economical and provide superior performance characteristics in various applications, but they present a potential hazard because they are not readily biodegradable and are environmentally toxic. During the past few decades, the level of public awareness of environmental issues has risen considerably and materials that do not meet accepted standards of biodegradability are disapproved of by environmentalists and government bodies. It is believed that federal directives in the U.S. will be strictly imposed in the next 2 to 5 years, eventually resulting in newer regulations on the development and application of environmentally friendly base stocks.

9.5.2.2 Synthetic fluids

Many of the alternatives to mineral oils are synthetic materials manufactured from various feed stocks by chemical processes. There are several types of synthetic oil, which differ from each other in performance and properties. Widely used synthetic oils include hydrocarbons, diesters, polyol esters, phosphate ester, silicones, polyglycols, polyphenyl ethers and perfluoroalkyl polyethers. Out of these fluids, low molecular weight polyalphaolefins (PAO 2, PAO 4, essentially 20:1 and 10:1 mixtures of hydrogenated dimers and trimers of α-decene), dialkyl adipates (isodecyl, isotridecyl), or polyol esters (mostly neopentyl glycol or trimethylol propane with fatty acids) are biodegradable synthetic base oils (Rudnick, 2002). The most important are PAOs, which are branched-chain paraffins, and resemble highly refined mineral oils in their structure, properties, and performance. Their inherent oxidation resistance is good, but their boundary lubrication is not as good as that of the highly refined mineral oils.

Synthetic oils offer improved performance but at a price. Most of the esters are biodegradable and offer superior thermal and oxidative stability. Prices for these niche products are higher than vegetable oils and significantly higher than petroleum base stocks. Although specialized synthetic lubricants have been successfully replacing mineral oil in various applications for many years, general-purpose synthetic lubricants have only recently been introduced on a large scale. They are generally more expensive, but have better oxidation and thermal resistance than mineral oils. Low resistance to oxidative degradation and poor low temperature behavior of vegetable base oils have triggered the development and rise in demand for biodegradable synthetic base stocks.

9.5.2.3 Natural oils

These include vegetable oils and animal fats. They are usually excellent boundary lubricants, but they are much less stable than mineral oils, and tend to break down to give sticky deposits. Vegetable oils are used in various industrial applications, such as emulsifiers, lubricants, plasticizers, surfactants, plastics, solvents, and resins.

The natural oils are mainly triacylglycerols (98%), diacylglycerols (0.5%), free fatty acids (0.1%), sterols (0.3%), and tocopherols (0.1%). The fatty acids exist mostly as esters of glycerol. They have a carbon chain length of 12 to 24 carbon atoms. The predominant carbon chain length of the fatty acids from plants is 18 carbon atoms. They are the fully acylated derivative of glycerol. The resulting structure is abundantly present in vegetable oils, and resembles a tuning fork in shape. Vegetable oils have 6 oxygen atoms and around 60 carbon atoms per molecule, compared to an average of 30 carbon atoms in mineral base oils. The fatty acid constituents of triacylglycerol molecules may be all identical (e.g., triolein in olive oil and tripalmitin in palm oil), two different, or all different. Tocopherols have important antioxidant properties. They are a series of benzopyranols with 1, 2, or 3 methyl groups attached to the phenolic ring along with a C_{16} side chain on the pyran ring. Antioxidant activity as a result of tocopherol content is high in corn oil, soybean oil, walnut oil, and cottonseed oil (Rossell et al., 1991 and Section 8.1). Fully refined oils (refined, bleached, and deodorized) have free fatty acid contents of less than 0.1% (normally 0.01 to 0.05% generally expressed as oleic acid). The quality of crude oils largely depends on the content of free fatty acids and quality generally deteriorates as the acid content rises. Free fatty acids are produced by hydrolysis of oils catalyzed by acids or enzymes.

9.5.2.3.1 Economics and availability

Until recently, mineral oil had a significant cost advantage over vegetable oils and so petroleum has been the base oil of economic choice. Recent rises in oil prices along with the low vegetable oil prices has narrowed the price difference to close to $0.05/lb (0.11/kg), and there is now more interest in vegetable oils base stocks (Table 9.11). Though most lubricants used currently originate from petroleum base stocks, vegetable oils have seen a promising increase as biodegradable fluids over the last decade. Environmental concerns as well as economics and performance issues will drive the market share for these oils and government legislation may force this issue. Today, less than 2% of the base stocks are products of the oleochemical and related industries with the primary area of their application in hydraulic fluids, which have the highest need for biodegradable lubricants (Padavich et al., 1995). This is consumed at approximately 5MMT/year in the U.S. market.

Because soybean oil provides nearly 80% of the seed oils produced annually in the U.S. and is the cheapest vegetable oil in the U.S. market, its relatively low cost and dependable supply make it one of the more important sources of lubricant base oil in the U.S. Another commonly used vegetable oil in lubricant applications is rapeseed oil due to its relatively good oxidative stability

TABLE 9.11 Cost of various base oils in 2004

Base Oils	Cost ($/lb[a])	Cost ($/kg)
Vegetable oils		
Soybean oil	0.22–0.25	0.48–0.55
Canola oil	~0.29	0.638
High-oleic sunflower oil	0.45–0.55	0.99–1.21
Synthetic oils		
TMP[b] trioleate	~1.00	~2.20
TMP[b] trioleate (high-oleic)	~1.25	~2.76
PAO8[c]	~0.90	~1.98
Mineral base oil		
(Group I and II)	~0.25	~0.55

[a] Price will vary based on quantity, customer supplier relationships, and market conditions.
[b] TMP, trimethylolpropane.
[c] PAO, polyalphaolefin.

compared to other vegetable oils, its reasonable cost, and its wide availability in Europe and North America (Whitby, 2004). Other vegetable oils used as lubricants include olive, sunflower, and castor oil.

9.5.2.3.2 Natural oil advantages

Environmental concerns over the use of petroleum-based products in activities such as forestry, farming, mining, boating, and others, has led to increased interest in the use of environmentally friendly fluids. The beneficial aspects of vegetable oils as lubricants are mainly their biodegradability and nontoxicity, which are not exhibited by conventional mineral base oils (Randles et al., 1992; Battersby et al., 1989). Their volatility is low due to the high molecular weight triacylglycerol structure and they have a narrow range of viscosity change with temperature (high viscosity index, VI) and high flash point. Lower volatility results in decreased exhaust emission and high VI means the oil is a naturally multigrade oil. The high VI index of vegetable oils eliminates the need for the polymeric VI improvers used with mineral oils, resulting in high shear stability of vegetable oils. The ester linkages deliver inherent lubricity on metallic surfaces due to their adhesive property. Higher lubricity or lower friction results in more power and better fuel economy. Vegetable oils also have superior solubilizing power for contaminants and additive molecules compared to mineral base fluids. The ester structures provide improved solvency for polar deposits and sludge containing worn metals. Further, vegetable oils have higher shear stability.

9.5.2.3.3 Natural oil disadvantages

Performance limitations of vegetable oil base stocks are poor oxidative stability due to *bis*-allylic hydrogen atoms in the fatty acyl chain, deposit-forming tendency, low temperature solidification, and low hydrolytic stability. Oxidation results in increased acidity, corrosion, and viscosity and volatility of the lubricant. The inherently narrow viscosity range limits their use in various viscosity grades, especially at lower viscosities. The polar nature of triacylglycerols contributes to air entrainment and problems of foaming. On the other hand, parameters like lubricity, antiwear protection, load carrying capacity, rust prevention, foaming, demulsibility, etc., are mostly additive-dependent. Antioxidant additives (Becker et al., 1996) have limited capability to improve on oxidative stability; therefore, other approaches are required to improve the above characteristics. The performance limitations of vegetable oil base stocks can be overcome by genetic modification, chemical modification, processing changes, and development in additive technology.

9.5.2.4 Chemically modified vegetable oils

Low temperature testing shows that vegetable oils solidify at –20°C on long-term exposure. Poor oxidative stability of vegetable oils is due to *bis*-allylic hydrogen atoms in the fatty acyl chain. Chemical modifications, therefore, are necessary to suppress or eliminate triacylglycerol crystallization and to improve oxidation stability. The inherent problems of poor low temperature performance and oxidation stability in vegetable oils can be partially improved by a variety of reactions at either the fatty acid carboxy groups or the hydrocarbon chain depending on end use applications. More than 90% of chemical modifications have been those occurring at the fatty acid carboxy groups, while less than 10% have involved reactions at fatty acid hydrocarbon chain. Without sacrificing favorable viscosity–temperature characteristics and lubricity, unsaturated vegetable oils can be converted into thermo-oxidatively stable products by saturation of carbon–carbon double bonds using alkylation, arylation, cyclization, hydrogenation, epoxidation, and other reactions. Chemical modifications at the carboxyl group of vegetable oils include transesterification, hydrolysis, etc. Reactions at double bond and carboxyl positions of vegetable oils are discussed by Erhan et al. (2005) and Hwang et al. (2002). With improvements in their low temperature performance and oxidation stability, they can be used in automotive and industrial lubricant applications with the additional advantage of being clean, biodegradable, nontoxic, and requiring lesser amounts of expensive additives (e.g., VI improvers are not required and lesser amounts of antiwear/antifriction additives are required).

9.5.2.5 Genetically modified vegetable oils

Monounsaturated fatty acids are more thermally stable than polyunsaturated fats and, therefore, are highly desired components in vegetable oils. Ideally, vegetable oils having high stability and low pour points contain only monounsaturated fatty acids. Advanced plant breeding and genetic engineering has enabled the development of vegetable oils with higher concentration of oleic acid and lower linoleic and linolenic acids. The oleic content of high-oleic varieties of rapeseed and soybean oil is 75 to 85%, while that of high-oleic sunflower oil is 80 to 92% (Whitby, 2004). The oxidative stabilities of these high-oleic oils are three to six times greater than normal vegetable oils. Specialty canola oil products that Monsanto (Monsanto Company, St. Louis, MO) expects to market include oil containing medium-chain fatty acids for lubricants as well as for nutritional and high-energy food products (Schmidt et al., 2005).

9.5.3 Lubricant additives

Lubricant additives are chemicals, nearly always organic or organo-metallic, that are added to oils in quantities of a few weight percent to improve the lubricating capacity and durability of the oil. Specific purposes of various lubricant additives are discussed below.

Wear and friction improvers are used to improve the wear and friction characteristics by adsorption and

extreme pressure (EP) lubrication. This includes adsorption or boundary additives, antiwear additives, and extreme pressure additives. The adsorption or boundary additives in current use are mostly the fatty acids and the esters and amines of these fatty acids. Common examples of antiwear additives are zinc dialkyldithiophosphate, tricresyl phosphate, dilauryl phosphate, diethyl phosphate, dibutyl phosphate, tributyl phosphate, and triparacresyl phosphate. The most commonly used EP additives are dibenzyldisulfide, phosphosulfurized isobutene, and chlorinated paraffin, sulfurchlorinated sperm oil, sulfurized derivatives of fatty acids and sulfurized sperm oil, cetyl chloride, mercaptobenzothiazole, chlorinated wax, lead naphthenates, chlorinated paraffinic oils, and molybdenum disulfide.

Antioxidants improve the oxidation resistance and, thus, prevent a gradual increase in the viscosity and acidity of oil. Widely used antioxidant additives are zinc dialkyldithiophosphate, metal deactivators, phenol derivatives, amines, and organic phosphates. Sulfur-based EP and antiwear additives are also quite effective as antioxidants. Sulfur and phosphorus in elemental form or incorporated into organic compounds are also effective as antioxidants and antiwear additives.

To control the corrosion of metal parts, corrosion inhibitors and rust inhibitors are used. Corrosion inhibitors are used to protect the nonferrous surfaces of bearings, seals, etc., against corrosive attack by oxidation products and additives containing reactive elements, such as sulfur, phosphorus, iodine, or chlorine. The commonly used corrosion inhibitors are benzotriazole, substituted azoles, zinc diethyldithiophosphate, zinc diethyldithiocarbamate, and trialkyl phosphites. Rust inhibitors are used to protect ferrous components against corrosion. Widely used rust inhibitors are metal sulfonates (i.e., calcium, barium, etc.), amine succinates, or other polar organic acids.

Contamination control additives restrict contamination by reaction products, wear particles, and other debris. The other possible contaminants are soot from inefficient fuel combustion, unburnt fuel, breakdown products of the base oil, corrosion products, dust from the atmosphere, organic debris from microbiological decomposition of the oil, etc. Without proper control of contamination, the oil will lose its lubricating capacity, become corrosive and become unsuitable for service. Additives, which prevent the development of all these detrimental effects, are known as "detergents" or "dispersants." Mild dispersants are typically low molecular weight polymers of methylacrylate esters, long chain alcohols, or polar vinyl compounds. Over-based dispersants are calcium, barium, or zinc salts of sulfonic, phenol, or salicylic acids.

Viscosity index (VI) improvers reduce excessive decrease of lubricant viscosity at high temperatures. They are usually high molecular weight polymers that are dissolved in the oil and change shape from spheroidal to linear as the temperature increases. Unfortunately these additives are easily degraded by excessive shear rates and oxidation. Under high shear rates, VI improvers can suffer permanent or temporary viscosity loss.

Pour point depressants (PPDs) enhance lubricant characteristics by reducing the pour point through interfering with the crystallization mechanism. PPDs do not prevent wax crystallization in the oil, but they are absorbed on the wax crystals and, thus, reduce the amount of oil occluded on the crystal. Reducing the crystal volume permits lubricant flow. Typically used PPDs are maleic anhydride-styrene copolymers and polymethacrylates. These can be used in mineral as well as vegetable oils, though a higher percentage is generally required in the latter.

Antifoam agents/foam inhibitors include silicones and miscellaneous organic copolymers.

The most common package of additives used in oil formulations contains antiwear and extreme pressure lubrication additives, oxidation inhibitors, detergents, dispersants, viscosity improvers, pour point depressants, and foam inhibitors.

9.5.4 Physico-chemical and performance properties of bio-based lubricant base oils

The physical and chemical properties of vegetable oils are determined mainly by the fatty acid (FA) profile. Table 9.12 shows the FA composition of some vegetable oils that are being used as potential lubricant base oils for industrial applications and also chemical properties associated with C = C unsaturation.

High unsaturation in the triacylglycerol molecule (and particularly high levels of 18:2 and 18:3) increases the rate of oxidation resulting in polymerization and an increase in viscosity (Brodnitz, 1968). On the other hand, high saturation increases the melting range of the oil (Hagemann et al., 1972). Therefore, suitable adjustment between low temperature properties and oxidative stability must be made when selecting a vegetable oil base stock for a particular industrial application. The fatty acids can be saturated or unsaturated resulting in a straight chain or bent chain configuration, respectively. With the increase of double bonds in the chain, ability to gain a close packed conformation is prevented and, therefore, the oil remains liquid. The higher the IV, the more unsaturated (the greater the number of double bonds) the oil and, therefore, the higher is the potential for the oil to polymerize through oxidation. Any attempt to increase the saturation content of the oil through hydrogenation will increase the melting range temperature of the oil as shown in Table 9.13 (Swern, 1970). An IV of less than 25 is required if the neat oil is to be used for long-term applications in unmodified diesel engines. Triacylglycerols in the range of IV 50 to 100 may result in decreased engine life and, in particular, will reduce the life of fuel pump and injector.

Most vegetable oils are unsuitable for lubricant applications due to their high saturated or polyunsaturated

TABLE 9.12 Analytical data of vegetable oils used as lubricant base oils

Vegetable Oil	Fatty Acid Composition[a] (%)					IV[b]
	16:0	18:0	18:1	18:2	18:3	
Soybean oil	11.1	4.8	24.2	53.6	6.3	131.0
High-oleic soybean oil	6.2	3.0	83.6	3.7	1.7	85.9
Sunflower oil	6.1	5.3	21.4	66.4	–	124.8
High-oleic sunflower oil	3.5	4.4	80.3	10.4	–	80.8
Safflower oil	6.4	2.5	17.9	73.2	–	135.2
High-oleic safflower oil	4.6	2.2	77.5	13.2	–	83.6
High-linoleic safflower oil	6.7	2.6	14.6	75.2	–	121.2
Rapeseed[c]	3.0	1.0	16.0	14.0	10.0	99.1
Corn oil	10.6	2.0	26.7	59.8	0.9	119.9
Cottonseed oil	18.0	2.0	41.0	38.0	1.0	109.1

[a] Gas chromatography analysis (16:0 palmitic, 18:0 stearic, 18:1 oleic, 18:2 linoleic, 18:3 linolenic), AACC Method 58-18, 1993.

[b] Iodine value (mg I_2/g), AOCS method, Cd 1-25, 1993.

[c] Rapeseed oil also contains other fatty acids, such as 1% of 20:0, 6% of 20:1, and 49% of 22:1.

TABLE 9.13 Melting point and iodine values of some vegetable oils

Oil	Iodine Value	Approx. Melting Point (°C)
Coconut oil	18	25
Palm kernel oil	16–19	24
Palm oil	54	35
Olive oil	81	–6
Castor oil	85	–18
Rapeseed oil	98	–10
Cotton seed oil	105	–1
Sunflower oil	125	–17
Soybean oil	130	–16
Linseed oil	178	–24
Tristearin (18:0)	0	74
Triolein (18:1)	86	5
Trilinolein (18:2)	173	–11
Trilinolenin (18:3)	261	–24

fatty acid content. The oxidation stability of polyunsaturated fatty acids can be improved significantly by converting them to saturated fats; this, however, will cause the low temperature behaviour of the material to deteriorate. Optimally, vegetable oils with high oxidation stability and low pour points are the high oleic varieties. The oxidation stability of such oils is three to six times greater than that of conventional vegetable oils. Such oils will provide both high thermo-oxidation stability and reasonably low temperature flow properties.

Bio-based lubricants that are accepted as environmentally friendly must pass bench tests designed to evaluate the potential performance as lubricants in addition to biodegradability and toxicity.

9.5.4.1 Viscosity

The primary consideration for any base oil to be used in lubricant formulation is its viscosity. Viscosities of base oils are mostly reported as kinematic viscosities measured at 40 and 100°C, according to ASTM (American Society for Testing and Materials) standard method D445-95 (Annual Book of ASTM Standards, 2000). The viscosity of any fluid changes with temperature, increasing as temperature decreases, and decreasing as temperature rises. Viscosity may also change with alterations in shear stress or shear rate. To compare base oils with respect to viscosity variations with temperature, ASTM Method D2270 provides a means to calculate a VI. This is an arbitrary number used to characterize the variation of kinematic viscosity of a base oil with temperature. The calculation is based on kinematic viscosity measurements at 40 and 100°C. For oils of similar kinematic viscosity, the higher the viscosity index, the smaller the effect of temperature. The benefits of higher VI are:

1. Higher viscosity at high temperature, which results in lower oil consumption and less wear.
2. Lower viscosity at low temperature, which for engine oil may result in better starting capability and lower fuel consumption during warm-up.

Other viscosity measurements are used to check properties at low or high temperatures. The Cannon minirotary viscometer is used to measure low temperature properties (yield stress and apparent viscosity) as per ASTM standard method D4684-97. ASTM method D4624-93 is used to determine if the test oils have the appropriate high-temperature characteristics required for an engine. This test is aimed at the performance of lubricants in the bearing systems of an engine.

The viscosities of vegetable oils, synthetic biodegradable lubricant base oils polyalphaolefin (PAO), trimethylolpropane (TMP) ester, and adipate, and mineral base oil (Erhan et al., 2000) are compared in Table 9.14. The mineral oil is a nonbiodegradable base oil mostly used for formulations of automotive lubricants. Except for natural antioxidants, these fluids do not have any additives. The vegetable oils have excellent VI. The linear fatty acids contribute to the high VI because the molecule is generally long.

TABLE 9.14 Viscosities and pour points of vegetable oil, synthetic oil, and mineral base oils

	KV[a] at 40°C, cST ASTM D445	KV at 100°C, cST ASTM D445	VI[b] ASTM D2270	Pour Points, °C ASTM D97
Vegetable oils				
Soybean	31.5	7.6	227	–9
Sunflower	31.6	7.7	226	–
High-oleic sunflower	39			–12
Canola	33			–18
Corn	31.9	7.7	223	–15
Rapeseed	40.3	9.1	217	–18
Olive	38.3	8.4	203	–9
Peanut	36.9	8.3	212	3
Castor	255.5	19.5	87	–24
Synthetic oils				
Diisotridecyl adipate	27	5.4	139	–51
TMP[c] trioleate	46.8	9.4	191	–39
PAO2[d]	5.54	1.8		–65
PAO4	16.8	3.9	129	–70
PAO6	31	5.9	138	–68
PAO8	45.8	7.8	140	–63
Mineral oil	65.6	8.4	97	–18

[a] Kinematic viscosity.
[b] Viscosity Index.
[c] Trimethylolpropane.
[d] Polyalphaolefin (kinematic viscosity 2 mm^2/s at 100°C).

9.5.4.2 Low temperature properties

Pour point measurement is most commonly used to check the low temperature properties of the oils. Pour points of various base oils are shown in Table 9.14. Overall, the data shows that low temperature properties of vegetable oils are inferior to those of synthetic base oils or even mineral oil. In vegetable oils, castor oil demonstrates PP notably lower than those of soybean, high-oleic sunflower, and canola oils, suggesting that hydrogen bonding between the hydroxy groups of ricinoleic acid interferes with the crystal growth. It must be noted that some fluids still pour after quite significant durations at slightly lower temperatures than their determined PP. A good example is castor oil, which pours after more than 24 hours storage at –25°C, although its PP appeared as –24°C in triplicate runs. Increasing molecular weight of fatty acids and full saturation contribute to the increase in PP, whereas *cis* unsaturation favors the decrease.

The relatively poor low temperature flow properties of vegetable oils arise from the appearance of waxy crystals that rapidly agglomerate resulting in the solidification of the oil. A vegetable oil is a complex mixture; therefore, the transition from liquid to solid state occurs over a wide temperature range involving several polymorphic forms (α, β', β) (Hagemann et al., 1983; Hagemann, 1988). Wax appearance and crystallization is a slow continuous process when the microcrystalline structures initially formed become macrocrystalline and rapidly change to a solid-like consistency. This results in a rapid viscosity increase leading to poor pumpability, lubrication, and rheological behavior. Wax crystallization at low temperature is also controlled by steric and geometrical constraints in these molecules. The fatty acid chains of triacylglycerol molecule have a "tuning fork" conformation and undergo molecular stacking during the cooling process. Another problem with vegetable oils is the manner in which they solidify. Mineral oil, which is a mixture of short- and long-branched chains, solidifies a little at a time under cold conditions, displaying a cloud point when the first solid appears in the liquid, and a pour point when the apparent viscosity of the solid/liquid mix is too high to allow immediate gravity flow. Vegetable oil molecules are mostly of the same size, so they solidify at very nearly the same temperature, giving vegetable oils a freezing point rather than a pour point. This can result in oil solidification after more than few days in cold winter weather. In vegetable oil triacylglycerols, the presence of double bonds in the fatty acyl chain influences the low temperature behaviour as shown in Table 9.13. The decreased melting temperatures of these compounds are a result of disorganization of the crystalline lattice by the presence of double bonds. It has been firmly established (de Jong et al., 1991; D'Souza et al., 1991) that presence of *cis* unsaturation, lower molecular weights, and diverse chemical structures of triacylglycerols favour lower temperatures of solidification. This demonstrates the contradiction of having both low temperature properties and the best possible oxidative stability in a given triacylglycerol molecule.

PPDs are used to suppress formation of large crystals during solidification, although the mechanism of PPD action on triacylglycerol crystallization remains undisclosed (Bentz et al., 1969). PPDs, like polymethacrylate, allow inclusion of the PPD molecule's branches into the growing crystal (Erhan, 2004). The effect of PPDs on pour points of vegetable oils (Table 9.15) shows that an amount of 0.4% by weight of PPD significantly reduces rates of solidification. Increased amount of PPD may slow down solidification, but further depression ceases quite rapidly. Low temperature and cold storage properties of vegetable oils, thus, do not respond appreciably to the PPD, as opposed to mineral oils (Asadauskas et al., 1999) and have

TABLE 9.15 Effect of PPD (polyalkylmethacrylate copolymer of 8000 amu, canola oil carrier 1:1.) on pour points (°C) of vegetable oils

	Amount of PPD % (w/w)			
Vegetable Oil	**0**	**0.4**	**1**	**2**
Soybean	–9	–18	–18	–18
High-oleic sunflower	–12	–21	–24	–24
Canola	–18	–30	–33	–33

shown unsatisfactory performance when exposed to low temperatures for longer durations (Antila et al., 1966).

Studies show that diluents have a significant role in lowering the pour point of the vegetable oils (Asadauskas et al., 1999). However, high dilution does not necessarily translate to proportionate depression of pour point and no synergism exists between diluents and PPD molecules. During the cooling process, the response to diluents and PPD molecules is dependent to some extent on the vegetable oil FA composition and its geometry. Pour point determinations of safflower, high-oleic safflower, and high-linoleic safflower in the presence of diluent and PPD are shown in Table 9.16.

The addition of a synthetic ester as a diluent to safflower and high-linoleic safflower oils showed a larger decrease in the pour point compared to the high-oleic oil. Due to the presence of multiple unsaturation in safflower and high-linoleic safflower oil, the triacylglycerol molecules encounter significant steric-hindrance from the "zigzag" nature of the FA chain during the cooling process. The presence of diluent molecules in the system enhances this effect by lowering the viscosity and by interfering with the stacking process during cooling. The addition of PPDs further lower the pour point. High-oleic and high-linoleic oils appear to show a better response in the presence of additive molecules.

In addition to exhibiting good low-temperature behavior, base oils should be stable over extended time at low temperature to qualify for any industrial and automotive applications. Although high-oleic oils exhibit good thermal-oxidative behaviour and acceptable PPD response, they fail in an industry-specified, low-temperature extended storage stability test. Table 9.16 shows the cold storage stability data of selected vegetable oils in the presence of diluents and PPD (Erhan et al., 2002). Using the optimized diluent and PPD concentration, safflower and high-linoleic safflower oils showed acceptable fluidity well beyond 7 days with some loss in optical clarity. Therefore, to meet the viscometric properties of vegetable oil-based lubricants for engine applications, PPDs, synthetic hydrocarbons, and synthetic fluids have to be used in various combinations to produce base fluids meeting SAE 30, 5W-30, and 10W-30 viscosity requirements as shown in Table 9.17. These requirements can be met using a proper selection and combination of various vegetable oils along with PPD and synthetic oil diluent. All of the blends shown in the table contain antioxidants and antiwear additives and are compared with commercially available 5W-30 oil. Similar lubricants can be prepared using combinations of soybean, castor, canola, high-oleic oils, esters, and synthetic hydrocarbons.

9.5.4.3 Oxidation stability

Oxidation is the single most important reaction of oils resulting in increased acidity, corrosion, viscosity, and volatility when used as lubricant base oils for engine oils. A number of tests are used to evaluate the oxidation stability of lubricants (Booser, 1997). The more common choices for synthetic and vegetable oil fluids are the TOST test (dry) ASTM D943, Rotary Bomb Oxidation Test (RBOT) ASTM D2272, Modified Thin Film Oxygen Uptake Test (TFOUT), Pressurized Differential Scanning Calorimetry (PDSC) ASTM method D6186-98, and Penn State thin-film Microoxidation (TFMO) test (Cvitkovic et al., 1979).

Several benchtop oxidation tests are available as screening tools for oxidative stability of vegetable oils. Evaluation of oxidation is complex and a fully acceptable protocol has yet to emerge. Estimation of peroxide value (PV) can be used as an index of oxidation if the peroxides formed are stable and do not decompose after formation, which in most cases is not true. The activation energy for the formation of peroxide is 146-272 kJ/mol (Labuza, 1971) and that of decomposition of lipid peroxide is 84-184.5 kJ/mol, suggesting peroxides are less stable than

TABLE 9.16 Response of diluents and PPD on pour point and cold storage stability of vegetable oils

	Pour Point in °C[a]			Number of Days at –25°C	
Fluid	**Neat Oil**	**Oil + Diluent[b]**	**Diluent + PPD[c]**	**Oil + Diluent[b]**	**Diluent + PPD[c]**
High-linoleic safflower oil	–21	–39	–48	7+	7+
Safflower oil	–21	–39	–45	7+	7+
High-oleic safflower oil	–21	–27	–36	1	1

[a] ASTM D97.
[b] Oil:diluent ratio of 65:35 (vol/vol).
[c] Pour point depressants (PPDs) concentration of 1%.

TABLE 9.17 SAE requirements and properties of typical environmentally friendly lubricants

Lubricant Oil	SAE Viscosity Grade	KV at 100°C	Pumping Viscosity[a] (cP)
SAE requirements		9.8–12.5 cSt	60,000 (max)
Rapeseed + castor	30 wt	9.72	Not required
High-oleic sunflower oil + PPD	10W-30	10.10	4651
Corn oil + synthetic ester + PPD	5W-30	10.34	26,287
Commercial lubricant	5W-30	10.42	25,664

[a] Measured using Cannon minirotary viscometer (MRV) as per ASTM D4684-97 method.

lipids (Swern, 1970). In the active oxygen method (AOM) (AOCS Official Method Cd-12-57, 1983), test oil is heated to 100°C and the oxidation is followed by measuring the PV of heated sample at regular time intervals until PV = 100 meq/kg is reached, which gives the AOM endpoint. A large amount of sample, numerous analysis, and critical control of airflow is required. With samples that form unstable peroxides, a PV = 100 meq/kg may never be reached and such measurements have no meaning. In the AOM method, consumption of O_2 may also be a measure for induction period.

The Rancimat method (Oxidationsstabilität, 1994; Laubli et al., 1988) is based on the fact that the volatile acids formed during oxidation (Loury, 1972; De Man et al., 1987) can be used for automated endpoint detection. Gordon and Mursi (1994) have shown good correlation of Rancimat results at 100°C with oil stability as measured by peroxide development during storage at 20°C. In another study, Jebe et al. (1993) pointed out the advantages of the Rancimat method at higher temperature. In the Sylvester test (Wewala, 1997), the sample is heated to 100°C in a closed vessel and pressure decrease due to O_2 consumption is monitored. The Oxidograph (Wewala, 1997) is an automated version of this method and the induction period is determined from the sudden decrease in the O_2 pressure. Oxidative status of oil can also be obtained by integrating the light curve during a chemiluminescence reaction (Matthäus et al., 1994). The method is highly sensitive for the measurement of lipid oxidation. Matthäus et al. (1993) described a linear correlation ($R^2 = 0.99$) between the iodimetric peroxide determination (DGF Einheitsmethoden; 1984) and the chemiluminescence method.

Another official method to measure induction period is the oil stability index (OSI) (AOCS Method Cd-12b-92, 1993). OSI values generally correspond well with AOM values if PV is 100 meq/kg or greater (Laubli et al., 1986). The method is automated and much easier compared to AOM. However, lengthy experimental time, large errors associated with small changes in O_2/air flow rate (Hill et al., 1995) and inability to differentiate between small changes in vegetable oil matrix are major disadvantages. PDSC is also popular for the determination of oxidative stabilities of vegetable oils (Shankwalkar et al., 1993; Kowalski, 1989; Kowalski, 1991). The TFMO method (Lee et al., 1993) is often the method of choice for studying vegetable oils because it is simple and reproducible. The test is especially effective when thermally induced volatility is low and insoluble deposit formation through polymerization is to be considered rather than rates of inhibitor depletion.

In vegetable oils, unsaturation, due to C = C from oleic, linoleic, and linolenic acid moieties, provides active sites for various oxidation reactions. Saturated FAs have relatively high oxidation stability (Brodnitz, 1968), but this decreases with increasing unsaturation in the molecule. The rate of oxidation depends on the degree of unsaturation of a fatty acyl chain. In general, the rate of oxidation of linoleic (18:2) is 10 (or more) times greater than oleic (18:1), while linolenic (18:3) is twice as great as the linoleic fatty acyl chain (Table 9.18).

TABLE 9.18 Oxidation rates of simple triacylglycerols

Triacylglycerols	Oxidation	Relative Rate
Tristearin (18:0)	Low	1
Triolein (18:1)	Moderate	10
Trilinolein 18:2)	High	100
Trilinolenin (18:3)	Very high	200

Oxidation usually takes place through a radical initiated chain mechanism (Murray et al., 1982).

Initiation $RH \rightarrow R^{\cdot}$

$R^{\cdot} + O_2 \rightarrow RO_2^{\cdot}$

Propagation $RO_2^{\cdot} + RH \rightarrow RO_2H + R^{\cdot}$

$R^{\cdot} + O_2 \rightarrow RO_2^{\cdot}$

Branching $RO_2H \rightarrow RO^{\cdot} + {}^{\cdot}OH$

$RO^{\cdot} + RH + O_2 \rightarrow ROH + RO_2^{\cdot}$

${}^{\cdot}OH + RH + O_2 \rightarrow H_2O + RO_2^{\cdot}$

Inhibition $In\ H + RO_2^{\cdot} \rightarrow In^{\cdot} + RO_2H$

Peroxide decomposition $RO_2H \rightarrow RO^{\cdot} + {}^{\cdot}OH$ + inert products

The free radicals generated during the initiation stage react with O_2 to form peroxy free radicals and hydroperoxides (Privett et al., 1962). During this period, O_2 is consumed in a zero-order process (Labuza et al., 1983), apparently leading to intermediates that are not well characterized, prior to the formation of peroxides (Privett et al., 1962). The latter undergoes further reaction to form alcohols, ketones, aldehydes, carboxylic acids (Shahidi, 1997), leading to rancidity and toxicity (Grosch, 1979). The formation of polar functionalities further accelerates the oil degradation process (Steinberg et al., 1989; Harman, 1982). These compounds have molecular weights that are similar to vegetable oils and, therefore, remain in solution. As the oxidation proceeds, the oxygenated compounds polymerize to form viscous material that, at a particular point, becomes oil insoluble leading to oil thickening and deposits. The extent of oxidation and formation of oxidation products are further complicated by the amount of unsaturation, structural differences in the various triacylglycerol molecules, and the presence of antioxidants. All these factors, together or individually, can change the specific compounds formed and the rates of their formation (Coates et al., 1986). In addition to unsaturation in the molecule, oxidative degradation and kinetics of oxidation is influenced by methylene chain length, *bis*-allylic methylene groups, etc. The cumulative effect of various structural parameters in the triacylglycerol molecule makes oxidation a highly complex process and no simple kinetic model alone would hold good for such systems.

Oxidative stabilities of various base oils using the Penn State TFMO test are compared in Table 9.19. In this test, a thin film of oil is oxidized in air high temperatures. The

TABLE 9.19 Oxidative degradation tendencies of various base oils using TFMO (30 min at 150°C)

Base Oils	Deposits (%)	Evaporation (%)
Soybean oil	48	2
High-oleic sunflower oil	13	0
Diisotridecyl adipate	3	5
PAO4[a]	6	45
Mineral oil	5	5

[a] Polyalphaolefin with viscosity of ~4 cSt at 100°C.

losses due to evaporation and oxidation and the deposit-forming tendencies (oxypolymerization) of the test sample are determined in the test as shown in Table 9.19. A temperature of 150°C and times of 30 to 60 minutes chosen for testing were high enough to cause a quantifiable polymerization in unsaturation-free base stocks, yet not too severe to result in oxidative gelation of vegetable oils. Therefore, the side processes, such as oxidative cleavage and formation of solids, were not too substantial. It appears from the data that vegetable oils oxypolymerize considerably faster than unsaturation-free fluids. Although high-oleic sunflower oil containing only 5% of linoleic acid shows higher resistance to oxypolymerization than soybean oil, its oxidative stability is still far less than those of PAO or adipate. Oxypolymerization proceeds much faster and slows down only when side processes, especially formation of solids, become more pronounced. It has been established that methylene-interrupted polyunsaturation is the key factor causing low oxidative stability of vegetable oils (Gardner, 1989). At higher temperatures, such as 175 and 200°C, evaporation was substantially higher in oils having high oleic content. Conversely, increase in polyunsaturation resulted in low evaporation of vegetable oil.

A similar trend was observed in safflower oil: high-oleic, and high-linoleic. The evaporative loss was greatest in high-oleic oil at 175 and 200°C. As the polyunsaturated FA content increased (as in high-linoleic safflower oil), the percent evaporation decreased at 175°C and remained comparable to safflower oil at 200°C. The deposit-forming tendency is the inverse of the evaporation trend with least deposit in high-oleic oils and more deposit in more polyunsaturated high-linoleic oils. The presence of polyunsaturation in the FA is the primary reason of low oxidative stability, as divinyl methylene hydrogen atoms are highly susceptible to free radical attack leading to substitution with O_2 molecule and consequent formation of polymeric oxy-polar compounds. These compounds are the precursors of oil insoluble deposits often encountered with high temperature oxidation of vegetable oils.

PDSC is another popular approach for rapid measurement of the oxidative stability of vegetable oils (Kowalski, 1993; Shankwalkar et al., 1993). The procedure is fast, requires only a small quantity of sample, and is extremely reproducible. A small amount of sample is placed in a hermetically sealed aluminum pan with a pinhole lid for interaction of the sample with the reactant gas (oxygen). A film thickness of less than 1 mm is required to ensure proper oil–O_2 interaction and eliminate any discrepancy in the result due to oxygen diffusion limitations (Kowalski, 1993; Adhvaryu et al., 1999). Oxygen gas (dry, 99% pure, obtained commercially) is pressurized in the module at a constant pressure of 3450 kPa and maintained throughout the length of the experiment. The sample is then heated at 10°C/min to 250°C. From the DSC thermogram, the onset temperature (OT) is determined. This represents the temperature when rapid increase in the rate of oxidation is observed in the system. This temperature is obtained from extrapolating the tangent drawn on the steepest slope of reaction exotherm. A high OT would suggest a high oxidative stability of the vegetable oil. The OT for various vegetable oils is shown in Table 9.20.

The OT is influenced by the degree of poly-unsaturation present in the vegetable oils. It is generally observed that a high polyunsaturation (linoleic and linolenic acid content) decreases, while high oleic content in the FA chain increases the OT. The increase in saturated fatty acids improves the resistance to initial thermal breakdown. The activation energy requirement for such system is considerably high. This results in delaying the onset of initial oxidation process where bond scission takes place to form primary oxidation products. The percentage of oleic acid (see Table 9.12) in the different vegetable oils explains the observed trends in oxidation stability. However, the polyunsaturated and saturated fatty acid contents do not conclusively explain the relative variation of OT among the vegetable oils. The role of different structural parameters obtained using ^{1}H and ^{13}C NMR on the oxidation behavior of unmodified and genetically modified vegetable oils has been explained elsewhere (Adhvaryu et al., 2000).

Improvements in oxidation stability are needed due to more stringent demands being placed on lubricant performance. Use of antioxidant (AO) additives along with high-oleic vegetable oils improves the oxidation stability. The AO package has to be optimized for vegetable oils. Typically a mixture of AO is required. Table 9.21 shows the effects of the AO package (commercial LZ7652) optimized for vegetable oils when evaluated in the ASTM D2272 RBOT and ASTM D943 TOST methods (Lawate, 2002; Rudnick, 2002). Also in these studies, it was found that high-oleic soybean oil is oxidatively more stable than conventional soybean oil, but less than mineral oil. In TFMO

TABLE 9.20 Oxidation stability of vegetable oils using pressurized differential scanning calorimetry

Vegetable Oil	OT (°C)
Cottonseed	150
Safflower	166
High-oleic safflower	178
High-linoleic safflower	166
Sunflower	145
High-oleic sunflower	177

TABLE 9.21 Oxidation stability of vegetable oils and synthetic ester using the RBOT method

Base Oils	RBOT (min)[a] No Antioxidant	RBOT (min)[a] 3.0 % Antioxidants	TOST, h[b]
Vegetable oil			
Canola	15	79	< 100
High-oleic canola	15	169	~ 350
High-oleic sunflower	15	232	~ 500
High-oleic soybean	15	280	
Synthetic oil			
TMP trioleate	15	170	
Mineral oil (200 N)		350	~ 2000

[a] Min to 25 lb pressure loss, ASTM D2272.
[b] Time to TAN = 2, ASTM D943.

tests the deposit-forming tendencies and oxidative volatility of vegetable oils are significantly reduced by the addition of AO additives (Perez et al., 2002). The proper combinations of high-oleic vegetable oils and additives have oxidative stability at par with off-the-shelf 10W-30 commercial mineral oil-based lubricant.

9.5.4.4 Friction-wear properties

Tests to evaluate the friction and wear characteristics of lubricants are numerous and range from bench tests to engine and pump stand tests (Annual Book of ASTM Standards, 2000; Booser, 1997). Pump stand tests include vane- (Dennison T-50, Vickers V104C) and piston-type tests (Dennison P-46). The DIN 51354 FZG test is popular both in Europe and the U.S. There are four different ASTM bench tests (Annual Book of ASTM Standards, 2000) used to evaluate wear and load-carrying ability: ASTM standard method D2266 (Four-Ball Wear), D2783 (Four-Ball EP), D2782 (Timken test), and D3233 (Falex EP). Each lab uses its own modification of the four-ball test, such as a sequential test correlated with the pump stand tests (Perez et al., 1986). In addition, the fluids are subjected to brake, clutch, and friction disc tests (Booser, 1997). Friction and wear performance of the vegetable oil based lubricants using a commercial additive package are acceptable for industrial and automotive lubricants.

9.5.4.5 Hydrolytic Stability

A serious threat to the stability of vegetable oils and synthetic esters is the presence of water. The reaction can yield organic acids that further catalyze the reaction resulting in further degradation of the base fluid and corrosive wear of metal surfaces. Additives can be used to improve hydrolytic stability (Booser, 1994), but good maintenance practices are the best insurance for long life. The stability of the fluids can be evaluated using the "coke bottle" test, ASTM standard method D2619 (Annual Book of ASTM Standards, 2000).

9.5.4.6 Biodegradability

There are two types of biodegradability tests: primary and ultimate biodegradation (Battersby, 2000; Product Review, 1996). Primary biodegradation involves the disappearance of the parent organic compounds under specific test conditions and may or may not indicate that the substrate will biodegrade completely. The primary biodegradation test method is CEC L-33-A-93 from the Coordinating European Council (CEC). This method measures the degree of degradation by the disappearance of specific hydrocarbons bands using IR (infrared) spectroscopy. Since the method does not identify the type of products produced, conclusions regarding the extent of degradation are limited. As regulations are tightened in Europe, the method may not be acceptable for certification of the fluid as environmentally friendly.

Total or ultimate biodegradation is measured by tests that result in the complete degradation to carbon dioxide and water by microbial action within 28 days. The two most widely used tests are ASTM standard method D5846 (Annual Book of ASTM Standards, 2000) and the Organization of Economic Cooperation and Development (OECD) test method OECD 301B. The OECD method is accepted worldwide and is the basis for the German chemical laws, dangerous substances legislation (UBA WGK Water hazard), and Eco-labeling (Blue Angel) (Laemmle, 2002).

The choice of base oil used is critical for environmentally friendly (EF) lubricants since it dictates the biodegradability and ecotoxicity of the finished lubricant. The biodegradability of different base oils is shown in Table 9.22. The use of PAO and mineral oils in EF lubricants is thus limited due to low biodegradability. Among the base oils listed, vegetable oils possess the highest biodegradability and lowest ecotoxicity.

9.5.4.7 Toxicity

Aquatic toxicity tests measure the extent to which a fluid will poison selected environmental species, such as algae (OECD 201), *Daphnia magna* (OECD 202-12), flathead

TABLE 9.22 Biodegradability of various base oils

Base Oils	% Biodegradability by CEC Method	% Biodegradability by OECD Method
Vegetable oils		
Soybean	100	>70
Canola	100	>70
High-oleic sunflower	100	>70
Synthetic oils		
TMP trioleate	90	>60
TMP trioleate (high-oleic)	90	>60
PAO8	<50	30
Mineral oil (150N)	15	5

minnows *Oncorhyuchus mykis* (OECD 203-13), and bacteria (OECD 209).

9.5.5 Suitability of natural oils as lubricants

The following vegetable oils are most suitable for use in lubricant applications. More details about these oils can be found in Chapter 2.

9.5.5.1 Soybean oil

Soy oil, extracted through pressing or via solvent extraction, is used for a number of industrial applications. Bioengineered (high oleic and/or low linoleic) soybeans may provide highly desirable improvements for fuels and other industrial products.

Current research priorities are focused on developing improved soy oil-based lubricants with improved oxidation stability and cold weather pour properties. Developments in the area of total loss lubricants, hydraulic fluids, and crankcase lubricants are showing promising results. Products, such as biodegradable grease and 2-cycle engine oils, based on soybean oil are currently available. These are environmentally friendly when used in outboard motors, lawnmowers and other small engines. Soybean oil used in crankcases must exhibit properties such as high lubricity, viscosity index, flash point and low evaporation loss.

9.5.5.2 Canola oil

Canola oil is typically referred to in the industry as a penetrating oil and generally has a higher level of linolenic fatty acid than soybean oil. High α-tocopherol content (19 mg/100gm), higher levels of oleic acid and lower levels of polyunsaturated acids contribute to the oil stability as compared to soybean oil. With the rapid development of high-oleic variety oils, various technical properties of the oil can be significantly improved, thereby meeting industrial specifications. Table 9.23 presents the comparison of natural and high oleic canola oil.

9.5.5.3 Sunflower oil

Traditional sunflower oil consists of 68% linoleic acid and about 20% oleic acid. Two varieties of the oil are currently available: normal and high-oleic. The high-oleic variety has higher oxidation stability and is suitable for industrial applications. Both canola and sunflowers are popular alternative oilseeds in the U.S. Sunflower oil is considered a premium oil due to its light colour, mild flavour, low level of saturated fats and ability to withstand high cooking temperatures. Although the sunflower has the potential for many industrial uses, in the U.S., it is mostly used for food or feed purposes.

TABLE 9.23 Fatty acid distribution and physical properties of natural and high-oleic canola oil

Fatty Acid Distribution/ Technical Properties	High-Oleic Canola Oil	Canola Oil
Palmitic acid (16:0)	3.5	3.5
Stearic acid (18:0)	1.5	1.5
Oleic acid (18:1)	84.0	61.5
Linoleic acid (18:2)	3.5	19.5
Linolenic acid (18:3)	4.0	10.5
Melting temperature (°C)	–5.5	–9.5
PDSC (minutes) at 130 °C	71	25

9.5.5.4 Safflower oil

There are two types of safflower varieties: one that produces oil that is high in monounsaturated fatty acids (oleic acid), and the other with high concentrations of polyunsaturated fatty acids (linoleic acid). The high-linoleic safflower oil contains nearly 75% linoleic acid, which is considerably higher than corn, soybean, cottonseed, peanut, or olive oils. High-oleic safflower variety may contain up to 80% oleic acid and is comparable to olive oil, and stable when heated. Regular safflower oil is considered as a drying or semidrying oil, with properties intermediate between soybean and linseed oils, and is currently used in manufacturing paints and other surface coatings. The oil is light in color and will not yellow with aging. This oil can also be used as lubricant base oil, but like most vegetable oils, is currently too expensive for this use. High-oleic safflower oil is rapidly gaining recognition as one of nature's most valuable vegetable oils with extensive industrial applications.

9.5.5.5 Meadowfoam oil

Meadowfoam oil contains three previously unknown long-chain fatty acids and resembles high erucic acid rapeseed oil in some respects (Bosisio, 1989). It is unusually high in long-chain fatty acids (over 90% C_{20} to C_{22} fatty acids) with very high levels of monounsaturation and very low levels of polyunsaturation. These characteristics make meadowfoam oil very stable, even when heated or exposed to air and crude meadowfoam oil is more oxidatively stable than other regular vegetable oils. Meadowfoam oil has the added benefit of enhancing the properties of other oils when mixed with them. Less expensive oils can be mixed with meadowfoam oil without the loss of the qualities of either oil and it can increase the stability of the oils to which it is added. The oil can be used as a lubricant, apart from other applications like light coloured premium grade solid wax, a sulfur compound valuable to the rubber industry, or used as a detergent or plasticizer.

9.5.5.6 Lesquerella oil

Lesquerella oil contains a hydroxy fatty acid and so resembles castor oil, which is an important raw material used by industry for making lubricating greases, resins, waxes, nylons, plastics, corrosion inhibitors, coatings, and cosmetics (Smith Jr. et al., 1961, 1962). Saturated hydroxy fatty acids produced by hydrogenation could be useful in the production of greases in the form of their lithium soaps. Many of the properties may be enhanced over those of castor oil because of the increased chain length of this new crop oil.

9.5.5.7 Cuphea oil

Cuphea oil contains high levels of short-chain saturated fatty acids, C_8, C_{10}, C_{12}, and C_{14}, which are used in the production of solvents, detergents, and emulsifiers. Other uses of the seed oil include cosmetics and motor oil.

9.5.5.8 Jojoba oil

Perhaps the most commercially advanced of the new crops is jojoba. Jojoba "oil" is a liquid ester wax rather than the familiar triacylglycerols produced in well-known oil seeds like canola. The principal oil structures contain 40 and 42 carbon atoms. The major market for jojoba oil continues to be within the cosmetic industry. An estimated 2000 metric tonnes per annum is consumed by this industry in the form of jojoba oil, hydrogenated jojoba oil, jojoba esters, hydrolyzed jojoba oil, ethoxylated jojoba oil, and other value-added jojoba oil derivatives. In lubricant applications, jojoba oil provides a market for approximately 100 tonnes annually. In general the price of jojoba is too high for this market compared to other available lubricant oils. The molecular structure of this oil is such that it is stable even at high temperatures and pressures unlike most of other lubricants.

9.5.5.9 Tallow and yellow grease

Tallow is inedible grease derived from animal fat renderings. Products such as lubricants, soap, cosmetics, and plastics are made from tallow. Tallow is gradually being replaced by yellow grease (waste vegetable oils) from used cooking oil from fast food restaurants. It contains a mix of unsaturated and saturated fatty acids (generally in a ratio of about 2.8:1). Other application areas of yellow grease are the manufacture of soap, makeup, clothing, rubber, and detergents.

9.5.5.10 Medium-chain triglycerides

Medium-chain triglycerides (MCT) are medium-chain fatty acid esters of glycerol. Medium-chain fatty acids are fatty acids containing from 6 to 12 carbon atoms. These fatty acids are constituents of coconut and palm kernel oils and are also found in camphor tree drupes. Coconut and palm kernel oils are called lauric oils because of their high content of the lauric acid (C_{12}). MCT used for nutritional and other commercial purposes are derived from lauric oils. They differ from other fats in that they have a slightly lower calorie content (Bach et al., 1996) and they are more rapidly absorbed and burned as energy, resembling carbohydrate more than fat (Bach et al., 1982). With mineral oil falling out of favour in use as a processing lubricant and as a mould release and polishing agent in hard candy production, MCTs may be an option. Many European countries are banning mineral oil usage in food applications, a trend that may spread to the U.S.

A recent development is an increase in use of castor oil and palm oil in manufacture of biodegradable- and food industry-grade lubricants.

9.5.6 Applications

Lubricants provide a well-established and highly competitive market, but growing only at an average rate of less than 1% per year (Padavich et al., 1995). More than 70% of total lubricant volume is used as motor oils for automotive engines and approximately 10% as hydraulic fluids. Other small areas of usage include: cutting oils, two stroke engine oils, chainsaw bar oils, wire rope oils, bicycle chain oils, railroad oils, pump oils, outboard engine oils, drilling oils, and other niche markets.

Nonfood uses of vegetable oils have grown very little during the past 40 years (with the exception of the burgeoning demand for biodiesel). Although some markets have expanded or new ones added, other markets have been lost to competitive petroleum products. Vegetable oils are currently being used in various industrial applications, such as emulsifiers, lubricants, plasticizers, surfactants, plastics, solvents, and resins. Research and development approaches take advantage of the natural properties of these oils for lubricant applications, namely amphiphilic character to deliver inherent lubricity, high molecular weight, low volatility, high viscosity index, good solubilizing power for additive molecules and being eco-friendly (Randles et al., 1992; Battersby et al., 1989) among others.

Vegetable oils and other lipid derivatives have shown significant increase in use as biodegradable lubricants over the last decade, but still contributing less than 2% of all base oils used in the market. A major application area is industrial hydraulic fluids, which represents a 222 million gallon market in the U.S., with potential use in waterways, farms, and forests. Vegetable base oils are suitable as metal cutting oils and fluids, and avoid the hazardous mist formation from mineral oils during use. Canola-based motor oils have rapidly evolved into a competitive product as a potential substitute for mineral oil-based products. In terms of pricing, they are highly competitive with synthetic motor oils. They are also the most "environmentally friendly" of the motor oils available maintaining properties of nontoxicity and biodegradability. In terms of functionality, they have exceeded expectations by surpassing both conventional and synthetic oils in the tests conducted. There has been significant reduction in tailpipe gas emissions of nitrogen oxides, carbon monoxide, and hydrocarbons, therefore providing an easy and effective way to reduce air pollution.

As crankcase oil, though vegetable oil-based lubricants have limited contact with the environment, active development work is in progress on base stocks (e.g., canola, corn, soybean oil) for use in air-cooled engines. Other significant niche market areas are cutting and drive chain oils, two stroke engine oils, chain saw bar oil, wire rope oil, marine oils and outboard engine lubricants, oil for water and underground pumps, rail flange lubricants, agricultural equipment lubricants, metal cutting oils, tractor oils, dedusting, and several others.

A recent product is a soy-based transformer dielectric fluid. This product is used to insulate and cool electrical distribution products, such as transformers, and is safe for the environment and the public. The fluid is biodegradable based on testing done by the U.S. Environmental Protection Agency (USEPA). The fluid also enhances the performance and life of a utility's transformer assets. The fluid extends paper insulation life five to eight times, lowering life cycle costs. The increased insulation life also translates to extended and enhanced transformer life or the ability to carry higher loads during peak demand periods without leading to premature insulation failure. The enhanced performance allows utilities to manage their assets more profitably and forestall costly capital expenditures. The fluid also has excellent fire resistant qualities. The fluid offers an ignition fire point of 360°C and flash point of 330°C, more than twice that of petroleum-based mineral oils. The soy-based fluid has been shown to enhance the loading performance of new transformers by up to 14% or extend their insulation life five to eight times. It also has a similar positive impact on larger units already in service, such as those found in electric substations. Although the cost of the fluid is slightly higher than mineral oils, its soy-based properties contribute to long-term savings that mineral oil cannot deliver. Accelerated aging tests have shown that this fluid extends transformer life well beyond that of units with mineral oil.

9.5.7 Future prospects of bio-based lubricants

Bio-based lubricants are becoming increasingly important in Europe, particularly for total loss lubricants. In Western Europe, bio-lubricants were 3.1% of total lubricant consumption in 2002 (Whitby, 2004). Their interest in biodegradable lubricants started in the mid 1980s. While the strongest environmental pressures and the resulting acceptance of biodegradable lubricants began in Northern Europe, the same pressures now appear in other European Countries. The first country in Europe to introduce bio-lubricants was Sweden, in 1988, when hydraulic fluids based on rapeseed oil were introduced. The City of Gothenburg published the first "Clean Lubrication" list in 1995, comprising 14 products from 10 lubricant suppliers. The list has been further expanded every year since then. Swedish standard SS 15 54 34 was revised in 1997 to include the environmental criteria in the "Clean Lubrication" list. By 2000, around 25% of all hydraulic fluids sold in Sweden were environmentally friendly, of which 80% were synthetic esters (Whitby, 2004). Almost all forestry operations now use environmentally friendly lubricants, including greases, gear oils, and chain bar oils, because this is now mandated for all forestry operators in Sweden.

Biobased lubricants have the potential to create new market opportunities for farmers while easing society's reliance on petroleum. Vegetable oils, however, offer some challenges that must be overcome in order to improve their usefulness as a sustainable alternative to petroleum base oils. These challenges are of such a scope that it is unlikely that one laboratory, or even one country's scientific community, will easily overcome them alone.

New applications of vegetable oil-based lubricants are constantly gaining predominance in areas where stiff regulations require expensive clean up and disposal, and environmental safety plays a major role, despite the higher costs involved. One such class of lubricants is total loss lubricants. The applications where lubricants are lost directly to the environment (railroad rails and switches, wire cables on cranes, the bars of chain saws, and other power equipment) are the most likely application areas to initiate the use of biodegradable lubricants. In these limited-life applications, the stability of the lubricant is not a factor, giving vegetable oil an advantage as the base oil. Widespread use of vegetable oils will depend on how well they perform with a wide range of factors, including temperature, pressure, metal surface, other functional fluids and existing technology. Chemically and genetically modified derivatives of vegetable oils have resulted in significant improvement in thermo-oxidative, low temperature stability, and lubrication properties, thereby increasing their use in a variety of industrial lubricant applications. Development of genetically modified vegetable oil is a lengthy process and it is currently not extensively available as an all-purpose less expensive material capable of delivering all performance qualities for nonfood uses. Nevertheless, it is evident that over the last decade there has been a significant progress in the research and development of lipid technology for innovative industrial uses.

In spite of all these technological advancements, an appropriate strategy is needed to convince users of lubricants. This drive toward the use of renewable resources was given a major boost in the U.S. government with the Federal Executive Order 13010 (which set a goal that 25% of all government purchases be biobased), which has been encouraging companies to make use of renewable, bio-degradable base stocks, rather than petroleum base stocks, in many applications (Patin et al., 2002). Achieving the U.S. federal government's goal of tripling use of bio-based products and bioenergy by 2010 could create $15 to 20 billion in new income for farmers and rural America and reduce fossil fuel emissions by an amount of up to 100 million metric tonnes of carbon (Soya and Oilseed Industry News, 2003). Another opportunity to accelerate the development of this market exists in the 2002 Farm Bill (Farm Security and Rural Investment Act, published January 11, Federal Register). Section 9002 includes language directing all federal agencies to give preference to bio-based products (lubricants is one of the categories specified in the guidelines), unless it is unreasonable to do so, based on price, availability, or performance.

The supply side of the market along with environmental pressure groups, agricultural companies and research institutes are more enthusiastic than the demand side of the market, mainly as a result of higher costs of bio-based lubricants. Users of lubricants are reluctant to use a more expensive product unless there are compelling economic or regulatory pressures to do so. Future outlook for vegetable oil-based lubricants, therefore, will be dictated by consumer awareness and regulations that mandate use of bio-based lubricants. The most encouraging development is OEM interest in these types of fluids, which provides an optimistic future to biobased lubricants.

References

Adhvaryu, A. et al., Application of quantitative NMR spectroscopy to oxidation kinetics of base oils using a pressurized differential scanning calorimetry technique, *Energ. Fuels*, **13**, 493, 1999.

Adhvaryu, A. et al., Oxidation kinetic studies of oils derived from unmodified and genetically modified vegetables using pressurized differential scanning calorimetry and nuclear magnetic resonance spectroscopy, *Thermochem. Acta*, **364**, 87, 2000.

American Oil Chemist's Society Official Method Cd 12-57, Fat Stability, Active Oxygen Method, *Am. Oil Chem. Soc.*, Champaign, IL, 1983.

American Oil Chemist's Society Official Method Cd12b-92, Oil Stability Index (OSI), *Am. Oil Chem. Soc.*, Champaign, IL, 1993.

Annual Book of ASTM Standards, *Section Five: Petroleum Products, Lubricants, and Fossil Fuels*, vol. 05.02, ASTM, West Conshohocken, PA, 2000.

Antila, V., Fatty acid composition, solidification and melting of Finnish butter fat, *Finn. J. Dairy Sci.*, **27**, 1, 1966.

Asadauskas, S. and Erhan, S.Z., Depression of pour points of vegetable oils by blending with diluents used for biodegradable lubricants, *J. Am. Oil Chem. Soc.*, **76**, 316, 1999.

Bach, A.C. and Babayan, V.K., Medium-chain triglycerides: an update, *Am. J. Clin. Nutr.,* **36**, 950–62, 1982.

Bach, A.C. et al., The usefulness of dietary medium-chain triglycerides in body weight control: Fact or fancy?, *J. Lipid Res.,* **37**, 708–26, 1996.

Battersby, N.S., The biodegradability and microbial toxicity testing of lubricants — some recommendations, *Chemosphere*, **41**, 1011, 2000.

Battersby, N.S. et al. A correlation between the biodegradability of oil products in the CEC L-33-T-82 and modified Sturm tests, *Chemosphere*, **24**, 1989, 1992.

Becker, R. and Knorr, A., An evaluation of antioxidants for vegetable oils at elevated temperatures, *Lubr. Sci.,* **8**, 95, 1996.

Bentz, A.P. and Breidenbach, B.G., Evaluation of the differential scanning calorimetric method for fat solids, *J. Am. Chem. Soc.*, **46**, 60, 1969.

Booser, E.R., *CRC Handbook of Lubrication and Tribology*, vol. III, CRC Press, Boca Raton, FL, 1994, 282.

Booser, E.R., *CRC Tribology Data Handbook*, CRC Press, Boca Raton, FL, 1997, 136.

Bosisio, M., Meadowfoam: pretty flowers pretty possibilities oilseed soaps limnanthes alba nutlets, *Agric. Res.*, USDA Res. Serv., 1989, **37**(2), 10.

Brodnitz, M.H., Autoxidation of saturated fatty acids. a review, *J. Agric. Food Chem.*, **16**, 994, 1968.

Coates, J.P. and Setti, L.C., Infrared spectroscopic methods for the study of lubricant oxidation products, *ASLE Trans.*, **29**, 394, 1986.

Cvitkovic, E. et al. A thin film test for measurement of the oxidation and evaporation of ester type l ubricants, *ASLE Trans.*, **22**, 395, 1979.

D'Souza, V. et al. Polymorphic behavior of high-melting glycerides from hydrogenated canola oil, *J. Am. Oil Chem. Soc.*, **68**, 907, 1991.

de Jong, S. et al. Crystal structures and melting points of unsaturated triacylglycerols in the β-phase, *J. Am. Oil Chem. Soc.*, **68**, 371, 1991.

de Man, J.M. et al. Formation of short chain volatile organic acids in the automated aom method, *J. Am. Oil Chem. Soc.*, **64**, 993, 1987.

DGF Einheitsmethoden zur Untersuchung von Fetten, Fettprodukten, Tensiden un verwandten Stoffen, Deutsche Gesellchaft für Fettwissenschaft e.V., Munster, Wissenschaftliche Verlagsgesellschaft GmbH Stuttgart, Method C-IV 6a, 1984.

Erhan, S.Z. and Adhvaryu, A., Vegetable-Based Base Stocks, in *Biobased Industrial Fluids and Lubricants*, Erhan, S.Z. and Perez, J.M., Eds., AOCS Press, Champaign, IL, 2002, Chap.1.

Erhan, S.Z. and Asadauskas, S., Lubricant basestocks from vegetable oils, *Ind. Crops Prod.*, **11**, 277, 2000.

Erhan, S.Z. et al. Chemically Functionalized Vegetable Oils, in *Synthetics, Mineral Oils, and Bio-Based Lubricants Chemistry and Technology*, Rudnick, L.R., Ed., CRC Press, Boca Raton, FL, 2005, Chap.22.

Erhan, S.Z., Vegetable oils as lubricants, hydraulic fluids, and inks, in *Bailey's Industrial Oil and Fat Products, Volume 6, Industrial and Nonedible Products from Oils and Fats*, 6th ed., Shahidi, F., Ed., John Wiley & Sons, New York, 2004, Chap.7.

Gardner, H.W., Oxygen radical chemistry of polyunsaturated fatty acids, *Free Rad. Biol. Med.*, **7**, 65, 1989.

Gordon, M.H. and Mursi, E., Comparison of oil stability based on the Metrohm Rancimat with storage at 20°C, *J. Am. Oil Chem. Soc.*, **71**, 649, 1994.

Grosch, W., Moll, C. and Biermann, U., Occurrence and formation of bitter-tasting trihydroxy fatty acids in soybeans, *J. Agric. Food Chem.*, **27**, 239, 1979.

Hagemann, J.W. and Rothfus, J.A., Computer modeling of theoretical structures of monoacid triglyceride alpha-forms in various subcell arrangements, *J. Am. Oil Chem. Soc.*, **60**, 1308, 1983.

Hagemann, J.W., Tallent, W.H., and Kolb, K.E., Differential scanning calorimetry of single acid triglycerides: effect of chain length and unsaturation, *J. Am. Chem. Soc.*, **49**, 118, 1972.

Hagemann, J.W., Thermal behavior and polymorphism of acylglycerols, in *Crystallization and Polymorphism of Fats and Fatty Acids*, Garti, N. and Sato, K., Eds., Marcel Dekker, New York, 1988, 9–95.

Harman, D., *Free Radical in Biology*, vol. 5, Academic Press, New York, 1982, 255.

Hill, S.E. and Perkins, E.G., Determination of oxidation Stability of Soybean oil with the oxidative stability instrument: operation parameter effects, *J. Am. Oil Chem. Soc.*, **72**, 741, 1995.

Hwang, H.S. and Erhan, S.Z., Lubricant base stocks from modified soybean oil, in *Bio-Based Industrial Fluids and Lubricants*, Erhan, S.Z. and Perez, J.M., Eds., AOCS Press, Champaign, IL, 2002, Chap. 2.

Jebe, T.A. et al. Collaborative study of the oil stability index analysis, *J. Am. Oil Chem. Soc.*, **70**, 1055, 1993.

Kowalski, B, Evaluation of activities of antioxidants in rapeseed oil matrix by pressure differential scanning calorimetry, *Thermochim. Acta*, **213**, 135, 1993.

Kowalski, B., Determination of oxidative stability of edible vegetable oils by pressure differential scanning calorimetry, *Thermochim. Acta*, **156**, 347, 1989.

Kowalski, B., Thermal-oxidative decomposition of edible oils and fats. DSC studies, *Thermochim. Acta*, **184**, 49, 1991.

Labuza, T.P. and Bergquist, S., Kinetics of oxidation of potato chips under constant temperature and sine wave temperature conditions, *J. Food Sci.*, **48**, 712, 1983.

Labuza, T.P., et al., Metal-catalyzed oxidation in the presence of water in foods, *J. Am. Oil Chem. Soc.*, **48**, 527, 1971.

Laemmle, P., Biodegradable Hydraulic Fluids and Utto-Lubricants, *SAE 2002-01-1455*, NCFP I02-20.3, 2002.

Laubli, M.W. and Bruttel, P.A., Determination of the oxidative stability of fats and oils: comparison between the active oxygen method (AOCS Cd 12-57) and the Rancimat method, *J. Am. Oil Chem. Soc.*, **63**, 792-795, 1988.

Laubli, M.W. and Bruttel, P.A., Determination of the oxidative stability of fats and oils: comparison between the active oxygen method (AOCS Cd 12-57) and the rancimat method, *J. Am. Oil Chem. Soc.*, **63**, 792, 1986.

Lawate, S., Environmentally friendly hydraulic fluids, in *Biobased Industrial Fluids and Lubricants*, Erhan, S.Z. and Perez, J.M., Eds., AOCS Press, Champaign, IL, 2002, chap.3.

Lee, C.J. and Klaus, E.E., Evaluation of deposit forming tendency of mineral and synthetic base oils using the Penn state microoxidation test, *Lubr. Eng.*, **49**, 441, 1993.

Loury, M., Possible mechanisms of Autoxidative Rancidity, *Lipids*, **7**, 671, 1972.

Matthäus, B. et al. Fast chemiluminescence method for detection of oxidized lipids, *Fat Sci. Technol.*, **96**, 95, 1994.

Matthäus, B. et al. Bestimmung von Hydroperoxiden in Fetten und Ölen durch Chemilumineszenz, *Lebensmittelchemie*, **47**, 85, 1993.

Murray, D.W. et al., Effect of basestock composition on lubricant oxidation performance, *Petrol. Rev.,* **36** (421), 36–40, 1982.

Oxidationsstabilität von Ölen und Fetten-Rancimatmethode, Metrohm AG, Herisau, Application Bulletin Metrohm Nr. 204/1 d, 1994.

Padavich, R.A. and Honary, L., A market research and analysis report on the vegetable oil based industrial lubricants, SAE Tech paper 952077, p. 13, 1995.

Patin, L.J. and James, D.K., Biodegradable and fire-resistant hydraulic fluids: now you can have both, *Lubric. World*, Nov. 26, 2002.

Perez, J.M. and Boehman, A.L., Environmentally friendly fuels and lubricants, in *Biobased Industrial Fluids and Lubricants*, Erhan, S.Z. and Perez, J.M., Eds., AOCS Press, Champaign, IL, 2002, Chap.6.

Perez, J.M. et al. Comparative evaluation of several hydraulic fluids in operational equipment, a full scale pump test stand and the four-ball wear tester–Part III. New and used hydraulic fluids, *Lubr. Engr.*, **52**, 416, 1986.

Privett, O.S. and Blank, M.L., The initial stages of autoxidation, *J. Am. Oil Chem. Soc.*, **39**, 465, 1962.

Product review on biodegradable fluids and lubricants, *Ind. Lub. & Tribol.*, **48**(2), 17, 1996.

Randles, S.I. and Wright, M., Environmentally considerate ester lubricants for the automotive and engineering industries, *J. Syn. Lubr.* **9**, 145, 1992.

Rossell, J.B. and Pritchard, J.L.R., *Analysis of Oil Seeds, Fats and Fatty Foods,* Elsevier, London, 1991.

Rudnick, L.R., A comparison of synthetic and vegetable oil esters for use in environmentally friendly fluids, in *Bio-Based Industrial Fluids and Lubricants*, Erhan, S.Z. and Perez, J.M., Eds., AOCS Press, Champaign, IL, 2002, Chap. 4.

Schmidt, M.A. et al. Biotechnological enhancement of soybean oil for lubricant applications, in *Synthetics, Mineral Oils, and Bio-Based Lubricants Chemistry and Technology*, Rudnick, L.R., Ed., CRC Press, Boca Raton, FL, 2005, Chap. 23.

Shahidi, F., *Natural Antioxidants Chemistry, Health Effects and Application*, AOCS Press, Champaign, IL, 1997, 1.

Shankwalkar, S. and Placek, D., Oxidation kinetics of tricresyl phosphate (TCP) using differential scanning calorimetry (DSC), *Lubr. Eng.*, **50**, 261, 1994.

Smith, C.R. et al., Lesquerolic acid, a new hydroxy acid from *Lesquerella* seed oil, *J. Org. Chem.*, **26**, 2903, 1961.

Smith, C.R. et al., Densipolic acid: a unique hydroxydienoid acid from *Lesquerella densipila* seed oil, *J. Org. Chem.*, **27**, 3112, 1962.

Soya & Oilseed Industry News, 'BioEnergy' conference aims to help power U.S. energy independence, Oct. 30, 2003.

Steinberg, D. et al., Beyond cholesterol: modifications of low-density lipoprotein that increase its atherogenicity, *New Engl. J. Med.*, **320**, 915, 1989.

Swern, D., *Organic Peroxides*, vol. 1, Wiley/Interscience, New York, 1970, 115.

Wewala, A.R., *Natural Antioxidants. Chemistry, Health Effects and Applications*, AOCS Press, Champaign, IL, 1997, 331.

Whitby, R.D., Market share of bio-lubricants in Europe and the USA, *Lipid Technol.*, **16**(6), 125–130, 2004.

9.6 Biofuels

9.6.1 What are biofuels and why are they attracting so much interest?

For the last century and more, man has exploited fossil fuels (coal, mineral oil, and gas) to provide energy for warmth (and cooling), transport, and to drive industrial machinery. The supplies of fossil fuels are large but finite and in recent decades and particularly in the last few years there has been a revived interest in alternative forms of energy among which are two based on plant matter — bioethanol and biodiesel (usually methyl esters derived from vegetable oils and animal fats). The reasons for this

new emphasis have included the rising cost of mineral oil, concern about security of supply and about environmental issues, and pressures from oil and fat producers and from governments. Europe has led the way in the production of biodiesel. North and South America have favoured bioethanol, but here too biodiesel is becoming more important. In 2004, the U.S. produced 4 billion gallons (19 million tonnes) of bioethanol, but only 30 million gallons (0.13 million tonnes) of biodiesel.

In 1973 and again in 1978 the price of crude petroleum rose very rapidly and unexpectedly. This had serious consequences for the economies of importing countries in Asia, Europe, and North America and led to a serious global economic downturn. The power of the mineral oil-producing countries, particularly in the Middle East, to control prices and supplies made the western countries concerned about security of supply and caused them to consider alternative sources of petroleum supply and alternative sources of energy. Those concerns remain and are deepened by current fluctuating prices that are frequently high and occasionally very high.

Environmental issues are immediate and local leading to pollution and also long-term and global leading to global warming. In cities, particularly, our dependence on cars, vans, and lorries (trucks) to transport goods and persons leads to poor atmospheric conditions (smog) and a consequent interest in fuels that are less polluting. There is also a growing concern about global warming that many consider to be manmade and arising in large part from the production of greenhouse gases of which carbon dioxide resulting from burning of fossil fuels is of greatest concern. There is also long-term worry about the depletion of our valuable but nonrenewable petroleum supplies.

Despite the increasing demand for oils and fats as food and for oleochemicals there are times of over-supply and pressures then come from producers of oils and fats to find new uses for these materials. There is also pressure from governments who, having set and accepted targets for the use of nontraditional forms of energy, are struggling to meet those targets. These alternative sources of energy are further seen as a way of avoiding the use of nuclear energy, considered by many as a less acceptable nonfossil fuel solution.

One way to meet some of these problems, in small part, is to replace fossil fuels with biodiesel (usually methyl esters derived from oils and fats). These materials have the advantage that they can be used neat or in various blends with petrodiesel without engine modification and with the approval of the vehicle manufactures. They can also be distributed easily through the existing fuel supply chain. Development and limited use of biodiesel goes back over 20 years, but these activities have accelerated in the last few years and many countries, both developed and developing, have plans to produce and use biodiesel if they are not already doing so. Several EU countries are in the lead, but they are being followed by rapid growth in Asia and in North and South America. However, biodiesel is probably more expensive to produce than petrodiesel and can only be economic with government assistance. In Europe this is provided in the form of lower taxation compared with conventional fuel. In the U.S. where fuels are less heavily taxed assistance can be provided via The Clean Air Act Amendments of 1990, the Energy policy Act of 1992, or by tax rebate. Currently this amounts to \$0.01 per 1% of biodiesel from soybean oil or \$0.005 from other sources. This is equivalent to 20 and 10 cents, respectively, for each litre of a 20% blend. The doubt about relative costs expressed earlier relates to the difficulty in determining the true cost of production free of taxes and subsidies and in the problem of assessing in financial terms the environmental and social benefits that result from producing and using biodiesel and the cost of global warming made worse by doing nothing. For example, in Brazil production of biodiesel from locally grown crops is seen not only as a way of reducing the environmental and economic cost of transporting fuel by road over long distances, but as a way of providing rural employment and slowing the drift of unemployed people to already overcrowded cities. Governments lend their support to the production and use of biodiesel for differing reasons including reduction in pollution, in carbon dioxide emissions, in the use of nonrenewable resources, and to encourage the use of domestically produced material.

Despite the popularity of biodiesel as a way of overcoming some environmental concerns, some serious questions have been raised (Dumelin, 2005). First it has to be recognised that biodiesel can only make a small contribution to the total requirement for energy. Until we develop new energy sources, such as hydrogen or atomic fusion, we will remain dependent on fossil fuels. The annual production of mineral oils is about 30 times larger than that of commodity oils and fats. Secondly, oils and fats are needed as an important source of energy-rich food and of essential nutrients. The demand for food increases with the growth in population and in income and there remains a substantial unmet need. Why then should we divert this irreplaceable food source? The Indian government has decreed that biodiesel cannot be made from any oil or fat that could be used for food and alternative supplies are being sought, such as the nonedible oil from *Jatropha curcas* seeds. There are claims already that the supply/demand system is being disturbed and this is reflected in pressure on prices. However, it is worth noting that Fry (2001) has forecast that this situation will change during the present century. Production levels will continue to increase through rising yields, population will level off about mid-century, and there is a limit to how much fat we can eat with the consequence that from around 2050 onwards there will be quite large supplies available for nonfood purposes including biofuels. A third concern is that biodiesel is only economic through manipulation of taxation. When the supply of biodiesel is small, this may not matter, but as it increases the downturn in tax revenues will have to be met in another way. Perhaps this is a way of transferring a part of the cost from

the customer to the taxpayer (frequently, but not always the same person).

Reaney et al. (2005) also take an optimistic view. They consider that biodiesel will become the largest market for oils and fats exceeding demands for food, feed, and the other nonfood uses discussed in this chapter. This will involve developments in agriculture (mainly higher yields and new biodiesel crops), better use of by-products (meal and glycerol), and improved production technology.

Although 100% biodiesel can be used in vehicles and is so used in Germany and Austria, it is more often offered as a blend with regular diesel. In the U.S. biodiesel generally contains 20% of added methyl esters, but in Europe 5% blends are more common. In addition to road vehicles, these products are being used in agricultural machinery, in boats — both for business and for pleasure — and in trains. Methyl esters can also be used in central heating systems and it is of interest that rapeseed methyl esters are used to heat the Reichstag building in Berlin, newly renovated after the union of West and East Germany into a single state in 1990. The methyl esters also serve in one route to fatty alcohols (Section 9.2.3).

9.6.2 From what material is biodiesel made?

Good quality biodiesel must meet certain criteria (see below) and this may limit the range of oils and fats that can be used for this purpose. It is desirable to avoid too high a content of saturated esters that may solidify at ambient temperature and high levels of polyunsaturated esters, especially those with more than two double bonds, which lead to undesirable oxidation and may cause problems during storage of the fuel or at the moment of use. Nevertheless a wide range of materials is available for use with convenience of supply being the most important. Rapeseed or canola oil (Europe), soybean oil (North and South America), palm oil (Malaysia), coconut oil (Philippines), and tallow (New Zealand) have been used or examined in the countries indicated. There is even a report of fish oil being used in Canada after removal of the valuable EPA and DHA. The fatty acid composition of some of these oils is shown in Table 9.24.

About 80% of the production cost of biodiesel lies in the price of the starting oil when this is an appropriately refined vegetable oil. Attempts to reduce cost have led to the use of animal fats (tallow, chicken fat, and animal fats unfit for human consumption), vegetable oils already used for frying and cooking, free acids removed during refining, and alternative vegetable oils, such as castor, palm, jatropha (*Jatropha curcas*), and karanja (*Pongamnia pinnata*) oils from India and Africa, babassu oil, jojoba oil (a wax that would yield methyl esters and fatty alcohols on methanolysis), and fatty acids recovered from refining processes (Table 9.25). The quality of these less common starting materials needs to be controlled and specifications for each

TABLE 9.24 Fatty acid composition of selected oils and fats

	Rapeseed	Sunflower	Soybean	Palm	Tallow
Palmitic	4–5	3–6	2–11	32–46	25–37
Stearic	1–2	1–6	2–6	4–6.3	14–29
Oleic	55–63	14–43	22–31	37–53	26–50
Linoleic	20–31	44–69	49–53	6–12	1–3
Linolenic	9–10		2–10		

Source: Adapted from Knothe, G. and Dunn, R.O., in *Oleochemical Manufacture and Applications*, Gunstone, F D., Hamilton, R.J., Eds., Sheffield Academic Press, Sheffield, U.K., 2001, Chap. 5.

TABLE 9.25 Fuel-related properties of selected oils and fats and of the methyl esters derived from them

	IV	CN	HG	Viscosity	CP	PP	FP
Oils and Fats							
Palm	35 61	42					
Rapeseed	94–120	37.6	39709	37.0 (37.8)	–3.9	–31.7	246
Soybean	117–143	37.9	39623	32.6 (37.8)	–3.9	–12.2	254
Sunflower	110–143	37.1	39575	37.1 (37.8)	7.2	–15.0	274
Tallow	35–48		40054	51.1 (40)			201
Petrodiesel		47	45343	2.7 (37.8)	–15.0	–33.0	52
Derived Methyl Esters							
Rapeseed		54.4	40449	6.7 (40)	–2	–9	84
Soybean		46.2	39800	4.1 (40)	2	–1	171
Sunflower		46.6	39800	4.2 (40)	0	-4	
Tallow			39949	4.1 (40)	12	9	96

Note: Iodine value, cetane number, gross heat of combustion (kJ/kg), kinematic viscosity min²/s) at the temperature indicated (°C), cloud point (°C), pour point (°C), flash point (°C).
Source: Adapted from Knothe, G. and Dunn, R.O., in *Oleochemical Manufacture and Applications*, Gunstone, F D., Hamilton, R.J., Eds., Sheffield Academic Press, Sheffield, U.K., 2001, Chap. 5.

would be required. Since large-scale production plants are generally preferred, it is necessary to secure a source of oil or fat available continually and in appropriate quantity.

9.6.3 How is biodiesel manufactured?

Despite the desire to use cheap starting materials for biodiesel production, the final product must meet defined specifications and this may be achieved more easily with good quality oil. The cost of upgrading a poor product may take up the saving in a cheaper starting material. For biodiesel production, crude oils should be degummed (removal of phospholipids) and neutralised (removal of free acids), but bleaching and deodorisation is not generally required. In general, oils and fats (triacylglycerols) are converted to methyl esters by reaction with methanol in the presence of an acidic, basic, or enzymatic catalyst (Section 9.2.2). Reaction with a basic catalyst (NaOH, KOH, or preferably NaOMe) is the preferred route, but is only appropriate with oils with low levels of free acid.

The other product of this reaction is glycerol (Section 9.2.5) and the economic value of this by-product is an important part of the economics of the whole process. Since many other oleochemical processes are dependent on the economic value of glycerol, there is concern about the over supply of this by-product with the consequence of this for prices (Section 9.2.5). It is important that the methanolysis reaction proceed as far as possible as partial glycerol esters as well as methanol, glycerol, and free acids are considered to be undesirable impurities in biodiesel. Walker (2004) has reported that one tonne of rapeseed can be expected to produce crude oil (0.41 tonnes) and meal (0.58 tonnes) and that the refined oil (0.40 tonnes) when reacted with methanol will furnish 0.04 tonnes of glycerol and 0.38 tonnes (432 litres) of methyl esters. With an oilseed yield of 3 t/ha (common in Europe, but not elsewhere) this means that 1300 litres of biodiesel can be obtained with the crop from one hectare of land. Obviously, the energy recovered when the esters are used as a biofuel must exceed that required to produce the fuel with calculations starting from the planted seed. Walker has cited energy output/input ratios for rapeseed methyl esters generally between 2.0 and 2.6 after allowing for the value of the meal and the glycerol. One of the most energy-demanding steps in the process is the fertiliser requirement. Walker (2004) has also discussed the economics of biodiesel production from rapeseed and produced figures of 30 to 60 pence/litre without allowance for distribution costs, taxation, or profit. The range of cost is related to the size of the operating plant.

Several manufactures have designed new or adapted existing plants for efficient biodiesel production. Inputs are methanol, oil (extracted and appropriately pretreated), and catalyst (NaOH, KOH, or NaOMe). The steps involved include interesterification, separation of the glycerol and ester phases, and recovery of glycerol (for further purification), methanol (for reuse), and biodiesel. Plants vary in size from 10 to 200 kt/year. The reaction is an equilibrium process involving two phases and interesterification may be carried out in two or three stages. At the end of each stage glycerol is removed and fresh methanol and a catalyst is charged to the reactor. Reaction proceeds at about 60°C with an overall residence time of about 2 hours. Plants are now available that are flexible enough to handle a range of different feed stocks (Meyer and Koerbitz, 2004). Information is available on the websites of companies, such as Ballestra, Bayer, Crown Iron Works, De Smet, Lurgi Life Science, and Westphalia available through Google.

9.6.4 Specifications for biofuels

Biodiesel must meet specifications (which may differ between countries and regions of the world) that have been accepted by most vehicle manufactures. The EU biodiesel specification (EN 14214) provides minimum and/or maximum values for ester content, density, viscosity, flash point, sulfur content, cetane number, water content, oxidation stability, acid value, iodine value, content of methanol, glycerol, monoacylglycerol, diacylglycerol, linolenate ester, phosphorus, and some other properties. These relate to rapeseed methyl esters and the iodine value with an upper limit of 120 would exclude biodiesel from sunflower oil or soybean oil.

ASTM standard D6751 in the U.S. covers flash point, water and sediment, carbon residue, sulfated ash, viscosity, sulfur, cetane, cloud point, acid number, and free and total glycerol. Iodine value is not included.

Cetane numbers are an important index of ignition quality. For biodiesel these are affected by chain length, unsaturation, and branching and generally have higher minimum values (47 in ASTM D6751 and 51 in EN 14214) than those specified for petrodiesel. Other important properties are viscosity, pour point, and cloud point, which, if not appropriate, can affect behaviour at low temperatures. Low temperature problems may be overcome by using isopropyl esters in place of methyl esters or by winterisation of methyl esters to remove higher melting components. The latter solution adds cost to the manufacturing process by virtue of the extra step involved and leaves a higher melting fraction for which an alternative use has to be developed. Because biodiesel contains unsaturated centres, it is liable to oxidation during storage and it is important to have adequate storage conditions and possibly to add antioxidant.

Reduction of sulfur dioxide emissions from vehicles has been achieved by use of ultra-low sulfur fuels, but this is disadvantageous insofar as lubricity is also reduced. However, lubricity in ultra-low sulfur diesel fuels can be restored by the addition of some 1 to 2% of biodiesel. Knothe and Steidley (2005) have shown that improved lubricity following addition of a little

biodiesel is a consequence mainly of the free acid and monoacylglycerols present in this material. These have been considered as undesirable contaminants and there is perhaps a case for considering minimum as well as maximum limits for these components.

Compared to regular diesel, biodiesel is considered to produce lower emissions of SO_2, CO, hydrocarbons, PAH (polycyclic aromatic hydrocarbons), particulates, and smoke. Depending on engine tuning, NO_x emissions are usually higher with biodiesel. Of course, carbon dioxide is produced as in the combustion of all organic products, but it is argued that this was trapped from the atmosphere during the growing season and, therefore, does not add to global supplies of this gas. Combustion of petrodiesel on the other hand liberates carbon dioxide trapped in fossil fuels millennia previously and, therefore, adds to the global carbon dioxide supply.

9.6.5 Present and future demand for biodiesel

Targets for the replacement of petrodiesel by biodiesel have been set in the EU, though not all member counties have accepted these. It is considered that in 2005 the level of replacement should be 2.5% of total vehicle consumption and that this should rise at the rate of 0.75% a year to 5.75% of total consumption. Targets set by individual countries for 2005 were 2.0% or above for Austria, Czech Republic, France, Germany, Italy, Latvia, Lithuania, Slovakia, Spain, Sweden, and the U.K., but not all these were met. Some other countries with targets of 1% or below are working actively to increase production and there are regular reports of new plants being planned and coming on stream. These levels of 2.5 and 5.75% will require about 2.5 and 6 tonnes of biodiesel. Currently about 80% of European biodiesel comes from rapeseed oil, but other sources will have to be found and this proportion must fall. Production of rapeseed in EU-25 in 2004/05 was around 5.5 million tonnes, 1 million tonnes higher than in the preceding year.

Interest in producing biodiesel is not confined to Europe and many other countries are beginning to produce this material. Where consumption targets have been set (and not met), a growing export–import trade in biodiesel methyl esters will probably develop. For example, Malaysia and Indonesia with their ample supplies of starting material should be a good source of biodiesel and coco-biodiesel is being developed in the Philippines for export to Japan. Malaysia is expected to meet about 10% of the world demand for biodiesel. It is expected that the U.S. will use 0.5 million tonnes of biodiesel in 2006 and that a similar amount will be produced and used in Brazil by 2010. These figures relate to biodiesel and do not include the burning of around 0.5 to 1.0 million tonnes of vegetable oils and animal fats to produce electricity (Anon., 2005). This use is driven in part by low prices for oils and fats (especially palm oil) and in part by pressure to meet manmade targets to reduce carbon dioxide emissions. The production of biodiesel in India is confined to nonedible oils by government decree (Kale, 2005a,b). Gunstone (2006) has forecast a demand for 40 to 50 million tonnes of biodiesel in 2020.

References

Anon., BioX plans palm oil use for green energy, *Inform*, **16**, 748, 2005.

Dumelin, E.E., Biodiesel — a blessing in disguise, *Eur. J. Lipid Sci. Technol.,* **107**, 63–64, 2005.

Fry, J., The world's oil and fat needs in the 21st century: lessons from the 20th century. Lecture presented in 2001 and cited by Gunstone, F.D., Ed., *Vegetable Oils in Food Technology*, Blackwell Publishing, Oxford, U.K., 2002, pp. 15–16.

Gunstone, F.D., Chang Lecture delivered at the AOCS Exhibition in St. Louis, May 2006.

Kale, V., Biodiesel gaining popularity in India, *Inform*, **16**, 466–468, 2005a.

Kale, V., Jatropha — India's crop for biodiesel production, *Inform*, **16**, 532–533, 2005b.

Knothe, G. and Dunn, R.O., Biofuels derived from vegetable oils and fats, in *Oleochemical Manufacture and Applications*, Gunstone, F.D., Hamilton, R.J., Eds., Sheffield Academic Press, Sheffield, U.K., 2001, Chap. 5.

Knothe, G., et al., Ed., *The Biodiesel Handbook*, AOCS Press, Champaign, IL, 2004.

Knothe, G. and Steidley, K.R., Lubricity of components of biodiesel and petrodiesel. The origin of biodiesel lubricity, *Energ. Fuels,* **19**, 11921200, 2005.

Meyer, S. and Koerbitz, W., Worldwide review of biodiesel production and studies on biodiesel production plants in Europe, *Malay. Oil Sci. Technol.,* **13**, 11–16, 2004.

Mittelbach, M. and Remschmidt, C., *Biodiesel — The Comprehensive Handbook*, (published by Mittelbach), Graz, Austria, 2004.

Reaney, M.J.T., Vegetable oils as biodiesel, in *Bailey's Industrial Oil and Fat Products,* 6th ed., Shahidi, F., Ed., Wiley Interscience, New York, 2005, Chap. 6.

Walker, K., Non-food uses, in *Rapeseed and Canola Oil,* Gunstone, F.D., Ed., Blackwell Publishing, Oxford, U.K., 2004, Chap. 7.

9.7 Surface coatings and inks

It has long been known that when thin layers of unsaturated fatty oils are exposed to air they harden to solid impervious films. Artists used this knowledge to provide a protective transparent film for their paintings. With incorporation of appropriate pigments, it is possible to produce a coloured layer. Surface coatings may be applied to wood, paper, metal and plastic surfaces to protect against moisture, oxygen, sunlight, radiation and pollutants, such as sulfur dioxide. They may also serve to decorate or disguise. About half the annual production of paint is used for internal and external use in buildings

(defined as architectural uses) and the remainder is used for coating cars, machines, and domestic appliances and for road markings, etc. It is estimated that about 1 million tonnes of vegetable oils are used in surface coatings each year. General information is provided by Turner (1980), Bentley (2001), Pilemand (2001), Erhan (2005), Van De Mark (2005), and Lin (2005).

Paints generally contain up to four components. The binder (or resin or vehicle) provides adhesion to the surface and cohesion within the paint film itself. It is this component that will contain any vegetable oil derivative that may be present in the paint. A pigment may be added to provide colour and opacity — paints without pigments are varnishes. Solvents or thinners, such as turpentine, white spirit or other hydrocarbons, are present to aid manufacture and application. These last components are now considered to be undesirable and attempts are being made to reduce the amount of these volatile organic compounds (VOC) and where possible to replace them with water or with nonvolatile material. Finally, the paint may contain additives, such as thickeners or wetting agents.

Traditionally paints were made with highly unsaturated oils, such as linseed and tung, but with the advent of alkyd resins in the 1950s, it has been possible to use semidrying oils of lower drying quality, such as soybean, safflower, and sunflower (Table 9.26).

Most binders today are alkyd resins and contain significant amounts of fatty acids. These are polyesters made from a polybasic acid and a polyhydric alcohol with unsaturated fatty acids acylated to additional hydroxyl groups. The polybasic acid is generally phthalic acid (benzene-1,2-dicarboxylic acid) used in the form of its anhydride. The polyhydric alcohol may be glycerol or pentaerythritol [$C(CH_2OH)_4$]. Other possible reactants are listed in Table 9.27. The fatty acids are mainly unsaturated and may be supplied as free acids, triacylglycerols, or often as monoacylglycerols derived from appropriate vegetable oils like linseed oil, soybean oil, or dehydrated castor oil. Castor, tung, coconut, safflower, sunflower, and tall oil may also be used. Alkyds usually contain 30 to 70% of fatty acids. Because the drying process involves oxidative polymerization, it is desirable that the vegetable oil serving as a source of the fatty acids contain as little natural antioxidant as possible. The fatty acids may also be modified to improve their properties as in reaction with maleic anhydride to increase functionality or by prior conjugation of the polyunsaturated system with a proprietary catalyst to speed drying.

TABLE 9.26 Fatty acid composition of oils used in alkyd resins

Oil	Sat	18:1	18:2	18.3
Linseed	9	19	20	47
Tung				69[a]
Soybean	15	23	52	8
Sunflower	11–13	20–30	60–70	
Safflower	10	14	75	
Tall[b]	3	30–46	36–45	

[a] Eleostearic acid (9c11t13t-18:3).
[b] Composition differs between oils from the U.S. and Scandinavia.

Alkyds are divided into three classes depending on their content of unsaturated oil, which may be >60% (long oil), 40 to 60% (medium oil), and <40% (short oil). Long oil alkyds are favoured for architectural purposes and short oil alkyds for coating equipment. The medium oil alkyds may be used for either purpose.

Paint films based on alkyd resins are produced in two stages. The first stage is production of the resin in bulk followed by hardening of the paint film after application. In a simple example, vegetable oil is heated with glycerol to give monoacyl glycerol and then with phthalic anhydride. This produces an intermediate, such as that formulated below. This reacts further at any free hydroxyl groups with more polybasic acid or with unsaturated acyl groups leading to compounds of high molecular weight. Further reaction can lead to branched-chain systems. Alternatively the polybasic acid, polyhydric alcohol, and unsaturated fatty acids are heated together in an inert atmosphere at 230 to 250°C. Products obtained in this way are reported to have a narrower molecular weight distribution.

TABLE 9.27 Polybasic acids or anhydrides and polyhydric alcohols used to produce alkyds

Acids or Anhydrides	Polyhydric Alcohols
Phthalic anhydride (benzene-1,2-dicarboxylic)	Glycerol (propane-1,2,3-triol)
Isophthalic anhydride (benzene-1,3-dicarboxylic)	Pentaerythitol $C(CH_2OH)_4$
Trimellitic anhydride (benzene-1,2,4-tricarboxylic)	Trimethylolpropane $CH_3CH_2C(CH_2OH)_3$
Maleic anhydride	Trimethylolethane $CH_3C(CH_2OH)_3$
Chlorendic anhydride (a)	Ethylene glycol $HOCH_2CH_2OH$
Fumaric acid	Neopentylglycol $HOCH_2C(Me)_2CH_2OH$
Adipic acid	
Sebacic acid	

[a] Derivative made from maleic anhydride and hexachlorocyclopentadiene.
Note: These are two separate lists and are not to be taken as indicating any association between materials on the same horizontal line.

```
–polyol–polybasic acid–polyol–
    |                    |
  acyl                 acyl
```

Other components are sometimes added to alkyds to produce some particular effect.

- Vinylated alkyds are resins into which vinyl monomers, such as styrene, vinyl toluene, or methyl methacrylate have been incorporated at levels of 20 to 60% along with a free radical initiator.
- Silicone alkyds are ethers containing Si-O-C bonds formed between alkoxy-polysiloxane oligomers and the free hydroxyl groups of alkyd resins. Silicone modifications give improved durability desired for exterior maintenance finishes.
- In urethane alkyds, part or all of the dibasic acid is replaced by diisocyanates, such as tolylene diisocyanate (highly toxic). Water must be absent from the reaction mixture since it destroys the isocyanate. The isocyanate function (R´N=C=O) reacts with hydroxyl groups (ROH) at low temperatures to form a urethane (R´NHCOR). Urethane alkyds have superior adhesion, hardness, abrasion and chemical resistance, and durability.
- Polyamide modified alkyds are thixotropic and are required to produce nondrip paints. Polyamides based on dimer fatty acids are introduced at a level around 10%. Ester interchange takes place and polyamide becomes chemically bonded to the alkyd.
- Another modification involves production of epoxides as sources of additional hydroxyl groups, which can be acylated with unsaturated acids.

The drying (hardening) process requires the liquid paint to harden to a solid film. This is achieved first though evaporation of the volatile thinner, followed by oxidation of the unsaturated acids in the binder, and sometimes by interaction with other added components, such as polyols and isocyanates. The drying or hardening process can be accelerated by the addition of cobalt salts of short chain acids that promote oxidation, by ultraviolet light, or by a pretreatment of the oil to promote its easy oxidation/polymerisation. The drying process continues after the film has hardened leading, eventually, to its breakdown (Mallegol et al., 2000a,b).

Paint mixtures require an accelerator and an antiskinning agent. Air drying involves formation of hydroperoxides and their subsequent breakdown and these processes are promoted by cobalt and magnesium naphthenates. However, it is desirable that drying occurs only in the spread film and not in the paint can during storage. Retardation can be achieved with an antiskinning agent. This is generally a cobalt complexing agent that sequestrates the cobalt in the can, but evaporates in the paint film so releasing the cobalt to act as accelerator.

Alkyds with linolenic acid dry quicker than those with linoleic acid, but the former also leads to more extensive oxidation with the undesirable result that the paint film yellows in an ammonia atmosphere. For white or light colour enamels, soybean, safflower oil, or dehydrated castor oil are preferred. These give films with excellent colour and gloss stability. Conjugated systems dry more quickly with less oxygen (~5%), probably by a different mechanism involving cyclic peroxides that break down to C-C and C-O-C dimers.

The ability to "dry" depends on the presence of linoleic acid and linolenic acid with one and two doubly allylic methylene groups, respectively. The drying index is calculated as lin + 2(len) and values of 120 (linseed), 69 (soybean), and 77 (safflower) have been cited. The value of 170 assigned to tung oil is related to the presence of eleostearic acid (conjugated triene).

Vegetable oil-based inks (Erhan, 2005), now widely used for newsprint, have several benefits over the more traditional petroleum-based products. These include superiority in rub resistance, production of a deeper black, providing outstanding colour, quicker adjustment to change in colour, and easier removal when recycling paper and using methyl esters as cleaning solvent. In addition, they have the usual range of environmental advantages: they are biodegradable (apart from the pigment), are low in VOC and PAH, permit easier cleaning of the press, and are based on a renewable resource. Such inks can be made from fatty oils, such as linseed, soybean, rape, palm, and others.

Inks are generally made with alkali-refined oils from which undesirable gums, waxes, free acids, and coloured impurities have been removed. Appropriate viscosities of 1.6 to 18P correspond to molecular weights of 2600 to 8900 and are achieved by heating to around 330°C, which leads to conjugation of polyene systems followed by cross linking and polymerisation (Erhan and Bagby, 1991).

References

Bentley, J., Surface coatings and inks, in *Oleochemical Manufacture and Applications,* Gunstone, F.D., Hamilton, R.J., Eds., Sheffield Academic Press. Sheffield, U.K., 2001, pp. 164–198.

Erhan, S.Z., Printing inks, in *Industrial Uses of Vegetable Oils,* Erhan. S.Z., Ed., AOCS Press, Champaign, IL, 2005, Chap. 9.

Erhan, S.Z. and Bagby, M.O., Lithographic and letterpress ink vehicles from vegetable oils, *J. Am. Oil Chem. Soc.,* **68**, 635–638, 1991.

Lin, K.F., Paints, varnishes, and related products, in *Bailey's Industrial Oil and Fat Products,* Shahidi, F., Ed., Wiley-Interscience, New York, 6th ed., 307–351.

Mallegol, J., et al., Long-term behaviour of oil-based varnishes and paints. Fate of hydroperoxides in drying oils, *J. Am. Oil Chem. Soc*. **77**, 249–255, 2000a.

Mallegol, J., et al., Long-term behaviour of oil-based varnishes and paints. Photo- and thermooxidation of cured linseed oil, *J. Am. Oil Chem. Soc*., **77**, 257–263, 2000b.

Pilemand, C., Use of vegetable oils in coatings in Europe, *Lipid Technol. Newsl.,* **7**, 37–41, 2001.

Turner, G.P.A., *Introduction to Paint Chemistry and Principles of Paint Technology,* 3rd ed., Chapman & Hall, London, 1980.

Van De Mark, M.R. and Sandefur, K., Vegetable oils in paints and coatings, in *Industrial Uses of Vegetable Oils,* Erhan. S.Z., Ed., AOCS Press, Champaign, IL, 2005, Chap. 8; also *Inform,* **16**, 478–481.

9.8 Castor oil products

9.8.1 Introduction

The castor plant, *Ricinus communis* L., is unique. It is thought to originate in Africa and is best suited to tropical and semitropical climates, where it may grow as a perennial tree or shrub. However, it is adapted to many areas, including northern parts of the temperate zone where it may grow as an annual. It is a monotypic species and, although there is considerable variation in characteristics, such as plant height, leaf and stem color, dehiscence, etc., only one species of *Ricinus* has been confirmed. The so-called *R. sanguinensis* appears to be a colorful red variant with no documentation to justify a difference in species name. The plant is classified in the Euphorbiaceae family (Wurdack et al., 2005).

Castor oil is derived from the seed of the castor plant. Castor seed contains up to 60% oil that is rich in a hydroxy fatty acid. The fatty acid is 12-hydroxyoctadec-*cis*-9-enoate, commonly known by the trivial name ricinoleate (Figure 9.7). The level of ricinoleate is up to 90% (Lin et al., 2003), although a mutant strain of castor is 14% ricinoleate and 75% oleate (Rojas-Barros et al., 2005). It is the high level of hydroxy fatty acid that imparts unique physical and chemical properties to castor oil, making it a key feedstock for industry (Naughton and Vignolo, 1992; Caupin, 1997).

The principal grower of castor and producer of castor oil is India, which accounts for over 70% of the export market (Section 2.2.2). Other nations that produce significant amounts of castor include Brazil, China, and Thailand. There is considerable interest in expanding castor production in the U.S., Central and South America, Africa, and the European Union. However, the presence of the toxin ricin and a potent sensitizing allergen in the seed meal have inhibited these plans (McKeon et al., 2002). The price for castor oil on the world market is approximately US $1.0 per kg, which compares to US $0.40 to 0.50 per kg for soy oil (Anon.).

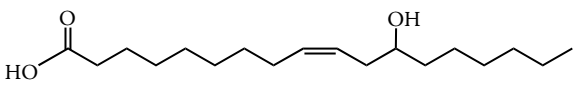


FIGURE 9.7 Ricinoleic acid.

9.8.2 Other sources of hydroxy fatty acids

Castor oil is currently the major source of hydroxy fatty acid, though there are other plant sources of hydroxy fatty acids. *Lesquerella fenderlii* is a "new crop" that has been developed as an alternate source of hydroxy fatty acid for U.S. production. Although this plant grows in desert climates and other marginal agricultural land, it has not yet reached the volume needed to be considered a commercial success or to meet industry needs. *Lesquerella* and *Physaria* species are Crucifereae and usually contain approximately 50% lesquerolate, 14-hydroxy-11-eicosenoate, the 20-carbon analog of ricinoleate, although some species contain up to 84% lesquerolate (Dierig et al., 2004). It arises naturally from ricinoleate by a two-carbon elongation. Its lubricant properties and cosmetic properties are similar to those of ricinoleate (Roethli et al., 1991). Despite this similarity, it does not induce peristalsis and does not have the same physiological effects that ricinoleate does (reviewed in McKeon et al., 2006). *Dimorphotheca pluvialis* produces an oil containing up to 54% of dimorphecolic acid, 9-hydroxy,-10-*trans*,-12-*trans*-octadecadienoate, and this oil can be dehydrated to produce an oil containing a fatty acid with conjugated double bonds and having nonyellowing, durable drying quality, similar to tung oil. Because of this desirable property, it is currently a crop of interest, but has not yet achieved commercial availability.

Until the late 1960s, castor was grown in the U.S. and supplied about half of the U.S. demand for castor oil. The rest of the need was filled by import of castor beans for processing. The loss of crop parity and the rising cost of energy needed to detoxify and de-allergenise castor meal, resulted in cessation of castor oil production in the U.S. by the early 1970s. The issuance of U.S. Presidential Executive Order 13134 in August 1999 supporting a drive to generate more products from biological sources provided considerable impetus to reintroduce castor as a U.S. crop. As a result, efforts to detoxify and de-allergenize castor seed using genetic engineering have developed to meet expanding needs for a safe source of castor oil (Auld et al., 2001).

9.8.3 Applications of castor oil

9.8.3.1 Medicinal and cosmetic

Castor oil is perhaps most commonly known for its laxative properties. Although more gentle laxatives have been developed, it is frequently used in medical studies on diarrhea and bowel function because of its potency. The basis for its laxative effect is physiological, as it acts as an agonist for nitric oxide synthase, inducing smooth muscle contractions in the bowels, and also in the esophagus and uterus (McKeon et al., 2006).

The physical properties of castor oil, including high viscosity and miscibility with polar and nonpolar compounds, make it a widely used ingredient in cosmetics, such as lipstick. Its noncomedogenic interaction with skin underlies

its widespread application in skin creams. Salts of ricinoleate, zinc ricinoleate in particular, also have deodorant properties and are used in personal deodorant applications (Kuhn et al., 2000). There have been reports of allergic reactions to these compounds (Mageri et al., 2001).

9.8.3.2 Lubricants

Greases and other lubricants must interact effectively with the components they are coating, retaining enough structure to remain bound to the parts for a reasonable span of time. Lithium soaps of ricinoleate and its hydrogenation product, 12-hydroxystearate, are effective thickening agents that provide prolonged coating properties to lubricating oils (Naughton and Vignola, 1992). The midchain hydroxyl as well as the carboxylate interact with metal surfaces and, in the soap form, are able to solvate the petroleum or other lubricating component, thus providing the structure needed to maintain the lubricating properties of the grease.

Esters of castor oil, ricinoleate, and products derived from them are widely used in lubricant formulation. Ricinoleate esters and castor oil estolides formed with ricinoleate and other fatty acids are used as viscosity enhancers for lubricant blends and have useful pour point properties, which can help to maintain acceptable lubricant performance at lower temperature. For example, the pour point of an oleate estolide of castor oil is –54°C (Ceremak et al., 2006).

9.8.3.3 Coatings

Castor oil can be dehydrated under various conditions, leading to a polyunsaturated oil that includes a significant proportion of conjugated linoleic acid, with the geometry 9-*cis*, 11-*trans*. Dehydration of ricinoleate leads to a higher proportion of conjugated linoleic acid (Naughton and Vignolo, 1992; Caupin, 1997). Certain naturally occurring oils, such as tung oil, contain a high proportion of fatty acids with conjugated double bonds; these oils are highly susceptible to oxidation and have excellent drying properties. Dehydrated castor oil is a generally acceptable substitute for the more expensive tung oil. These drying oils may be used alone or in combination with other architectural coating components.

9.8.3.4 Surfactants

Because of the midchain hydroxyl group in ricinoleate, soaps made from castor oil have very good emulsifying properties. Chemical modification of the hydroxyl group can be used to alter these properties (Caupin, 1997). The first synthetic surfactant was the sulfated product of castor oil, known as Turkey Red Oil. It is still produced and is used in the tanning process, textile manufacture, and in cosmetic applications. Since it is completely soluble in water, it is an effective emulsifying agent for water-immiscible substances. The hydrogenated form is solid and has no harmful effect on the skin, so it is used in skin creams as an emulsifying agent (Naughton and Vignolo, 1992).

9.8.3.5 Biodiesel

Biodiesel (Section 9.6) represents an alternative to petroleum-derived diesel fuel and is considered especially desirable as it comes from renewable sources. In general, biodiesel, which is usually formulated as methyl esters of fatty acids, enhances the performance of petroleum diesel by adding lubricity and reduces particulate pollution due to the presence of oxygen in the ester moiety (Goodrum and Geller, 2005). Furthermore, since synthetic lubricity agents are sulfur-based, replacement of these compounds by fatty acid methyl esters can eliminate the source of sulfur oxide pollution from diesel fuel. It has been shown that methyl esters derived from transmethylation of castor oil are superior in imparting lubricity when added to diesel fuel (Goodrum and Geller, 2005; Geller and Goodrum, 2004). Methyl esters derived from castor oil and from *Lesquerella* oil (50% lesquerolate) behave similarly, and impart greater lubricity at a relatively low level (0.18%) compared to 1% canola or soy oil methyl esters. Thus, it appears that the mid-chain hydroxyl group is a key contributor to lubricity. Paradoxically, the pure fatty acid methyl ester, ricinoleate, is not as effective as esters generated from the oil (Yaganeh and Mehdizadeh, 2003). The presence of a third oxygen in methyl ricinoleate, with a mid-chain location, may also be significant in enhancing oxygenation, resulting in a cleaner burning fuel.

9.8.3.6 Polymers

Castor oil contains 70% triricinolein (Figure 9.8), with the remainder almost entirely di-ricinoleins (Lin et al., 2003). As a polyhydroxy compound, it readily forms polymers with cross linking agents that react with hydroxyl groups (Naughton and Vignolo, 1992). In reaction with di-isocyantes, castor oil forms polyurethanes that are used in foaming applications, as thermoplastics and as electrical insulators. Polyurethanes formed from the reaction of castor oil with toluene di-isocyanate produce millable polyurethane elastomers. These elastomers are useful in engineering applications, such as O-rings, hose and automotive belts (Yaganeh and Mehdi-zadeh, 2003). The polyurethanes derived from castor oil are also used in coatings and flooring, as well as electrical insulators (Hofer et al., 1997). The polyol nature of castor oil makes it a renewable resource that can substitute for petroleum-derived polyols, such as polyethers, in some applications.

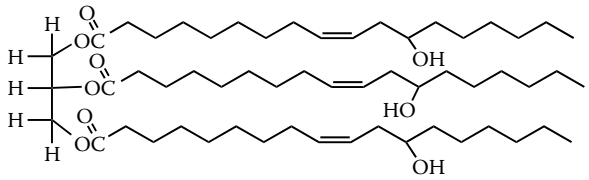


FIGURE 9.8 Triricinolein: This is the major component of castor oil, comprising 70% of the triacylglycerol.

In addition to use in production of polymers, castor polyols are also used in "blends" (Guhanathan et al., 2004). These interpenetrating networks combine two or more polymer materials, which act synergistically to provide a product with properties otherwise unavailable from any of the components. In these materials, castor oil may be involved as a monomer cross linking agent, as an oligomer or as a polymer.

Ricinoleate can be condensed to form lactones, which are susceptible to polymerization by ring opening with a suitable catalyst (Slivniak and Domb, 2005a). Using a purified form of the dilactone, polymers up to 4400 MW have been obtained. Co-polymerization with lactide leads to co-polyesters up to of 16,000 MW. Polylactic acid is a useful bio-based polymer, but is usually modified to improve its properties. Co-polymerization of lactide and ricinoleate lactone leads to a co-polymer with a reduced melting point (Slivniak and Domb, 2005b).

The development of nanotechnology holds the promise of better engineered and "smarter" products. Given the chemical properties of castor oil and ricinoleate, these natural products are very likely to be used in production of nano-engineered polymers. Dendritic polymers of castor oil have been synthesized, using heat or lipases (Bodalo-Santoyo et al., 2005). These dendrimers are oligoricinoleates esterified sequentially, forming chain lengths up to 4 whose properties can be controlled by the level of acylation. Composite materials incorporating castor oil-based polyurethane foams have found use in fabrication of floor coverings (Naughton and Vignolo, 1992; Shikanov et al., 2005), insulators (Naughton and Vignolo, 1992; Qu et al., 2005) and structural components (Naughton and Vignolo, 1992. Caupin, 1997).

9.8.3.7 Ricinoleate-derived chemicals

The combination of a midchain hydroxyl group and a double bond imparts unique chemical reactivity that is unavailable in typical vegetable oils or fats. As a result, ricinoleate provides many useful chemicals and chemical intermediates used by industry for a variety of applications.

Alkali fusion of castor oil yields 10-hydroxy-decanoic acid or sebacic acid (Naughton and Vignolo, 1992) depending on the molar amount of alkali and the temperature used for the fusion reaction (Figure 9.9). In either case, the co-product is 2-octanol, a component of flavors and esters. Sebacic acid is the more valuable product. It is used in the synthesis of polyesters, lubricants, plasticizers, and polyamides, such as Nylon 6,10, which has better solvent and water resistance than Nylon 6,6 (Caupin, 1997). Co-polymers of sebacic acid and ricinoleic acid show promise as biodegradeable drug carriers and drug delivery systems (Altafim et al., 2003). 10-Hydroxy-decanoic acid obtained from more limited alkali fusion is a natural pheromone for bees and, since it is useful in regulating sebaceous secretions, is used in some cosmetic preparations (Clay et al., 2000).

The single largest use of castor oil is in production of 11-amino-undecanoic acid, the monomer for the polyamide Nylon 11, also known as Rilsan (Naughton and Vignolo, 1992). The monomer is produced by steam pyrolysis of methyl ricinoleate at 550°C, with production of methyl 10-undecylenate (Figure 9.9), which is hydrolyzed, brominated, and subsequently aminated to produce the monomer. Nylon 11 is used as engineering plastic, as it is stable to wet conditions and retains mechanical strength over extended use (Caupin, 1997). As a result, it is also useful as a coating on metal to prevent corrosion. During

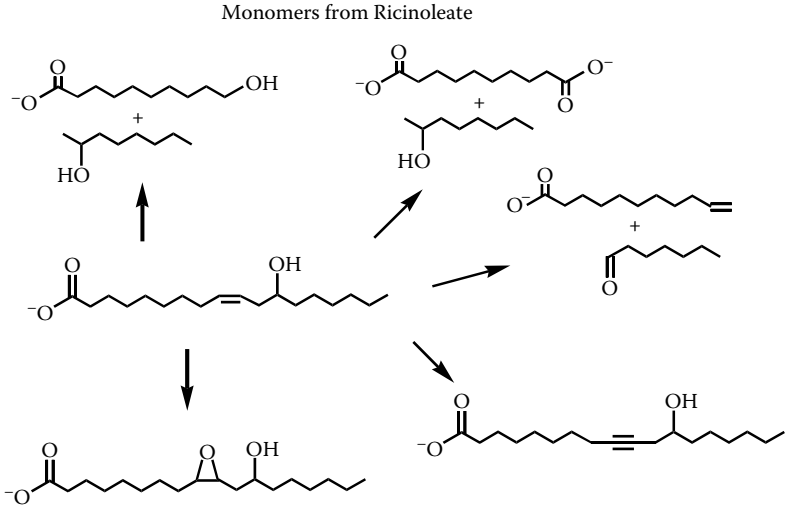


FIGURE 9.9 Monomers derived from ricinoleic acid. From the top left, in clockwise direction: >10-hydroxy-decanoic acid and 2-octanol, resulting from caustic fusion with 1 mole of NaOH at 180-200°C; > sebacic acid and 2-octanol, resulting from caustic fusion with 2 moles of NaOH at 250-275°C; >10-undecylenic acid and heptaldehyde, resulting from pyrolysis in superheated steam at 550°C; >12-hydroxy-octadec-9-ynoic acid resulting from bromination and dehydrobromination of ricinoleate; and >9,10-oxy-12-hydroxy-octadecanoic acid, resulting from epoxidation or hot air oxidation.

production of undecylenate, heptaldehyde is also produced, and is used in flavors and aromas. It is also oxidized to heptanoic acid. Esters of heptanoate remain liquid at low temperatures, retain viscosity, and are not volatile, so they are useful in such demanding applications as jet engine lubrication (Caupin, 1997). Esters of heptanoate are also used as plasticizers.

Methyl ricinoleate can be converted to a hydroxyacetylenic fatty acid (Figure 9.9) by bromination, then dehydrobromination by sonic irradiation in ethanolic KOH (Lie Ken Jie et al., 1996). This compound presents three reactive moieties for polymerization reactions and has potential for more advanced applications, for example, in building nanomaterials.

Ricinoleate can be epoxidized by hot air oxidation or by oxidation with peroxyacetic acid (Figure 9.9). The product formed can be used as a plasticizer and in paints or other coatings (Naughton and Vignolo, 1992) as a low volatile organic carbon (VOC) component.

9.8.4 Summary

Castor oil is a unique natural product, used in many industries and many types of products. It is generally thought that the uses of castor oil in industry are limited by the availability of the oil. Certainly, the presence of a midchain hydroxyl group and the polyol nature of castor oil provide nearly endless possibilities for chemists. More widespread cultivation of castor is very likely to expand utilization of castor oil by industry.

References

Altafim, R.A.C. et al. The effects of fillers on polyurethane resin-based electrical insulators. *Mat. Res.*, **6**, 187–191, 2003.

Anon., USDA Agricultural Statistics, Government Printing Office, Washington, D.C., 2005.

Auld, D.L. et al. Development of castor with reduced toxicity. *J. New Seeds*, **3**, 61–69, 2001.

Bodalo-Santoyo, A. et al. Enzymatic biosynthesis of ricinoleic acid estolides. *Biochem. Eng. J.*, **26**, 155–158, 2005.

Caupin H.J. Products from castor oil: past, present, and future. In Gunstone, F.D., Padley, F.B., Eds., *Lipid Technologies and Applications*. Marcel Dekker, New York, pp. 787–795.

Cermak, S.C. et al. Synthesis and physical properties of estolides from lesquerella and castor fatty acid esters. Ind. Crops Prod., 23, 54–64, 2006.

Clay, A.F. et al. Hydroxydecanoic acid for greasy and acne-prone skin. *COSSMA*, **1**(14), 12–14, 2000.

Dierig, D. et al. Improvement in hydroxy fatty acid seed oil content and other traits from interspecific hybrids of three *Lesquerella* species: *Lesquerella fendleri*, *L. pallida,* and *L. lindheimeri*. *Euphytica*, **139**, 199–206, 2004.

Geller, D.P. and Goodrum, J.W. Effects of specific fatty acid methyl esters on diesel fuel lubricity. *Fuel*, **83**, 2351–2356, 2004.

Goodrum, J.W. and Geller, D.P. Influence of fatty acid methyl esters from hydroxylated vegetable oils on diesel fuel lubricity. *Biores. Technol.*, **96**, 851855, 2005.

Guhanathan, S. et al. Studies on castor oil-based polyurethane/polyacrylonitrile interpenetrating polymer network for toughening of polyunsaturated polyester resin. *J. Appl. Polymer Sci.*, **92**, 817–829, 2004.

Hofer, R. et al. Oleochemical polyols — a new raw material source for polyurethane coatings and floorings. *J. Coatings Tech.*, **69**, 65–72, 1997.

Kuhn, H. et al. Mechanism of the odor-absorption effect of zinc ricinoleate. A molecular dynamics computer simulation. *J. Surf. Deterg.*, **3**, 335–343, 2000.

Lie Ken Jie, M.S.F. et al. Ultrasound-assisted synthesis of santalbic acid and a study of triacyl-glycerol species in Santalum album (Linn) seed oil. *Lipids,* **31**, 1083–1089, 1996.

Lin, J.T. et al. Identification and quantification of the molecular species of castor oil by HPLC and ELSD. *J. Liq. Chrom. Rel. Tech.*, **26**, 773–780, 2003.

Magerl, A. et al. Allergic contact dermatitis from zinc ricinoleate in a deodorant and glyceryl ricinoleate in a lipstick. *Cont. Derma.*, **44**, 119–121, 2001.

McKeon, T.A. et al. Developing a safe source of castor oil. *Inform* **13**, 381–385, 2002.

McKeon, T.A. et al. Hydroxy fatty acids. In *Nutraceutical and Specialty Lipids and Their Co-Products*, F. Shahidi, Ed., CRC Press, Boca Raton, FL, 2006.

Naughton, F.C. and Vignolo, R.L. The chemistry of castor oil and its derivatives and their applications. *Int. Castor Oil Assoc.*, Westfield, NJ, 1992.

Qu, J. et al. Synthesis of castor oil water-borne polyurethane-acrylate hybrid emulsions. *J. Chem. Ind. Eng.*, **56**, 168–173, 2005.

Roethli, J.C. et al. Lesquerella as a Source of Hydroxy Fatty Acids for Industrial Products, USDA-CSRS Office of Agricultural Materials. Growing Industrial Materials Series (unnumbered), Washington, D.C., 1991.

Rojas-Barros, P. et al. Inheritance of high oleic/low ricinoleic content in the seed oil of castor mutant OLE-1. *Crop Sci.*, **45**, 157–162, 2005.

Shikanov, A. et al. Poly(sebacic acid co-ricinoleic) biodegradeable carrier for paclitaxel — effect of additives. *J. Cont. Rel.*, **105**, 52–67, 2005.

Slivniak, R. and Domb, A.J. Macrolactones and polyesters from ricinoleic acid. *Biomacromolecules*, **6**, 1679–1688, 2005a.

Slivniak, R. and Domb, A.J. Lactic acid and ricinoleic acid based copolyesters. *Macromolecules*, **38**, 5545–5553, 2005b.

Wurdack, K.J. et al. Molecular phylogenetic analysis of uniovulate Euphorbiaceae (Euphorbiaceae sensu stricto) using plastid *rbcL* and *trnL-F* DNA sequences. *Am. J. Bot.*, **92**, 1397–1420, 2005.

Yeganeh, H. and Mehdizadeh, M.R. Synthesis and properties of isocyanate curable millable polyurethane elastomers based on castor oil as a renewable resource polyol. *Eur. Polymer J.*, **40**, 1233–1238, 2003.

10

LIPID METABOLISM

J.L. Harwood

10.1 Fatty acids

10.1.1 Fatty acid thiolesters

For most of the reactions in which fatty acids participate in cells, they have to be "activated" (Gurr et al., 2002). This usually involves the generation of a thiolester, such as acyl-CoA or acyl-ACP. Because of the importance of thiolesters, the synthesis of CoA (coenzyme A) and ACP is critical and, indeed, the CoA biosynthetic pathway has been suggested as a possible target for antibacterials (Leonardi et al., 2005). Activation of fatty acids to coenzyme A esters is discussed by Watkins (1997) and, for a general discussion of the production of acyl-thioesters in different organisms, refer to chapters in Vance and Vance (2002).

Because of the poor aqueous solubility of fatty acids, even as their acyl-thioesters, and because the latter may have potentially harmful (detergent-like) effects on cells, there are binding proteins present in cells. These include fatty acid-binding proteins (FABP) as well as acyl-CoA binding proteins (ACBP) (Veerkamp and Maatman, 1995; Glatz and van der Vusse, 1996; Bernlohr et al., 1997).

10.1.2 *De novo* synthesis

De novo synthesis of fatty acids requires the concerted action of two multiprotein complexes or multifunctional proteins. These are acetyl-CoA (coenzyme A) carboxylase and fatty acid synthase. Acetyl-CoA carboxylase (ACCase) is a type I biotin-containing carboxylase. ACCases can have a multiprotein structure or exist as multifunctional proteins (Table 10.1). ACCase catalyses the first committed step of fatty acid and, hence, acyl lipid synthesis. Bacteria and most plant chloroplasts (Harwood, 1996) contain a multisubunit form that is readily dissociated into its component proteins. By contrast, mammals, fungi, and plant cytosols contain large (200 to 250 kDa) multifunctional polypeptides. The structures, regulation, and enzymatic mechanisms of the multisubunit ACCases are reviewed by Cronan and Waldrop (2002), while Kim (1997) has described mammalian ACCases and their regulation.

In the first part of the ACCase reaction, the biotin moiety of BCCP (biotin carboxyl carrier protein) is carboxylated in an (adenosine 5-triphosphate) ATP-mediated process. Recent evidence has implicated carboxyphosphate as an intermediate. The carboxyl moiety is then transferred to the acceptor acetyl-CoA, probably by the use of carbon dioxide as the electrophile. Study of the half-reactions of acetyl-CoA carboxylase has allowed proposals concerning the mechanism of biotin participation to be made (De Titta et al., 1980). The whole subject of the mechanism of biotin-containing enzymes has been excellently reviewed (Knowles, 1989).

Acetyl-CoA carboxylase has been purified to homogeneity from various animal tissues including rat liver (Wakil et al., 1983), chicken liver (Wada and Tanabe, 1983), rat and rabbit mammary gland (Wakil et al., 1983), and goose uropygial gland (Rainwater and Kollattukudy, 1982). It is a multifunctional protein of mass 220 to 260 kDa (kilodaltons).[1] By means of combined protein, chemical, and molecular cloning techniques, Takai et al. (1987) elucidated the primary structure around the biotin-binding site of

[1] The dalton (Da) is equal to 1/12 of the mass of an atom of ^{12}C. Many biologists use the dalton as a unit of mass, although it is not a "recognised" unit. It is especially convenient for structures where the word "molecule" is incorrect, but is used more generally. One should write that the molecular *mass* (not weight) of a protein, for example, is 220,000 Da or 220 kDa.

TABLE 10.1 Examples of different acetyl-CoA carboxylases

Species	Protein structure	Details
E. coli	Multiprotein complex	Four proteins: biotin carboxylase, BCCP and carboxyltransferase (a heterodimer); transcription of all four acc genes is under growth rate control
Yeast	Multifunctional protein	190–230 kDa; activated but not polymerised by citrate
Dicotyledon plants	Multiprotein complex	In chloroplasts; similar properties to *E. coli* enzyme; probably three of the subunits coded by nucleus, one by chloroplast
	Multifunctional protein	Presumed to be cytosolic; concentrated in epithelial cells in pea; molecular mass 200–240 kDa; functions as dimer Graminicide insensitive
Grasses (Poaceae)	Multifunctional proteins	Two isoforms, both 200–240 kDa, which function as dimers Chloroplast isoform is graminicide sensitive, but cytosolic form (concentrated in epithelial cells) is insensitive Both are nuclear encoded
Animals	Multifunctional proteins	Cytosolic; about 250 kDa, but functions as polymer of up to 10^7 kDa; aggregation increased by citrate; also regulated by phosphorylation in response to hormones

Source: From Gurr, M.I., Harwood, J.L. and Frayn, K.N. (2002). *Lipid Biochemistry*, 5th ed., Blackwell Scientific, Oxford, U.K. With permission.

chicken liver acetyl-CoA carboxylase, and they (in 1988) described the complete amino acid sequence of the chicken liver enzyme by cloning and sequencing the DNA complementary to the appropriate messenger RNA. The chicken liver acetyl-CoA carboxylase was found to be composed of 2324 amino acid residues with a calculated molecular mass of 262,706 Da. The BCCP domain was in the middle region of the polypeptide. The amino terminal portion showed a primary structure homologous to that of carbamyl phosphate synthase, and was thought to be the site of the biotin carboxylase for which carboxyl phosphate is the postulated reaction intermediate (see Knowles, 1989).

Although animal acetyl-CoA carboxylases have molecular masses of about 250 kDa, they function as polymers of molar masses 4×10^6 to 8×10^6 g/mol (Wakil et al., 1983). The regulation of mammalian acetyl-CoA carboxylase has been well studied owing to the key role of this enzyme in controlling overall fatty acid (and fat) synthesis. Control occurs in two ways: short-term control, which involves allosteric regulation and covalent enzyme modification, or long-term control, where the amounts of the carboxylase are changed (Gurr et al., 2002). Metabolite control is due to citrate (Moss and Lane, 1971) and long-chain acyl-CoAs. The latter cause depolymerisation of mammalian carboxylases. Palmitoyl-CoA, stearoyl-CoA, and arachidoyl-CoA are the most effective at inhibiting the carboxylase (Nikawa et al., 1979). Other effectors have been reported to regulate mammalian acetyl-CoA carboxylase (cf. Wakil et al., 1983), but their physiological importance is less certain.

Mammalian acetyl-CoA carboxylase is also subject to regulation by phosphorylation/dephosphorylation (Kim, 1997). Phosphorylation with one mole of phosphate per mole of rat liver carboxylase subunit causes complete inactivation. The reactions have also been studied using preparations from the rat epididymal fat pad. Long-term regulation of the carboxylase is caused by diet, thyroxine, and insulin. Total enzyme amounts also change during cell differentiation and development (reviewed by Volpe and Vagelos, 1976). Use of antibodies to rat liver acetyl-CoA carboxylase revealed unexpectedly that the mitochondrial outer membrane contained a major pool of rather poorly active enzyme. It was suggested that this protein represented a reservoir of acetyl-CoA carboxylase that could be released and activated under lipogenic conditions (Allred and Roman-Lopez, 1988). However, the role of ACCase-generated malonyl-CoA in the control of mitochondria β-oxidation (see below) should be noted. The control of mammalian acetyl-CoA carboxylases has been summarised well by Goodridge (1991).

Animal acetyl-CoA carboxylases have been recently reviewed by Rangan and Smith (2002), who give further information about domain organisation. They also draw attention to the two major isoforms of ACCase, α and β, which are found in animals. The α-isoform is found in the cytosol of lipogenic tissues (e.g., adipose) where it is important for *de novo* synthesis of fatty acids. The β-isoform is present in tissues, which generally have a low lipogenic activity and is associated with the outer mitochondrial membrane. The malonyl-CoA produced by ACCase-β functions primarily as a negative regulator of carnitine palmitoyltransferase I and, therefore, regulates the flux of fatty acids into mitochondria for β-oxidation.

Until fairly recently the molecular nature of plant acetyl-CoA carboxylase was unclear. However, it now seems that the enzyme is present as a multifunctional protein of mass 220 to 240 kDa (see Harwood, 1996) in all plant cytosols. Reference to the purification of higher molecular mass forms of the enzyme from parsley and oilseed rape as well as earlier work will be found in Harwood (1988). A similar form has also been purified to near homogeneity from the diatom *Cyclotella cryptica* (Roessler, 1990). In most plants (the gramineae are an exception), the major ACCase isoform,

however, is a multiprotein complex located in the chloroplast where it is used for *de novo* fatty acid synthesis. In gramineae (grasses), the chloroplast isoform is a multiprotein complex. In addition to acetyl-CoA carboxylase, cell-free extracts from several monocotyledonous (monocot.) and dicotyledonous (dicot.) plant species have been found to contain three additional biotin-containing enzymes — 3-methylcrotonyl-CoA carboxylase, propionyl-CoA carboxylase and pyruvate carboxylase (Wurtele and Nikolau, 1990) — the presence of which can complicate purification protocols making use of avidin affinity columns. However, propionyl-CoA carboxylase activity is also present in many purified plant acetyl-CoA carboxylases. Regulation of the plant acetyl-CoA carboxylase is not as well understood as it is for mammals. Tricarboxylic acids do not seem to function at all, and the enzyme in leaves may be activated, at least in part, through changes in the stroma medium as photosynthesis occurs (see Harwood, 1988). Nevertheless, recent measurement of the pool sizes of intermediates during light-activated fatty acids synthesis in spinach leaves shows clearly that the activity of acetyl-CoA carboxylase is a key regulatory component (Post-Beittenmiller et al., 1991).

Recent updates on plant acetyl-CoA carboxylase and its regulation are given by Schmid and Ohlrogge (2002) and by Harwood (2005). These refer to the different forms of ACCase found in graminaceae (grasses) compared to dicotyledons and the importance of ACCase in the regulation of lipid synthesis (see Section 10.8).

In addition to the animal and some plant acetyl-CoA carboxylases, that from the yeast *Saccharomyces cerevisiae* also seems to be a multifunctional protein of about 250 kDa. It appears to be regulated by nutrient supply and coordinately with the fatty acid synthase genes (Trotter, 2001).

In contrast to the above, the acetyl-CoA carboxylase from *Escherichia coli* contains four separate proteins: BCCP, biotin carboxylase and a heterodimer, carboxyl transferase (Cronan and Waldrop, 2002). In this regard, *E. coli* shares the same characteristics as the transcarboxylase from *Propionibacterium shermanii* (Samols et al., 1988). Early work on the structure of the component proteins of *E. coli* acetyl-CoA carboxylase has been summarised (see Volpe and Vagelos, 1976; Cronan and Waldrop, 2002). The DNA sequence of the gene encoding the biotin carboxylase subunit has been reported and encodes a protein of 449 residues. The sequence is strikingly similar to the amino terminal sequence of two biotin-dependent carboxylase proteins, yeast pyruvate carboxylase and the subunit of rat propionyl-CoA carboxylase (Li and Cronan, 1992). Neither citrate nor phosphorylation plays any role in regulating the bacterial enzyme. Instead, the guanosine nucleotides, guanosine 3′-diphosphate 5-di (and tri-) phosphate, were suggested for use. However, these observations have been questioned (see Cronan and Waldrop, 2002). On the other hand, it is clear that nutrient supply is important in controlling total ACCase activity.

Fatty acid synthases can be categorised as Type I, Type II, and Type III. The distribution and some of the properties of the Type I and Type II enzymes are summarised in Table 10.2. The Type I synthases tend to occur in higher organisms, and all of the purified synthases of eukaryotes, with the exception of plants, are of this type. Two bacterial genera, *Mycobacterium* and *Corynebacterium,* also contain Type I synthases. These synthases are large molecular mass multifunctional proteins containing covalently bound acyl carrier protein (ACP) (see Wakil et al., 1983). On the other hand, Type II synthases, such as that from *E. coli,* consist of individual enzymes that can be isolated in an active form. The ACPs of Type II synthases also readily dissociate and can be purified and characterised (see Volpe and Vagelos, 1973). Type III synthases elongate preformed acyl chains and will be dealt with later.

The partial reactions of fatty acid synthases are shown in detail in Table 10.3, where assay details for measuring activities in plants are given. A comparison of some features of the Type I and Type II fatty acid synthases is made in Table 10.4.

The Type I fatty acid synthases from rat liver, adipose and lactating mammary glands, rabbit mammary glands, and uropygial glands have been purified and found to have many common features (Table 10.4). The two subunits appear to be identical and of molecular mass 200 to 250 kDa

TABLE 10.2 Distribution and major properties of Type I and Type II fatty acid synthases

	Distribution	Major characteristics
Type I	Animals	High-molecular-mass multifunctional proteins.
	Birds	Covalently bound ACP[a].
	Yeast	Release unesterified fatty acids (animals) or acyl-CoA (yeast).
	Euglena gracilis (cytoplasm)	
	Fungi	
	Mycobacterium smegmatis	
	Corynebacterium diphtheriae	
Type II	Plants	Dissociable enzymes in a complex with ACP.
	Euglena gracilis (chloroplasts)	Dissociable ACP.
	Most bacteria	Release acyl thioesters or transfer final products directly to lipid.
	Cyanobacteria	

[a] ACP, acyl carrier protein.

Source: From Harwood, J.L. and Russell, N.J. (1984) *Lipids in Plants in Microbes,* Allen and Unwin, Hemel Hempstead, U.K. With permission.

TABLE 10.3 Partial reactions of fatty acid synthase (FAS) and assay principles for measuring their activity in plants

Enzyme	Method
Activities common to all FASs	
Acetyl-CoA:ACP transacylase [^{14}C]Acetyl-CoA + ACP $\rightleftharpoons$ [^{14}C]Acetyl-CoA + CoA	Precipitate acetyl-ACP Count
Malonyl-CoA:ACP transacylase [^{14}C]Malonyl-CoA + ACP $\rightleftharpoons$ [^{14}C]Malonyl-CoA + CoA	Precipitate malonyl-ACP Count
β-Ketoacyl-ACP synthase[a] Acetyl-ACP + Malonyl-ACP $\rightleftharpoons$ Acetoacetyl-ACP + CO_2 + ACP	Measure absorbance at 303 nm (acetoacetate formation)
or *n*Acyl-ACP + Malonyl-ACP $\rightleftharpoons$ (*n* + 2)Ketoacyl-ACP + ACP + CO_2	In presence of NADPH[b] and other FAS enzymes, measure absorbance at 340 nm With [^{14}C]malonyl-CoA, measure counts in acyl chains Use of NaH[^{14}C]CO_2 permits a CO_2 exchange assay
β-Ketoacyl-ACP reductase β-Ketoacyl-ACP + NAD(P)H $\rightleftharpoons$ β-Hydroxyacyl-ACP + NAD(P)	Change in absorbance at 340 nm
β-Hydroxyacyl-ACP dehydrase β-Hydroxylacyl-ACP $\rightleftharpoons$ Enoyl-ACP + H_2O	Back-reaction followed with crotonyl-ACP and decrease in absorbance at 263 nm
Enoyl-ACP reductase Enoyl-ACP + NAD(P)H $\rightleftharpoons$ Acyl-ACP + NAD(P)	Change in absorbance at 340 nm
Additional activities present in *E. coli* and higher plants	
Acetoacetyl-ACP synthase [^{14}C]Acetyl-CoA + Malonyl-ACP $\rightleftharpoons$ [^{14}C]Acetoacetyl-ACP + CoA + CO_2	Reaction in presence of cerulenin Precipitate acetoacetyl-ACP and count
β-Ketoacyl-ACP synthase II Palmitoyl-ACP + [2-^{14}C]Malonyl-ACP $\rightleftharpoons$ [2-^{14}C] β-ketooctadecanoyl-ACP + ACP + CO_2	Reaction products reduced and counts in acyl chain measured

[a] β-Ketoacyl-ACP synthase I in plants.
[b] NAD(P)(H), nicotinamide adenine dinucleotide (phosphate), (reduced).
Source: From Harwood, J.L. et al. (1990).

TABLE 10.4 Types of fatty acid synthases in different organisms

Source	Subunit types	Subunit (mol. mass)	Native (mol. mass)	Major products
Type I: Multicatalytic polypeptides				
Mammalian, avian liver	α	$220–270 \times 10^3$	$450–550 \times 10^3$	16:0 free acid
Mammalian mammary gland	βα	$200–270 \times 10^3$	$400–550 \times 10^3$	4:0–16:0 free acids
Goose uropygial gland	α			2,4,6,8-tetramethyl-10:0
M. smegmatis	α	290,000	2×10^6	16:0-, 24:0-CoA
S. cerevisiae	α, β	185,000, 180,000	2.3×10^6	16:0, 18:0-CoA
Dinoflagellates	α	180,000	4×10^5	
Type II: Freely dissociable enzymes				
Higher plant chloroplasts	Separate enzymes	–	–	16:0-, 18:0-ACP
E. gracilis chloroplast	Separate enzymes	–	–	12:0-, 14:0-, 16:0-, 18:0-ACP
E. coli	Separate enzymes	–	–	16:0-, 18:1-ACP

Source: From Gurr, M.I., Harwood, J.L. and Frayn, K.N. (2002). *Lipid Biochemistry,* 5th ed., Blackwell Scientific, Oxford, U.K. With permission.

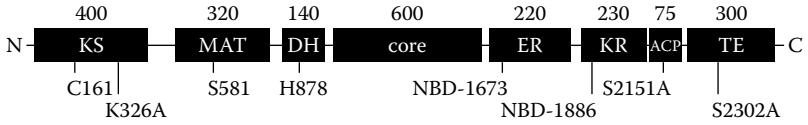


FIGURE 10.1 Linear domain map of the animal FAS. The approximate number of residues in each domain is indicated above the map. Below are shown the locations of nucleophilic residues involved in the formation of covalent acyl-O-serine, acyl-S-cysteine, and acyl-S-phosphopantetheine intermediates, the dehydrase active-site histidine residue, and the beginning of the two glycine-rich motifs in the nucleotide-binding domains (NBD). The introduction of mutations at the critical sites indicated compromise activity of the specific domains. Residue numbering is for the rat FAS. Abbreviations: ACP, acyl carrier protein; DH, dehydrase; ER, enoyl reductase; KR, β-ketoacyl-ACP reductase; KS, β-ketoacyl-ACP synthase; MAT, malonyl acetyl transferase; TE, thioesterase. (From Smith, S., Witkowski, A. and Joshi, A.K. (2003) *Prog. Lipid Res.* **42,** 289–317. With permission.)

(cf. Wakil et al., 1983). In contrast, the Type I synthase of yeast is not only larger (2.4×10^6 Da) than those of animals (0.4×10^6 to 5×10^6 Da), but contains two non-identical subunits of molecular masses 208 and 220 kDa. The native yeast synthase is, thus, an $\alpha_6\ \beta_6$ complex (cf. Schweizer et al., 1973).

The 208 kDa α subunit of the yeast FAS, encoded by the *FAS2* gene, is trifunctional and contains domains for β-ketoacyl-ACP synthase, β-ketoacyl-ACP reductase, and acyl carrier protein. The 220 kDa β subunit, encoded by the *FAS1* gene, possesses acetyl-, malonyl-, and palmitoyl transferase, dehydratase, and enoyl-ACP reductase activities (Trotter, 2001).

The partial reactions of the yeast Type I FAS were elucidated in a classic series of experiments by Lynen (1967). Since then we have much more information, including the mechanism of reaction termination by which the acyl chain is transferred from ACP on the α-chain of FAS to CoA (Schweizer, 1984). For a general summary, see Gurr et al. (2002).

Animal FAS complexes consist of homodimers of native molecular masses of 450 to 550 kDa. The earliest attempts to generate a domain map of FAS utilised limited proteolysis. As the entire sequences of several animal FASs were deduced, a more detailed domain order emerged. The order of domains has now be confirmed by the use of mutants, compromised in specific activities and is shown in Figure 10.1 (Smith et al*.,* 2003). Various models for animal FAS have been proposed with two identical polypeptide chains arranged antiparallel that together form two centres for palmitate synthesis at the subunit interface. By the use of modified FASs in which the activity of one of the functional domains was specifically compromised by mutations, details of the dimeric structure began to be revealed (Smith et al., 2003). In a recent update, Astrurias et al. (2005) carried out cryo-EM analysis of single FAS particles and showed that the images were of two coiled monomers in an overlapping arrangement. Only limited local rearrangements were needed for catalytic interaction among different functional domains. Monomer coiling was suggested to be useful for FAS. The above papers give important information of animal fatty acid synthase (FAS), which is summarised by Rangan and Smith (2002).

The Type I fatty acid synthase of *Mycobacterium smegmatis* is unusual in several respects. First, the two reductases have different reduced pyridine nucleotide specificities: the β-ketoacyl-ACP reductase requires NADPH and the enoyl-ACP reductase requires NADH, whereas the reductases of other Type I synthases only use NADPH. The products of the *M. smegmatis* synthetase have a bimodal distribution with peaks at C16 and C24, and the overall rate of fatty acid synthesis is increased by two types of polymethylsaccharides found in the mycobacterial cell wall (Bloch, 1977). Mycobacteria also contain a Type II FAS and details of the action of both types of FAS will be found in Barry et al. (1988).

The Type II synthase of *E. coli* was the first FAS studied. The individual proteins have all been isolated, purified, and characterised (see Volpe and Vagelos, 1973) and the genes coding for these proteins identified (White et al., 2005). The malonyl group of malonyl-CoA (produced by ACCase) is transferred to ACP by malonyl-CoA: ACP transacylase (FabD). All of the subsequent intermediates in *E. coli* FAS are attached to the terminyl sulfhydryl of ACP, which is one of the most abundant proteins in the bacterium. Acetoacyl-ACP is then formed by acetoacetyl-ACP synthase (FabH), which, it should be noted, uses acetyl-CoA as the other substrate in contrast to the usual priming reaction for FAS (in Type I enzymes) (Figure 10.2). The remaining cycle of reactions continues via reduction (FabG), dehydration (FabA or FabZ), and a second reduction (FabI) to create a saturated fatty acid. Condensation then takes place with malonyl-ACP and is catalysed by FabB (β-ketoacyl-ACP synthase I) or, at later stages, with FabF (β-ketoacyl-ACP synthase II). There are two other isoforms of enoyl reductase formed in bacteria termed FabK and FabL. The FabK protein of Gram-positive bacteria has an additional flavin cofactor (White et al., 2005).

The *E. coli* FAS is also interesting in that it can produce monounsaturated (mainly *cis*-vaccenic) acids as well as saturated products. The ratio of the three principal products — palmitate, palmitoleate, and *cis*-vaccenate — is controlled by the activity of the enzymes shown in Figure 10.3. Regulation of the pathway is discussed well by Jackowski et al. (1991).

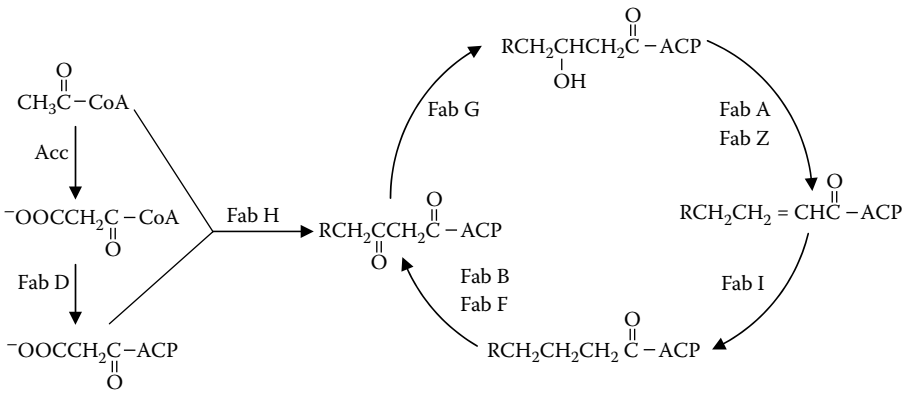


FIGURE 10.2 Pathway of fatty acid synthesis in *E.coli.* The initial reaction is condensation between acetyl-CoA and malonyl-ACP, catalysed by FabH. After that a cycle of reduction, dehydration, and a second reduction generates a fatty acid. Later condensation utilises the gradually lengthening fatty acid and uses FabB except for the final stages when FabF is used (see text and Heath et al. (2001) for more details.) Abbreviations: Acc, acetyl-CoA carboxylase; Fab A, β-hydroxyacyl-ACP dehydratase isomerase; Fab B, β-ketoacyl-ACP synthase I; Fab D, malonyl-CoA: ACP transacylase; Fab F, β-ketoacyl-ACP synthase II; Fab G, β-ketoacyl-ACP reductase; Fab H, β-ketoacyl-ACP synthase III; Fab I, enoyl-ACP reductase; Fab Z, β-hydroxyacyl-ACP dehydratase.

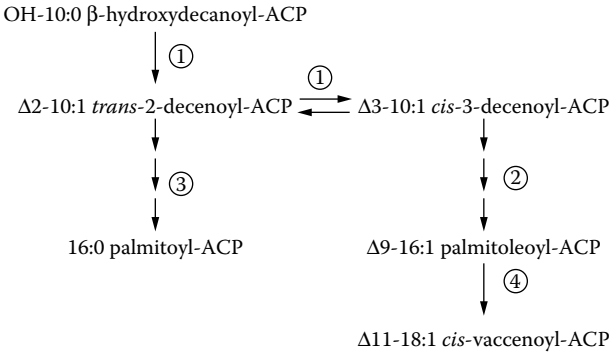


FIGURE 10.3 Product diversification in fatty acid biosynthesis. Three main fatty acids are produced by the *Escherichia coli* fatty acid synthase system. The ratio of these fatty acids is controlled by the activity of three enzymes: (1) 3-hydroxydecanoyl-ACP dehydrase (encoded by the *fabA* gene) is a specific dehydrase that introduces the double bond into the acyl chain; (2) 3-ketoacyl-ACP synthase I (encoded by the *fabB* gene) catalyses an essential step in the unsaturated fatty acid elongation pathway; (3) both 3-ketoacyl-ACP synthases I and II can elongate saturated fatty acids; and (4) 3-ketoacyl-ACP synthetase II (encoded by the *fabF* gene) is responsible for the elongation of 9-16:1 to 11-18:1. (From Jackowski et al. (1991).)

The fatty acid synthase of *Brevibacterium ammoniagenes* has a high molecular mass (like Type I synthases), but produces both saturated and unsaturated acids (like *E. coli* type II synthetase). In most Gram-positive and some Gram-negative bacteria, branched-chain acids are made by Type II synthases that use short-chain branched acyl-CoA primers instead of acetyl-CoA and so produce *iso* or *anteiso* fatty acid products (Kaneda, 1977). The topic of lipid synthesis (and, especially, fatty acid synthesis) in relation to existing and new antibacterials has been reviewed by Heath et al. (2001, 2002) who give a summary of fatty acid synthesis in *E. coli* with reference to some other bacteria.

Plants also contain a Type II FAS. The enzymology of the complex has been reviewed (Stumpf, 1987; Harwood, 1988, 2005). Like *E. coli*, there are several condensing enzymes. β-Ketoacyl-ACP synthase I (KAS I) is responsible for forming keto acids of up to 16 carbons. KAS II then allows the condensation of palmitoyl-ACP with malonyl-ACP and, therefore, controls the production of stearate. These enzymes differ in their sensitivity to cerulenin and arsenite (see Harwood, 1988). A third condensing enzyme, KAS III, has recently been discovered and, like its counterpart in *E. coli,* is responsible for acetoacetyl-ACP formation (Jaworski et al., 1989; Walsh et al., 1990).

Although it is believed generally that *de novo* synthesis of fatty acids in plants is concentrated in plastids (see Harwood, 1988), a report of acyl-ACP in plant mitochondria (Chuman and Brody, 1989), together with fatty acid synthesis mediated by ACP in *Neurospora crassa* mitochondria (Mikolajczyk and Brody, 1990), raise the possibility for some activity in this organelle.

Much attention has been paid to plant ACP. Earlier work on its purification and properties has been well reviewed (Ohlrogge, 1987). ACP is synthesized in the cytosol and posttranslationally imported into plastids, where

proteolytic processing to the mature protein takes place. The attachment of the prosthetic group needs holo-ACP synthetase, which is cytosolic (El Hussein et al., 1988). Further aspects of the biochemistry of plant ACP are covered by Ohlrogge et al. (1991) and by Slabas and Fawcett (1992).

All of the proteins catalysing the partial reactions of plant FAS have been purified and, in many cases, genes coding for them have been identified and sequenced. Most of these proteins occur as isoforms (see Harwood, 1988). Updates on the genetics of fatty acid synthesis (Ohlrogge et al., 1991) and its molecular biology (Slabas and Fawcett, 1992) have been published. Fatty acid synthesis in plants has been summarised by Schmid and Ohlrogge (2002) and reviewed in some detail by Harwood (2005).

Regulation of fatty acid synthesis in plants has been discussed by Harwood (1996), Ohlrogge and Jaworski (1997), and, more recently, by Harwood (2005). The regulation of fatty acid synthesis in animals is described by Rangan and Smith (2002).

Euglena gracilis is an interesting organism because, when it grows heterotrophically (when it is "animal-like"), it contains a Type I synthase. When it is grown photoautotrophically, it contains, in addition to its cytoplasmic Type I enzyme, a Type II synthase in its chloroplasts (Ernst-Fonberg and Bloch, 1971). See also Worsham et al. (1988).

10.1.3 Elongation systems

As mentioned in Section 10.1.2, the elongation of fatty acids is generally carried out by Type III fatty acid synthases, which use malonyl-CoA as the source of C_2 units for addition.

In plants, the elongation of stearate to form very long-chain saturated fatty acids (which are precursors of the various components of waxes, cutin, and suberin; see Kolattukudy, 1980, 1987) takes place via several chain length-specific systems (Walker and Harwood, 1986). Malonyl-ACP and NADPH are required (see Harwood, 1988, 1996) and a cycle of condensation, reduction, dehydration, and second reduction reactions using acyl-CoAs are established. Proteins catalysing these partial reactions have been demonstrated in leek (Lessire et al., 1989) and *Lunaria annua* (Fehling et al., 1992). In the latter case, monounsaturated acyl-CoAs are also substrates, and the elongation of such acids is important in brassicas, such as rapeseed, where erucate (Δ13-22:1) is a major component of the seed oil (Section 2.2). The genetics of the rape elongation system are well described by Ohlrogge et al., (1991). Elongation in jojoba (which accumulates lipid as wax esters) uses a system with oleoyl-CoA and malonyl-CoA as substrates, and this plant has been studied in some detail (Pollard and Stumpf, 1980).

Formation of the very long-chain saturated fatty acids involved in the surface coverings of plants is subject to inhibition by thiocarbamate herbicides (Harwood, 1990), probably as their sulfoxide metabolites (Abulnaja and Harwood, 1991; see Section 11.8). Plant fatty acid elongation was updated by Harwood (1996) and summarised in Schmid and Ohlrogge (2002).

Elongation products in yeast have been extensively studied and three separate elongase genes ELO1, ELO2, and ELO3 identified in *Saccharomyces cerevisiae.* All three genes are for condensing enzymes in the elongation systems (Dittrich et al., 1998). More recently, progress has been made in identifying other components. The yeast systems elongate saturated and monounsaturated fatty acids with different chain-length specificities (Leonard et al., 2004).

Although there are some animal elongases, which have substrate preferences for saturated or monounsaturated fatty acids, many of them are used to elongate polyunsaturated fatty acids (PUFAs). This is not surprising because most dietary PUFA are 18C molecules, whereas most biologically effective metabolites are 20 or 22C (see Sections 10.1.8 and 11.1). Very long-chain PUFAs are particularly important components of mammalian brain (especially 22:6n-3) and it is not surprising that some of the first studies identified different elongases in this tissue (Goldberg et al., 1973; Bourre et al., 1975). Details of the ELOV (very long-chain elongase) genes from humans, rats, and mice are given in Leonard et al., (2004). Like the plant enzymes, the mammalian elongases are microsomally located (endoplasmic reticulum) and use malonyl-CoA in four steps (like FAS) to produce products 2C longer (see Cook and McMaster, 2002; Leonard et al., 2004).

Fatty acids can also be elongated in mammals by a mitochondrial system, which uses acetyl-CoA as the unit for C_2 addition and NADH for reduction. In general, the mitochondrial system elongates fatty acids in the range C_{10} to C_{14}, whereas the microsomal system uses C_{16} and longer acids. At first it was thought that the mitochondrial elongation system could operate by a reversal of β oxidation, but this is now not considered thermodynamically feasible. Indeed, the (flavin adenine dinucleotide) FAD-dependent acyl-CoA dehydrogenase of oxidation is substituted by a more thermodynamically favorable enzyme, enoyl-CoA reductase, which is rate-limiting for the overall process in mitochondria (Cook and McMaster, 2002).

In contrast to animals and plants, bacteria do not usually contain significant amounts of acids longer than C_{18}. A notable exception is the long-chain (up to C_{56}) fatty acids found in mycobacteria. These are formed by an elongation system using acetyl-CoA and NADH, which may be a reversal of β oxidation (Harwood and Russell, 1984).

Fatty acid elongation systems in lower eurkaryotes (the nematode *Caenorhabditis elegans,* fungi, microalgae, moss) are described by Leonard et al. (2004).

10.1.4 Desaturases

Unsaturated fatty acids can be produced by anaerobic or aerobic pathways — the latter being the most usual mechanism. Aerobic desaturases have been studied in a large number of cases, although successful purifications have only been made a few times. A general summary is found in Gurr et al. (2002).

The anaerobic method, which is used by many members of the Eubacteriales, including all the anaerobes as well as some aerobes and facultative aerobes, has already been summarised in Section 10.1.2. It relies on the activity of the β-hydroxy-decanoyl-ACP β, γ-dehydrase whose isomerase activity allows the synthesis of a *cis*-3-decenoyl-ACP from the usual *trans*-2-decenoyl-ACP. *cis*-3-Decenoyl-ACP cannot be reduced by the enoyl-ACP reductase, but can be condensed by the β-keto-acyl-ACP synthase. This reaction, therefore, allows chain lengthening, but preserves the *n*-7 *cis* double bond and results in the synthesis of a series of monoenoic fatty acids (Figure 10.4).

Details of the enzymes and the position of the genes involved in their synthesis are summarised in Heath et al. (2002). The ratio of the three main products of *E. coli* FAS is controlled by the relative activity of three enzymes, as illustrated in Figure 10.3.

Aerobic desaturation involves the stereospecific removal of two hydrogen atoms from an acyl chain. Along with reducing equivalents from NAD(P)H or other reductants, the hydrogen atoms are used to reduce molecular oxygen to water. The desaturases are membrane-bound multienzyme complexes, with the exception of the stearoyl-ACP desaturase found in chloroplasts. The basic details of aerobic desaturation appear to be the same in all organisms in that oxygen and a reduced cofactor are necessary, although the nature of the carriers varies in different systems (Figure 10.5) as well as their susceptibility to cyanide

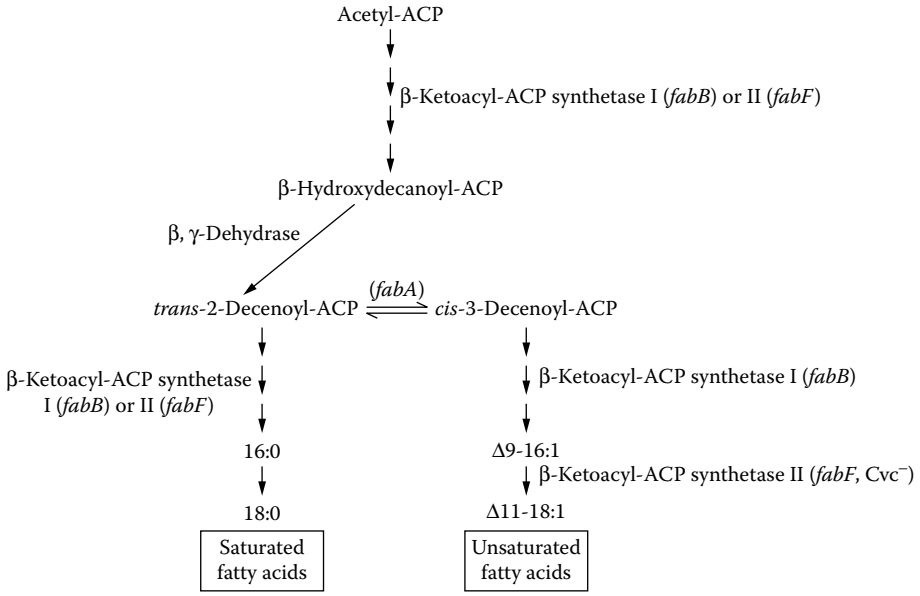


FIGURE 10.4 Anaerobic pathway of fatty acid biosynthesis in bacteria showing mutants of *Escherichia coli.* Here *fab* refers to deficient mutants described for *E.coli.* (From Harwood, J.L. and Russell, N.J. (1984) *Lipids in Plants in Microbes,* Allen and Unwin, Hemel Hempstead, U.K. With permission.)

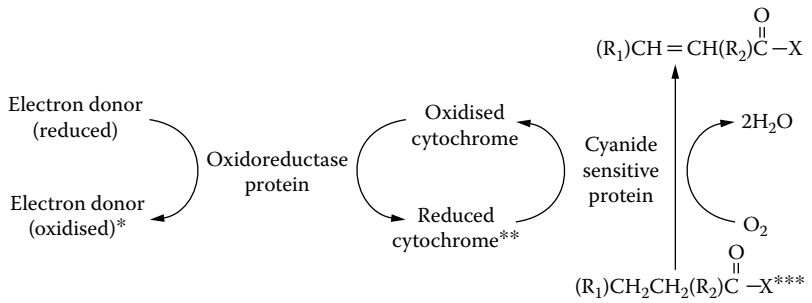


FIGURE 10.5 A generalized scheme for aerobic fatty acid desaturation: *e.g., NADH, NADPH, reduced ferredoxin; **e.g., cytochrome b_5; ***e.g., acyl-ACP (stearoyl-ACP Δ9-desaturase in plants); acyl-CoA (stearoyl-CoA Δ9-desaturase in animals); oleoyl-phosphatidylcholine (Δ12-desaturase in yeast or plants); linoleoyl-monogalactosyldiacylglycerol (Δ15-desaturase in plant chloroplasts). (From Gurr, M.I., Harwood, J.L. and Frayn, K.N. (2002). *Lipid Biochemistry*, 5th ed., Blackwell Scientific, Oxford, U.K. With permission.)

inhibition. There is also some controversy as to whether the two hydrogen atoms are removed sequentially (with the involvement of a hydroxy intermediate) or in a concerted mechanism. Evidence with *Corynebacterium diphtheria* using tritiated substrates suggested a stepwise mechanism (Schroepfer and Bloch, 1965), whereas experiments with ^{2}H-Iabelled substrates and *Chlorella vulgaris* indicated concerted hydrogen removal (Morris, 1970).

The first double bond inserted into an acyl chain is usually Δ9. The stearoyl-CoA Δ9-desaturase was first purified from rat liver (Strittmatter et al., 1974). The complex consists of three major proteins: NADH-cytochrome b_5 reductase, cytochrome b_5, and a terminal desaturase (or cyanide-sensitive) protein. Usually the activity of the terminal desaturase limits the overall speed of desaturation. The NADH-cytochrome b_5 oxidoreductase is a flavoprotein of mass 43 kDa, cytochrome b_5 has a molecular mass of 16.7 kDa, and the terminal desaturase is 53 kDa (see Cook, 1991). The approximate location of the three components in the endoplasmic reticulum has been deduced from their amino acid sequence and the use of specific chemical reagents. A useful summary of fatty acid desaturation in animals is given in Cook and McMaster (2002).

The Δ9-desaturases from plants and algae (and *Euglena gracilis*) use stearoyl-ACP (the final product of their FASs) as substrate. The enzyme was first purified substantially from developing safflower seeds by McKeon and Stumpf (1982). The 9-(stearoyl) desaturase has been cloned from castor seeds (Knutzon et al., 1991) and from safflower (Thompson et al., 1991). The cDNA from safflower includes a 33 amino acid transit peptide. Modulation of the steroyl-ACP desaturase level, using antisense technology in transgenic rapeseed, resulted in a marked decrease in oleate with a commensurate increase in stearate (Kridl et al., 1991). For summaries of the stearoyl-ACP desaturases from plants, refer to Harwood (1996). Mutations affecting the levels of oleic acid in crop plants are discussed in Schmid and Ohlrogge (2002).

In contrast to animals and plants, bacteria are unique in producing Δ10-monoenoic fatty acids. Bacilli commonly possess Δ5- or Δ10-desaturases, and *Bacillus licheniformis* contains both enzymes when it grows at low temperatures (Fulco, 1974). Under these conditions, it synthesizes small amounts of Δ5,10-16:2. It will be noted that, although this acid is polyunsaturated, it does not possess the usual methylene-interrupted structure. In fact, a general distinction is often made between bacteria and other organisms in that the former are unable to synthesize methylene-interrupted polyunsaturated fatty acids. This is not always true. For example, the filamentous gliding bacteria (*Flexibacter* spp.) contain considerable amounts of (*n*-3)-20:5, which in some species are the major fatty acids (Johns and Perry, 1977). Morever, marine bacteria may be able to produce methylene-interrupted PUFAs (see below).

Other features of aerobic desaturation in bacteria are discussed by Schweizer (1989). However, it should be noted that the "anaerobic" pathway utilizing fatty acid synthase is characteristic of many bacteria, particularly those of the orders Pseudomonadales and Eubacteriales.

It is now established that several species of Gram-negative bacteria, mainly marine, can produce PUFAs (Tocher et al., 1998). Attention has been particularly focused on *Shewanella* spp. (Metz et al., 2001) where a polyketide synthase seems to be used (see also Valentine and Valentine, 2004).

Most mammalian tissues can modify acyl chain composition by introducing more than one bond. Like Δ9-desaturation, further desaturation requires molecular oxygen and an associated electron transport system. Animal systems cannot generally insert double bonds beyond the Δ9 position. Consequently, double bonds are inserted at the Δ6, Δ5, and Δ4 positions. However, synthesis of linoleate from oleoyl-CoA has been demonstrated in some insects (de Ronobales et al., 1987). Protozoa, such as *Tetrahymena* (Umeka and Nozawa, 1984) and *Acanthamoeba castellanii,* also contain desaturases capable of producing linoleate. The latter system has been studied in some detail and shown to take place on phosphatidylcholine and probably is an *n*-6 (rather than a Δ12-) desaturase (Jones et al., 1993; Rutter et al., 2002). Interestingly, the *Acanthamoeba n*-6 desaturase is induced by low temperature (Avery et al., 1995) and, independently, by oxygen changes (Thomas et al., 1998).

Polyunsaturated fatty acids in all organisms usually contain methylene-interrupted double bonds and conjugated systems are rare. However, so-called conjugases (that produce conjugated PUFAs) have been found in a number of plants and their genes have been cloned (e.g., Cahoon et al., 1999; Iwabuchi et al., 2003).

The animal desaturases are also used to further modify polyunsaturated fatty acids from the diet (e.g., linoleate, α-linolenate), and the major pathways are depicted in Figure 10.6. Cook and McMaster (2002) have summarised the metabolism of acids of the *n*-6 family (linoleate, etc.) and the *n*-3 family (α-linolenate) and competition between the pathways.

In contrast to the stearate desaturases discussed above, the enzymes forming linoleate and α-linolenate in lower and higher plants use complex lipids as substrates. The use of phosphatidylcholine in this way was proposed originally following experiments with the yeast *Candida albicans* (Pugh and Kates, 1975) and with the green alga *Chlorella vulgaris* (Nichols et al., 1967). However, some yeasts contain both oleoyl-CoA and oleoyl-phospholipid desaturases (Pugh and Kates, 1975).

More recent work covering the Δ9, Δ12, and Δ6-desaturases from various yeasts or fungi is summarised by Tocher et al. (1998). In the fungus, *Mortierella alpina,* a number of desaturases including a Δ5 enzyme must be present because this organism can produce arachidonate. Other species can also produce 20:5n-3 (EPA) and, therefore, must have a Δ15 (n-3) desaturase as well (Tocher

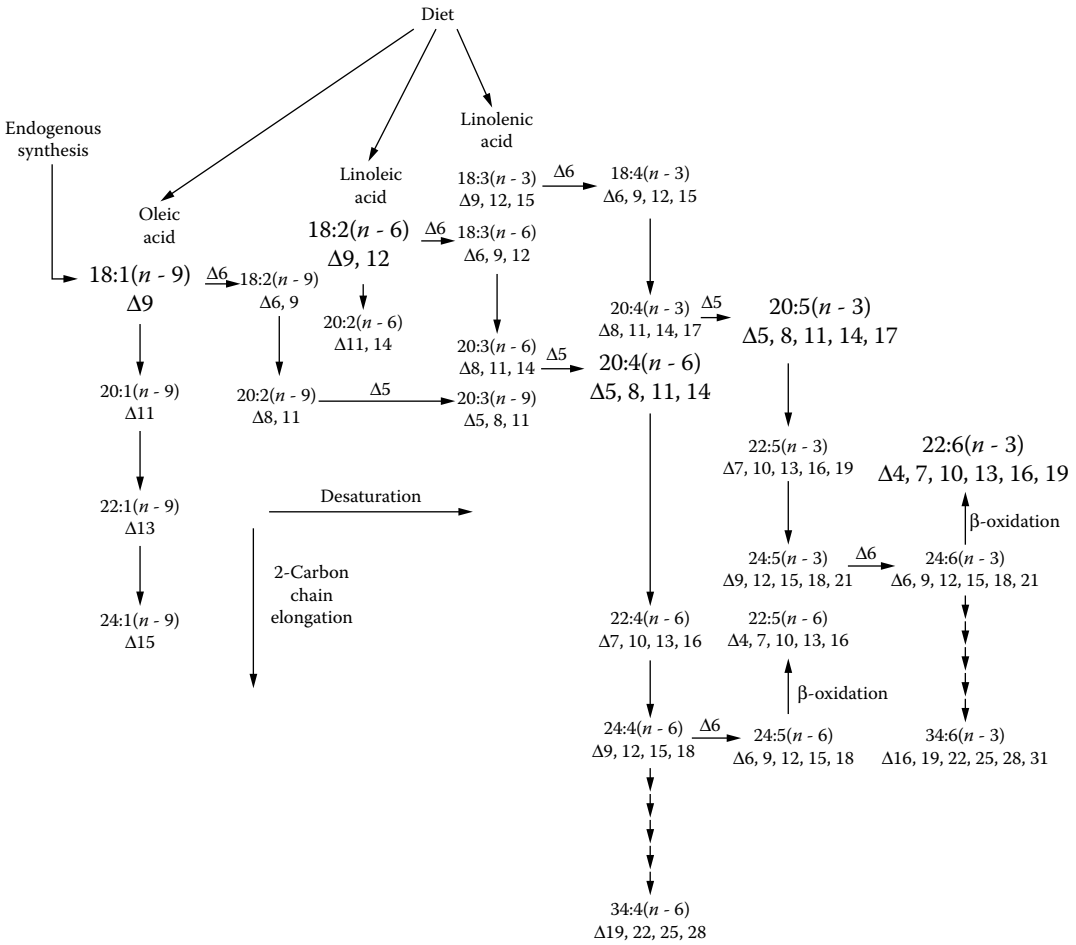


FIGURE 10.6 Major pathways for polyunsaturated fatty acid synthesis in animals. Note the alternating sequence of desaturation in the horizontal direction and chain elongation in the vertical direction in the formation of polyunsaturated fatty acids from dietary essential fatty acids. Type size for individual fatty acids reflects, in a general way, relative accumulation in tissues. (From Gurr, M.I,. Harwood, J.L. and Frayn, K.N. (2002). *Lipid Biochemistry*, 5th ed., Blackwell Scientific, Oxford, U.K. With permission.)

et al.,1998). Genes isolated from *Mortierella,* as well as from various algae, have proven useful in the genetic manipulation of higher plants to produce very long-chain PUFAs (see Section 11.8).

In plants, desaturation of oleate to linoleate uses phosphatidylcholine (in the endoplasmic reticulum) as the major substrate. The enzyme in safflower has been shown to use cytochrome b_5 as an electron source (Kearns et al., 1991; Smith et al., 1992). However, oleate desaturation in chloroplasts uses ferredoxin (Schmidt and Heinz, 1991). Other complex lipid substrates probably are also used (see Harwood. 1988), especially as demonstrated for chloroplast lipids (see Jones and Harwood, 1980). Further desaturation of linoleate to α-linolenate can take place either using monogalactosyldiacylglycerol (Jones and Harwood, 1980) or with phosphatidylcholine. The relative importance of these two substrates depends on the type of tissue: leaves use monogalactosyldiacylglycerol mainly, but seeds (which have poorly developed plastids) use the nonchloroplastic phosphatidylcholine as the main substrate. These aspects are discussed by Harwood (1996) and by Browse and Somerville (1991). The use of complex lipids for substrates, the cooperation of extraplastidic with plastid pathways and the site of Arabidopsis mutations in the pathways are shown in Figure 10.7. A recent update of plant fatty acid desaturation is by Hildebrand et al. (2005).

The fatty acid desaturation systems in cyanobacteria have been studied in detail by Murata and colleagues. This work was particularly in relation to chilling sensitivity. The research has been summarized in Murata and Wada (1995). The possible roles of signaling pathways in desaturase induction are reviewed by Mikami and Murata (2003).

10.1.5 Hydroxylation

Hydroxy fatty acids are formed as intermediates in various metabolic sequences (e.g., fatty acid biosynthesis, β-oxidation) as a result of specific hydroxylation reactions, and following other activities, such as those of lipoxygenase (see Section 10.1.7). The hydroxyl group is usually introduced close to one end of the acyl chain

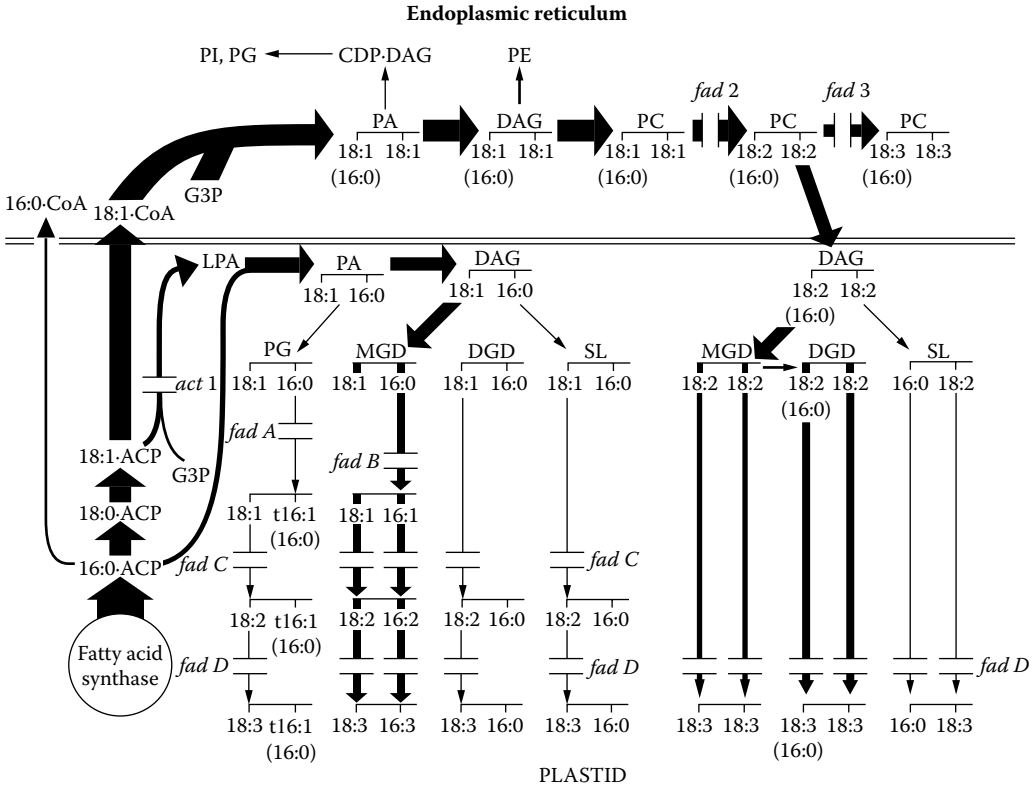


FIGURE 10.7 An abbreviated diagram of the two-pathway scheme of glycerolipid biosynthesis in the 16:3 plant *Arabidopsis*. Widths of the lines show the relative fluxes through different reactions. (From Browse and Somerville, 1991.)

(e.g., α,β) and less commonly in the middle of the chain. A good example of the latter in plants is the formation of ricinoleic acid (the major acid of castor oil, Section 2.2.2 and Section 9.8) by hydroxylation of oleoyl-phosphatidylcholine substrate using NADH and oxygen as co-factors (Moreau and Stumpf, 1981; Smith et al., 1992).

Few of the hydroxylases have been characterised, so it is difficult to compare their properties. However, they appear to be mixed-function oxidases and require molecular oxygen and a reduced pyridine nucleotide. Most of the enzymes appear to require cytochrome P_{450}, although ferredoxin (or a related haem protein) may substitute in some bacterial or plant systems (see Gaillard, 1980). Cell-free hydroxylating systems have been studied extensively in *Bacillus megaterium* and *Pseudomonas oleovorans* and some enzymes have been purified (see Schweizer, 1989).

α-Oxidation systems producing 2-hydroxy fatty acids have been demonstrated in yeasts, bacteria, plants (see Harwood and Russell, 1984), and animals, while ω-oxidation systems introduce a hydroxyl group to the methyl end of the acyl chain. These oxidations are described more fully in Section 10.1.6.

The major hydroxy fatty acids in plants have an ω-OH and an in-chain OH group (e.g., 10,16-dihydroxypalmitic acid). Their synthesis seems to involve ω-hydroxylation with NADPH and O_2 as cofactors, followed by in-chain hydroxylation using the same co-substrates. If the precursor is oleic acid, then the double bond is converted to an epoxide, which is then hydrated to yield 9,10-hydroxy groups. These conversions involve CoA esters. In-chain plant hydroxylase is sensitive to inhibition by *O*-phenanthroline and by CO in a reaction that is reversed by 420 to 460 nm light (Kolattukudy, 1980, 1987).

It should be noted that a few hydroxylations occur without a mixed-function oxidase enzyme. For example, the ergot fungus *Claviceps purpurea* forms 12-hydroxyoleic (ricinoleic) acid by hydration of linoleic acid (Harwood and Russell, 1984).

10.1.6 Oxidation of fatty acids

Oxidation of fatty acids can occur in a number of ways, depending on the position of oxidation (e.g., α-, β-, or ω-oxidation) and the nature of the substrate (e.g., lipoxygenase attack on polyenoic fatty acids).

10.1.6.1 α-Oxidation

The removal of a single carbon atom from the carbonyl end of a fatty acid is carried out by α-oxidation. This process is particularly active in plants, but is also found in mammals (notably brain tissue) and bacteria. The removal of a single carbon may be important when degradation of a fatty acid by β-oxidation (see below) is blocked by the presence of a methyl branch at position 3, such as in phytanic acid. Defects in α-oxidation of

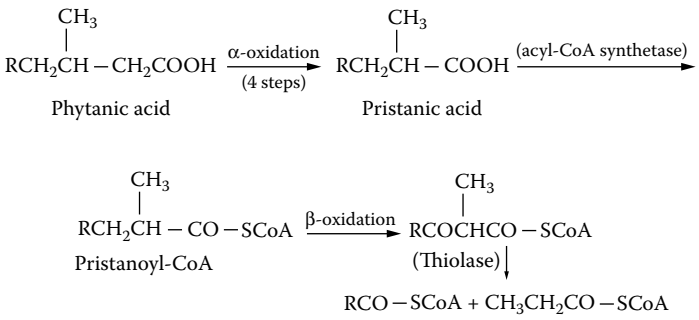


FIGURE 10.8 Phytanic acid metabolism.

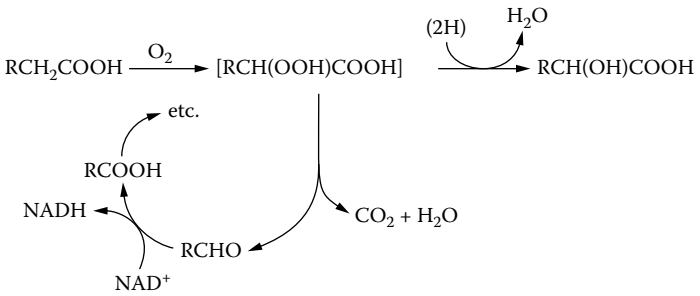


FIGURE 10.9 The α-oxidation of fatty acids in plants. Adapted from Galliard (1980).

phytanic acid give rise to Refsum's disease and there are other disorders giving rise to defects in α-oxidation (Mukherji et al., 2003). Human metabolism of phytanic and pristanic acids is reviewed by Verhoevan and Jakobs (2001) (see Section 11.6) and is shown in simplified form in Figure 10.8.

α-Oxidation is also important because α-hydroxy fatty acids are intermediates in the process and these acids are components of certain sphingolipids (Bowen et al., 1974). Thus, brain cerebroside fatty acids are highly enriched in cerebronic acid (α-OH 24:0). Furthermore, tissues that accumulate large amounts of fatty acids almost invariably also contain significant amounts of odd chain-length fatty acids (e.g., see Mcllwain, 1966).

In plants the mechanism of α-oxidation in leaves and seeds is identical. The fatty acid substrate is nonesterified (cf. β-oxidation) and is usually C_{12} to C_{18}. It is attacked by molecular oxygen to generate an unstable 2-hydroperoxy intermediate (Figure 10.9), which decomposes to an aldehyde with release of carbon dioxide. Under certain conditions (e.g.,, in the presence of an enzyme, such as glutathione peroxidase, which will reduce peroxides), then a D-2-hydroxy fatty acid may be produced, which cannot be metabolized easily. Under normal conditions, though, the aldehyde is oxidized in the presence of a source of reducing power (pyridine nucleotide or flavoprotein depending on the tissue) to give a fatty acid one carbon atom shorter than the original fatty acid. A significant breakthrough to our understanding of α-oxidation in plants has come from the discovery of a pathogen-inducible oxygenase, which has significant homology to prostaglandin endoperoxide H synthase (see Section 10.1.8) (Graham and Eastmond, 2002). This has led to the suggestion that a major role for α-oxidation in plants is in defence responses to pathogens (Hamberg et al., 1999).

The occurrence of bacterial fatty acid α-oxidation is not firmly established and, if present, represents a pathway of minor importance (Finnerty, 1989). It has been studied in few bacterial species. However, in *E.coli,* the D-2-hydroxy fatty acid is preferentially decarboxylated, unlike in plants and animals where it is the L form that is metabolised preferentially. Thus, the 2-hydroxy acids tend not to accumulate in bacteria (Lekakis, 1977).

10.1.6.2 β-Oxidation

The β-oxidation of fatty acids was the first metabolic process in which labeled compounds were used for its investigation. Knoop's classic experiments at the turn of the century were later confirmed and extended by others to reveal the details of the process (for references, see Greville and Tubbs, 1968; Wakil, 1970; Kunau et al., 1995; Schulz, 2002). Key steps in the pathway are the activation of the fatty acid to a coenzyme A thioester, the α,β-dehydrogenation of the acyl-CoA, the hydration of the resultant double bond, oxidation of the β-hydroxyacyl-CoA, and thiolytic cleavage of the β-ketoacyl-CoA (Figure 10.10).

The mechanism of fatty acid uptake by animal cells has not been fully elucidated. Plasma membrane carrier proteins responsible for saturable high-affinity uptake have been identified, but some workers argue that spontaneous and nonspecific diffusion of fatty acids across the

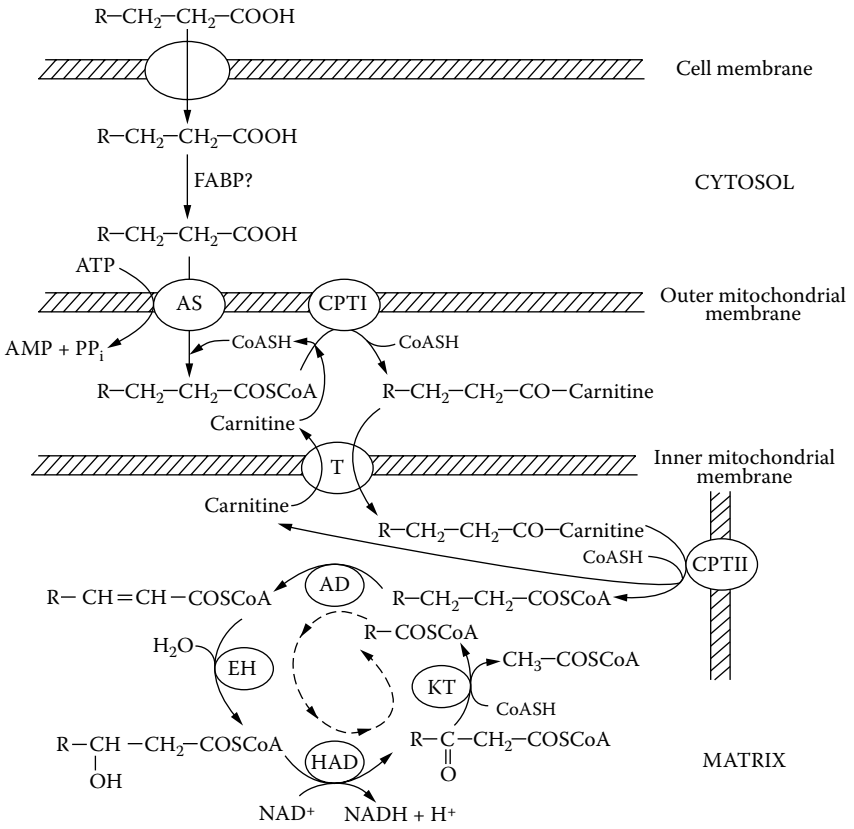


FIGURE 10.10 Pathway of mitochondrial fatty acid oxidation. Enzymes of the pathway are AS, acyl-CoA synthetase; CPT I, carnitine palmitoyltransferase I; T, carnitine:acylcarnitine translocase; CPT II, carnitine palmitoyltransferase II; AD, acyl-CoA dehydrogenase; EH, enoyl-CoA hydratase; HAD, L-3-hydroxyacyl-CoA dehydrogenase; KT, 3-ketoacyl-CoA thiolase. Other abbreviation: FABP, fatty acid binding protein. (From Schulz, 1991b.)

plasma membrane is also important (see Schulz, 2002). β-Oxidation is preceded by activation of fatty acids to their coenzyme A (CoA) thioesters. A group of acyl-CoA synthetases have been identified that differ in their chain-length specificities, but which catalyse the same type of reaction:

$$\text{R-COOH} + \text{ATP} + \text{CoASH} \rightleftharpoons \text{RCO-SCoA} + \text{AMP} + \text{PP}_i$$

The individual acyl-CoA synthetases are grouped as short-chain, medium-chain, or long-chain synthetases, depending on their substrate specificities. Short-chain synthetases, which act on acetate or propionate, are present in the mitochondria of most animal tissues, but, in addition, are found in the cytosol of lipogenic tissues, such as liver, intestine, adipose tissue, and mammary gland. Medium-chain synthetases are also located in the mitochondrial matrix. By contrast, long-chain acyl-CoA svnthetases in animals are membrane-bound and associated with the endoplasmic reticulum, mitochondrial outer membrane or peroxisomes (see Schulz, 2002).

Acyl-CoAs that need to be taken up by mitochondria do so via a carnitine-dependent mechanism. Two carnitine palmitoyltransferases and a carnitine:acylcarnitine translocase are needed, as illustrated in Figure 10.10.

Three enzymes (differing in chain-length specificity) that catalyse α,β-dehydrogenation of acyl-CoAs have also been purified. The overlapping specificities of these enzymes allow efficient oxidation of fatty acids in the C_4 to C_{20} range (Greville and Tubbs, 1968). The acyl-CoA dehydrogenases (EC 1.3.99.3) are flavoproteins containing FAD (flavin adenine dinucleotide) as a prosthetic group. They have molecular masses of 170 to 190 kDa and are composed of four identical subunits, each of which carries a noncovalently bound FAD. Nucleotide sequences for several rat liver and human acyl-CoA dehydrogenases have been determined, with a greater than 90% homology observed for the same enzyme from rat compared to human (see Schulz, 2002).

Hydration of the α,β-*trans* double bond is catalysed by enoyl-CoA hydratase (EC 4.2.1.17; crotonase). This enzyme will also attack *cis* double bonds and 3-enoyl-CoAs. An L-OH fatty acid is produced (Δ4, in the latter case, and Δ3, in β-oxidation) from the *trans* isomers. For more details, see Schulz (2002).

The L-3-hydroxyacyl-CoA obtained in the above step is oxidized by NAD^+ in the presence of 3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35). The enzyme is specific for the L form and usually has little chain-length specificity (Wakil, 1970). The enzyme has been purified from pig

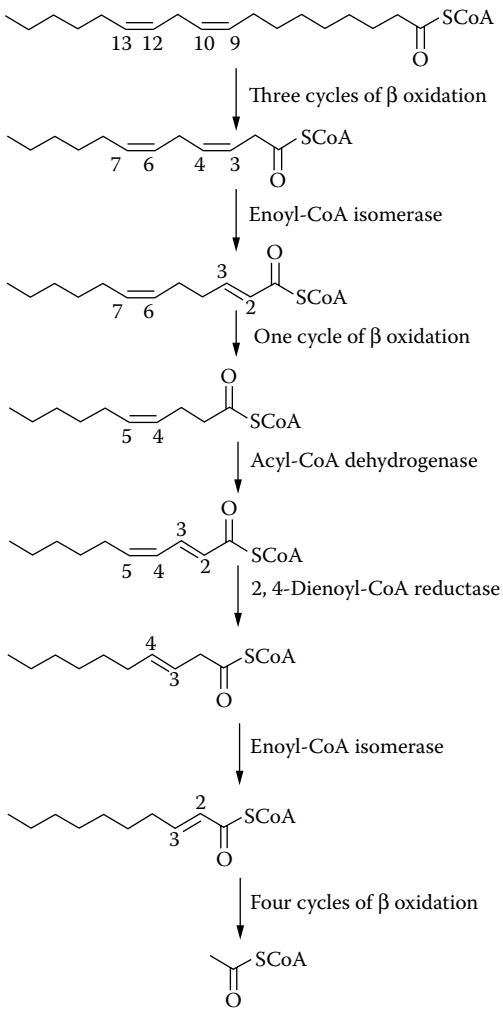


FIGURE 10.11 The β-oxidation of linoleoyl-CoA. (From Schulz, 1991b.)

heart and rat liver. It has a molecular mass of 65 kDa, is composed of two identical subunits, and its conformation at 2.8 Å resolution has been determined (Schulz, 1991). Some enzymes will utilize $NADP^+$ more slowly and these dehydrogenases will also oxidize *S*-3-hydroxyacyl-*N*-acylthioethanolamine and *S*-3-hydroxyacyl-hydrolipoate.

Thiolytic cleavage of the 3-ketoacyl-CoA is catalysed by acetyl-CoA acyltransferase (EC 2.3.1.16), which liberates acetyl-CoA and an acyl-CoA two carbons shorter than the original substrate (see Figure 10.10). The above enzyme has a broad chain-length specificity, but a second enzyme, acetyl-CoA acetyltransferase (EC 2.3.1.9), catalyses the same reaction with acetoacetyl-CoA as substrate. Kinetic and exchange studies indicate that these thiolase reactions occur in two stages (see Greville and Tubbs, 1968). Both types of thiolases are homotetramers of mass about 170 kDa. While the 3-ketoacyl-CoA thiolase with its broad chain-length specificity is essential for β-oxidation, the acetoacetyl-CoA thiolase probably functions mainly in ketone body metabolism (Schulz, 1991).

All mitochondrial enzymes of β-oxidation are synthesised in the cytosol on free polysomes (see Ozasa et al., 1984).

Oxidation of saturated straight-chain fatty acids proceeds smoothly using the above series of enzymes, but there are problems in the breakdown of other fatty acids. For example, odd-chain acids finally liberate propionyl-CoA, which is converted to succinyl-CoA for further metabolism. Unsaturated fatty acids must also be modified because their double bonds may be in the wrong position and because of incorrect configuration for normal catabolism. Epimerase and isomerase enzymes are present to cope with these problems (see Gurr et al., 2002 and Schulz, 2002; Figure 10.11).

Apart from β-oxidation in mitochondria, peroxisomes are also important. The reactions and enzymes involved have been characterised by Hashimoto (1990), with the research being aided by the use of drugs, such as clofibrate or di(2-ethyl-hexyl)phthalate, which cause peroxisome proliferation and also induce the enzymes of peroxisomal β-oxidation (Lazarow and Moser, 1989). Although the intermediates of peroxisomal β-oxidation are the same as in mitochondria, there are differences in the enzymes concerned. The first reaction is catalysed by acyl-CoA oxidase, which generates hydrogen peroxide as a product.

The latter is rapidly destroyed by peroxisomal catalase activity. The hydration and second dehydrogenation steps are catalysed by a trifunctional enzyme. The essential features are illustrated in Figure 10.12. Because the acyl-CoA oxidase is almost inactive towards octanoyl-CoA (or shorter chains), peroxisomes are incapable of complete oxidation of fatty acids. For the oxidation of unsaturated fatty acids, such as linoleate, a 2,4-dienyl-CoA reductase is present and a Δ^3-*cis,* Δ^2-*trans*-enoyl-CoA isomerase (as the third activity of the trifunctional enzyme) is also needed (see Schulz, 1991; Osmundsen et al., 1991). The main function of peroxisomal β-oxidation in animals seems to be in the chain shortening of very long-chain fatty acids, prostaglandins, dicarboxylic acids, and various xenobiotic compounds (see Schulz, 1991).

In plants, it was observed a number of years ago that peroxisomes, such as the glyoxysomes of germinating oil seeds, contained enzymes necessary for fatty acid β-oxidation (see Galliard, 1980; Kindl, 1987). Early aspects of the work were discussed by Beevers (1980). A thorough review has covered aspects of β-oxidation in plants (Gerhardt, 1992). The author concluded that there was little evidence for any other location for β-oxidation apart from in peroxisomes. Nevertheless, the possibility that mitochondria could be involved for the oxidation of straight chain acids is still not resolved and these organelles seem to be utilized for β-oxidation of short branched-chain 2-oxo acids (Graham and Eastmond, 2002). The latter authors have made a thorough review of peroxisomal β-oxidation in plants and its regulation.

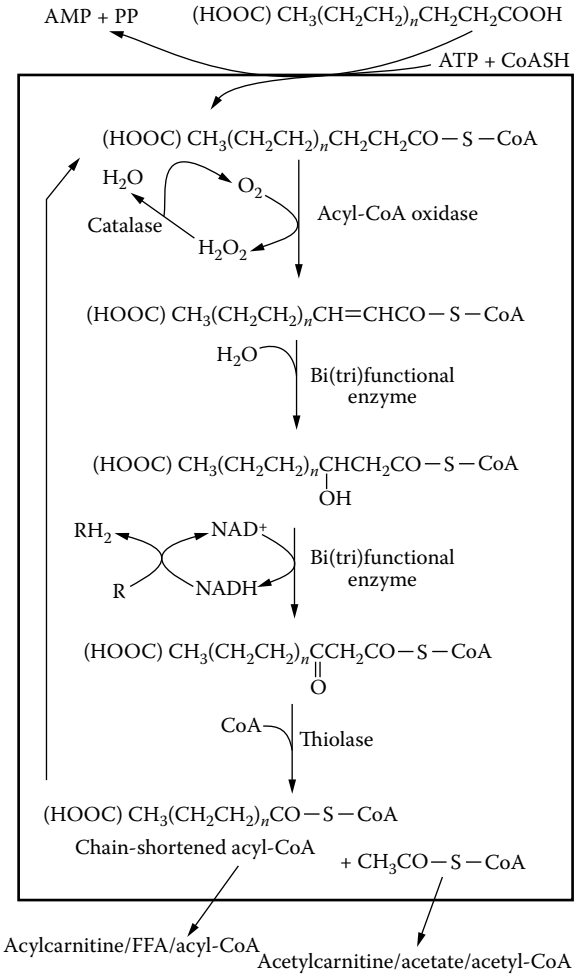


FIGURE 10.12 Essential features of peroxisomal β-oxidation. A schematic representation of the main metabolic features of peroxisomal - oxidation. The enzymes of peroxisomal β-oxidation are contained within the peroxisomal membrane (rectangular box). Possible end products of β-oxidation are indicated at the bottom. The [HOOC] to the left of the substrate acyl-CoA ester indicates that mono-CoA esters of dicarboxylic acids are also substrates for peroxisomal β-oxidation. An incorrect stoichiometry of catalase-dependent decomposition of H_2O_2 is used for the sake of brevity. The arrows pointing down to Acylcarnitine/FFA/acyl-CoA, or to Acetylcarnitine/acetate/acetyl-CoA are meant to indicate possible alternative forms of export of chain-shortened fatty acids out of the peroxisome (FFA = free fatty acid). The oxidant (R) of NADH active *in vivo* remains to be established. With isolated peroxisomes, pyruvate (and lactate dehydrogenase) can function in this capacity. (From Osmundsen et al., 1991.)

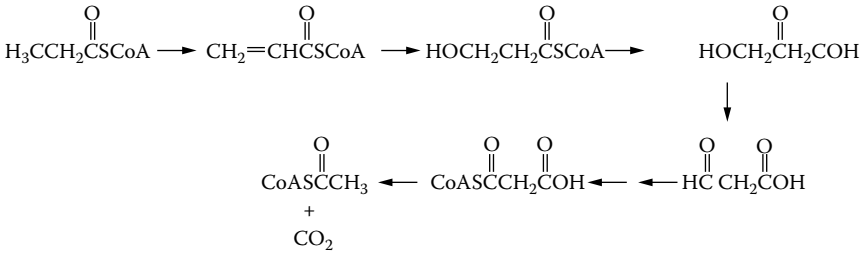


FIGURE 10.13 Propionate metabolism in plants.

Plants operate a different mechanism from animals when removing the propionate liberated from odd chain-length fatty acid oxidation. The plant system involves a hydratase and two hydrogenase enzymes, and the overall scheme is summarized in Figure 10.13. The pathway is superficially similar to modified β-oxidation in bacteria, but the two systems differ in the fate of the individual carbon atoms (Stumpf, 1970).

Bacteria take up nonesterified fatty acids from the growth medium by a process that probably involves the formation of acyl-CoA. The fatty acid taken up, therefore, is in a form directly available for β-oxidation. In *E. coli* there has been some study of the genes coding for fatty acids degradation *(fad)*. These genes, which code for the enzymes of uptake/activation and β-oxidation, are located in three sites on the chromosome and comprise a regulon (Klein et al., 1971). Several types of *fad* mutants are known and another class of mutants, *fadR,* are constitutive for β-oxidation and are probably repressor mutants. In addition to being a repressor for *fad* genes, the *fadR* gene product may also control isocitrate lyase and malate synthetase (Maloy et al., 1980), which are key enzymes of the glyoxylate shunt. There are some differences in the details of β-oxidation enzymes in *E. coli* compared to those from animals. For example, only one acyl-CoA synthetase is present and this can activate both medium-chain and long-chain fatty acids (Schulz, 2002). Even though *E. coli* does not synthesize polyunsaturated fatty acids, it can easily oxidize them by the reductase-dependent pathway (above). Details of the enzymes involved in *E. coli* fatty acid β-oxidation and available mutants that are defective in individual steps are given by Finnerty (1989). The same author also discusses the features of peroxisomal β-oxidation in microbial eukaryotes, such as *Candida tropicalis.* Other sources of information on *E. coli* are Nunn (1986) and Black and Dirusso (1994) and for other bacteria (Kunau et al., 1995).

The control of mitochondrial β-oxidation flux is reviewed by Eaton (2002).

10.1.6.3 Inherited diseases of β-oxidation

A number of diseases that compromise the functions of liver, muscle, and other organs have been ascribed to deficiencies of β-oxidation. These include myopathic carnitine deficiency; deficiencies of short-chain, medium-chain, or long-chain acyl-CoA dehydrogenases; and deficiencies in the electron transferring flavoprotein or its dehydrogenase. Several disorders (e.g., Zellweger syndrome) associated with impairment of peroxisomal β-oxidation have also been described. See Schulz (2002) and Section 11.6 for more details.

10.1.6.4 Ketone bodies

In mammalian liver, the excessive amounts of acetyl-CoA liberated by β-oxidation of fatty acid are converted to various ketone bodies. This conversion takes place at high rates when an elevation in the glucagon/insulin ratio occurs, such as during fasting or in uncontrolled diabetes. Several enzymes are involved, as shown below. These enzymes are also found in extrahepatic tissues, such as heart, kidney and intestine.

$$2\ \text{Acetyl-CoA} \xrightarrow[\text{thiolase}]{\text{Acetoacetyl-CoA}} \text{Acetoacetyl-CoA} + \text{CoA}$$

$$\text{Acetoacetyl-CoA} + \text{Acetyl-CoA} \xrightarrow[\text{synthase}]{\text{HMG-CoA}} \text{Hydroxymethylglutaryl-CoA} + \text{CoA}$$

$$\text{Hydroxymethylglutaryl-CoA} \xrightarrow[\text{lyase}]{\text{HMG-CoA}} \text{Acetoacetate} + \text{Acetyl-CoA}$$

$$\text{Acetoacetate} + \text{NADH} + \text{H}^+ \xrightarrow[\text{dehydrogenase}]{\text{D-3-Hydroxybutyrate}} \text{3-Hydroxybutyrate} + \text{NAD}^+$$

The ketone bodies produced by various tissues can readily diffuse into the blood and be taken up by other extra hepatic tissues and converted back to acetyl-CoA for complete combustion in the tricarboxylic acid cycle. A thorough review of these reactions and of the regulation of ketogenesis (Figure 10.14) has been provided by McGarry and Foster (1980) and see also Gurr et al. (2002) for a summary.

Ketone bodies provide important alternative fuels to body tissues when carbohydrate is in short supply or cannot be efficiently utilised. A particular example is the central nervous system, which cannot utilise plasma fatty acids for energy. Thus, in prolonged starvation, ketone bodies become more important than glucose as a fuel source. The possibility of the utilisation of ketone bodies obviates the harmful degradation of muscle protein for gluconeogenesis. In addition, acetoacetate and 3-hydroxybutyrate are thought to be important precursors for lipid synthesis in neonatal brain (Webber and Edmond, 1979).

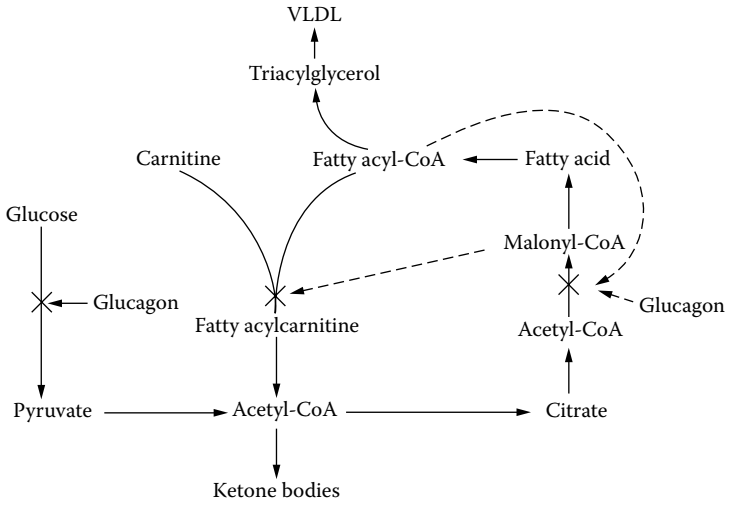


FIGURE 10.14 Interactions between fatty acid synthesis and oxidation in liver. In the fed state, malonyl-CoA levels are high. This allows rapid fatty acid synthesis and inhibits β-oxidation by lowering carnitine acyltransferase I activity. If triacylglycerol synthesis is impaired, then acyl-CoA will feed back to inhibit acetyl-CoA carboxylase. In the fed state, this does not normally happen, and triacylglycerols are incorporated into very low density lipoprotein (VLDL) for export to extrahepatic tissues. Glucagon excess in fasting leads to a suppression of glycosis, cessation of lipogenesis, and activation of β-oxidation and ketogenesis. (From McGarry, J.D. and Foster, D.W. (1980). *Annu. Rev. Biochem.* **49**, 395–420. With permission.)

Although ketone bodies serve useful functions, excessive accumulation of such compounds in blood can cause clinical problems, such as ketoacidosis. This may be severe in diabetes or alcoholism. A comprehensive review of clinical aspects of ketone bodies is that of Soling and Seufert (1978).

10.1.6.5 ω-Oxidation

Microsomal preparations from many tissues can oxidise fatty acids in the ω-position. An ω-hydroxy fatty acid is formed first, and this can be oxidised by NAD^+ and cytoplasmic enzymes to yield a dicarboxylic acid, which can be further attacked by β-oxidation (Greville and Tubbs, 1968). The enzyme(s) responsible for the oxidation of the ω-hydroxy fatty acid have been studied much less than the ω-hydroxylation system. There may be an ω-hydroxy acid dehydrogenase, although liver alcohol dehydrogenase can convert such acids to semialdehyde derivatives. Further oxidation of the latter would be catalysed by an NAD-limited aldehyde dehydrogenase (cf. Greville and Tubbs, 1968). ω-Oxidation is important for the further metabolism of fatty acids that are di-substituted in the 2- or 3-positions as well for the catabolism of various xenobiotics that have alkyl chains (see Lenk, 1972). The role of peroxisomes in the ω-oxidation of fatty acids and xenobiotics is discussed by Osmundsen et al. (1991).

The ω-hydroxylase system (alkane 1-mono-oxygenase, EC 1.14.15.3) has nonhaem irons as, apparently, the only prosthetic groups directly involved in the hydroxylation reactions. The system from *Pseudomonas oleovorans* has been isolated by Coon and coworkers (Kusunose et al., 1964). The system was fractionated into three components: a nonhaem iron protein (similar to the rubredoxins), a flavoprotein, and a final component required for hydroxylase activity called the ω-hydroxylase. The latter has a molecular mass of 42,000 Da with 1 atom of iron. It also contains a large amount of phospholipid, and activity is lost if this lipid is removed. Other studies have included electron paramagnetic resonance (EPR) experiments on the spectra shown by the nonhaem ironprotein (for reviews, see Coon et al., 1972; Gunsalus et al., 1975).

In plants, the ω-hydroxylase system is responsible for synthesis of ω-hydroxy fatty acyl components of cutin and suberin (see Sections 1.1.2.10 and 1.2.12). The reaction has been studied in preparations from *Vicia faba* with NADPH and oxygen as the required cofactors (Kolattukudy, 1980). The true substrate for ω-hydroxylation of palmitate is the free acid, and the active subcellular preparation is the microsomal fraction. The reaction showed the properties of a classic mixed-function oxidase, being inhibited by *O*-phenanthroline, 8-hydroxyquinoline (metal-ion chelators), sodium azide, and thiol-directed reagents. The involvement of cytochrome P_{450} in the *V. faba* system is unproven. Although the hydroxylation is inhibited by carbon monoxide, this inhibition was not reversed by light at 420 to 460 nm. Thus, if a cytochrome P_{450} is involved in the system, it must have unusual properties when compared to other cytochrome-P_{450}-containing enzymes (Kolattukudy, 1980).

The recent description of three long-chain fatty acid oxidase genes from *Candida* has led to the identification of a gene family involved in ω-oxidation in yeast with homologues in bacteria and plants (Van Hanen et al., 2000). In *Candida* the omega carbon is oxidised successively by a cytochrome P_{450} alkane/fatty acid oxidase, a H_2O_2-generating alcohol oxidase, an aldehyde dehydrogenase producing

TABLE 10.5 Major products of ruminant biohydrogenation of C_{18} unsaturated fatty acids

Substrate	Products
Oleic acid	18:0
Linoleic acid	9*c*, 11*t*-18:2
	11*t*-18:1
	18:0
α-Linolenic acid	9*c*, 11*t*, 15*c*-18:3
	11*t*, 15*c*- and 9*c*, 11*t*-18:2
	11*t*-18:1
	18:0

ω-alcohols, ω-aldehydes, and ω-fatty acids. Four genes with high homology to *Candida* ω-oxidation genes are present in the Arabidopsis genome (Graham and Eastmond, 2002).

10.1.6.6 Fatty acid catabolism in ruminants

A specialized situation for lipid breakdown is the rumen of such animals as sheep and ox. A large number of microorganisms, such as bacteria of the genera *Ruminococcus, Bacteroides,* and *Butyrivibrio,* as well as protozoa, play a part in the breakdown of leaf lipids (see Section 2.10). Thus, α- and β-galactosidases are present to cleave the galactose residues of galactosylglycerides. Active lipases hydrolyse the acyl residues, and the liberated fatty acids are often biohydrogenated to give various mixtures of conjugated fatty acids containing *trans* as well as *cis* double bonds (Table 10.5) (see Garton, 1977; Harwood and Russell, 1984; and Gurr et al., 2002). Fermentation results in the production of large quantities of acetic, propionic, and butyric acids — the proportions of which vary with the ruminants' diet. These water-soluble, short-chain fatty acids are absorbed well, and much of their total quantity is metabolised to ketone bodies in the rumen wall. Other features of fatty acid metabolism in ruminants are discussed by Garton (1977).

10.1.7 Lipoxygenase

Lipoxygenases (LOXs) (linoleate: oxygen oxidoreductase (EC 1.13.10.12)) constitutes a large family of nonheme, iron-containing fatty acid dioxygenases, which are ubiquitous in plants and animals (Brash, 1999). LOXs catalyse the regio- and stereo-specific dioxygenations of PUFAs containing a 1Z, 4Z-(*cis, cis*-1,4) pentadiene system to produce conjugated hydroperoxydiene derivatives:

$$\mathrm{RCH = CHCH_2CH = CHR'}$$

$$\downarrow \mathrm{O_2}$$

$$\mathrm{RCH = CHCH = CHCH(OOH)R'}$$

Thus, typical substrates for lipoxygenases would be the PUFAs linoleic, α- linolenic, arachidonic and eicosapentaenoic acids.

10.1.7.1 Lipoxygenases in plants

Because 20C PUFAs are only minor fatty acids in plants, the plant LOXs are classified according to their positional specificity of linoleate oxygenation, either at the 9-(9-LOX) or the 13-carbon (13-LOX) (Feussner and Wasternack, 2002).

Lipoxygenase activity is widespread in the plant kingdom, often in very high amounts. The enzymes are particularly important in the food plants, where they destroy the essential polyunsaturated fatty acids to produce derivatives with characteristic tastes and flavours. They are also used for bleaching natural pigments, such as wheat flour carotenoids or alfalfa chlorophyll. General reviews on lipoxygenases are those by Gaffney (1996) and Piazza (1996). Plant lipoxygenases are discussed by Galliard and Chan (1980) and by Siedow (1991), while the lipoxygenase pathway is reviewed by Grechkin (1998) and Feussner and Wasternack (2002). Other useful reviews cover the commercial importance of lipoxygenases (Eskin et al., 1977), their role in olive oil quality (Harwood and Aparicio, 2000) and in plant defence (Blée, 1988). The animal lipoxygenases are dealt with separately in section 10.1.8.

Plant tissues containing high levels of lipoxygenase activity are shown in Table 10.6. Leguminous seeds generally contain lipoxygenase, but the absence of measured

TABLE 10.6 Plant tissues containing high levels of lipoxygenase activity

Plant	Family	Tissue	Lipoxygenase activity (μl O_2 consumed in 10 min/gram fresh weight)
Yellow bean	Leguminosae	Seed	6480
Potato	Solanaceae	Tuber	4560
Eggplant	Solanaceae	Fruit	4320
Soybean	Leguminosae	Seed	4150
Artichoke	Compositae	Heart	3360
Pea	Leguminosae	Seed	1769
Cauliflower	Cruciferae	Floret	1440
Avocado	Lauraceae	Fruit	720
Tomato	Solanaceae	Fruit	360
Lettuce	Compositae	Leaf	120

activity in certain plant tissues does not mean that the enzyme is absent, since there are often inhibitors present. The enzyme has been detected in particulate, cytosolic, and vacuolar fractions (Feussner and Wasternack, 2002).

Several types of lipoxygenase have been described. These differ in various properties, and the "classic" soybean lipoxygenase (Theorell et al., 1947) may be atypical in several respects. Two main types are present in soybeans. The Theorell enzyme acts on free acids only and is sometimes called an "acid" lipoxygenase (Verhue and Francke, 1972). The same enzyme is referred to as an "alkaline" lipoxygenase by Grosch et al. (1977) because its optimal activity is found at pH 9. It is better referred to as a Type I enzyme. Similarly, a second lipoxygenase with an optimum pH of 6.5 has been referred to as an "ester" enzyme a "neutral" enzyme, or a "b" enzyme and it is better referred to as the Type II enzyme (Galliard and Chan, 1980).

Isoenzymic forms of lipoxygenase have been purified to homogeneity from many different plants (see Eskin et al., 1977). Most of the enzymes have properties similar to the soybean Type II enzyme. Tissues that have been used for study include wheat, alfalfa, potato, barley, pea, and various other legumes. Most lipoxygenases appear to be single polypeptides of molecular masses in the region 70,000 to 100,000 Da. A single atom of nonhaem iron per molecule is found (Chan, 1973) and the pH optima are in the range of 5.5 to 7.0. Because lipoxygenase lacks cofactors other than nonhaem iron, the number of inhibitors is small. The *trans*-unsaturated fatty acids, acetylenic fatty acids, α-bromo fatty acids, and fatty alcohols will all inhibit, and antioxidants or oxygen scavengers are effective in certain cases (see Hamberg et al., 1974; Eskin et al., 1977).

The remarkable increase in sequence information has allowed phylogenetic tree analysis of multigene families. For LOXs, the Type I and Type II enzymes, as well as those classified as the 9- or 13-LOXs, form individual groups in separate branches of the tree (Feussner and Wasternack, 2002).

Soybean Type I lipoxygenase is particularly stable. Other lipoxygenases are less stable and activity is lost during purification. Heat treatment is often used in the food industry to cause inactivation so as to prevent off-flavours. The purified lipoxygenases are generally unstable at 70°C, but higher temperatures may be necessary for inactivating the enzymes in foodstuffs.

A number of assay methods have been used for measuring lipoxygenase. These techniques use radioisotopes or the oxygen electrode and colorimetric methods. These assays and necessary precautions are discussed by Galliard and Chan (1980).

Lipoxygenases will also catalyse co-oxidation reactions. This is used both in assay methods for the enzyme and in commercial applications. An example of the latter is the addition of soybean or broad bean flours (both rich in lipoxygenase activity) to wheat flour in order to bleach pigments for white bread production. Enzymes from different sources differ in their co-oxidation ability, e.g., soybean Type I enzyme has poor activity in this regard while soybean Type II enzyme has high co-oxidation activity. The reaction probably proceeds by a free radical process (Veldink et al., 1977) and requires the presence of a substrate (e.g., linoleic acid) as well as the cosubstrate. The extent of the co-oxidation may depend on the lifetime of the radical intermediates and the relative efficiency of the lipoxygenase-mediated radical reduction (Weber and Grosch, 1976).

Whereas in animals, arachidonate is a major substrate for LOX attack, in plants α-linolenate is the major substrate. Breakdown of this acid is known variously as the α-linolenic acid cascade or lipoxygenase pathway (see Figure 10.15). The fatty acid hydroperoxides produced by LOXs can be converted to three main types of products (see Figure 10.15) with important functions (Gurr et al., 2002) (Fig. 10.16):

1. Co-oxidative reactions with peroxygenase give a mixture of epoxy and hydroxyl fatty acids (depending on the nature of the acceptor fatty acid for the monooxygenation) and the products have roles in cutin biogenesis and defence against pest attack (Blée, 1988).
2. Hydroperoxide lyase cleaves the hydroperoxide into an aldehyde and an oxo-unsaturated fatty acid. The products have a role in pest defence and appear to act as pollinator and herbivore attractants, especially for flowers and fruits. They are also important flavour and aroma components in foods, drinks, and perfumes (see e.g., Sanchez and Salas, 2000).
3. Allene oxide synthase gives rise to the precursor of jasmonic acid. The latter and associated compounds are commonly known as jasmonates although some workers prefer the term "jasmonins" to differentiate from jasmonic acid esters. The jasmonins have important effects on plant growth, development, and senescence.

Hydroperoxide lyase activity under 2 (above), usually occurs with the 13-hydroperoxide derivative thus giving rise to 6C compounds, which are volatile. Combinations of the volatiles produced are effective attractants for both pollinators and herbivores and the easily detected smells of various fruits, cut grass, cucumber, etc. derive from them. Profiles of the volatiles produced can be easily analysed by headspace gas chromatography and even used for identification purposes (see Harwood and Aparicio, 2000).

Physiological roles for lipoxygenase-derived products in plants are shown in Table 10.7 (Gurr et al., 2000). For more detail, refer to Seidow (1991), Blée (1998), Feussner and Wasternack (2002) and Rosahl and Feussner (2005).

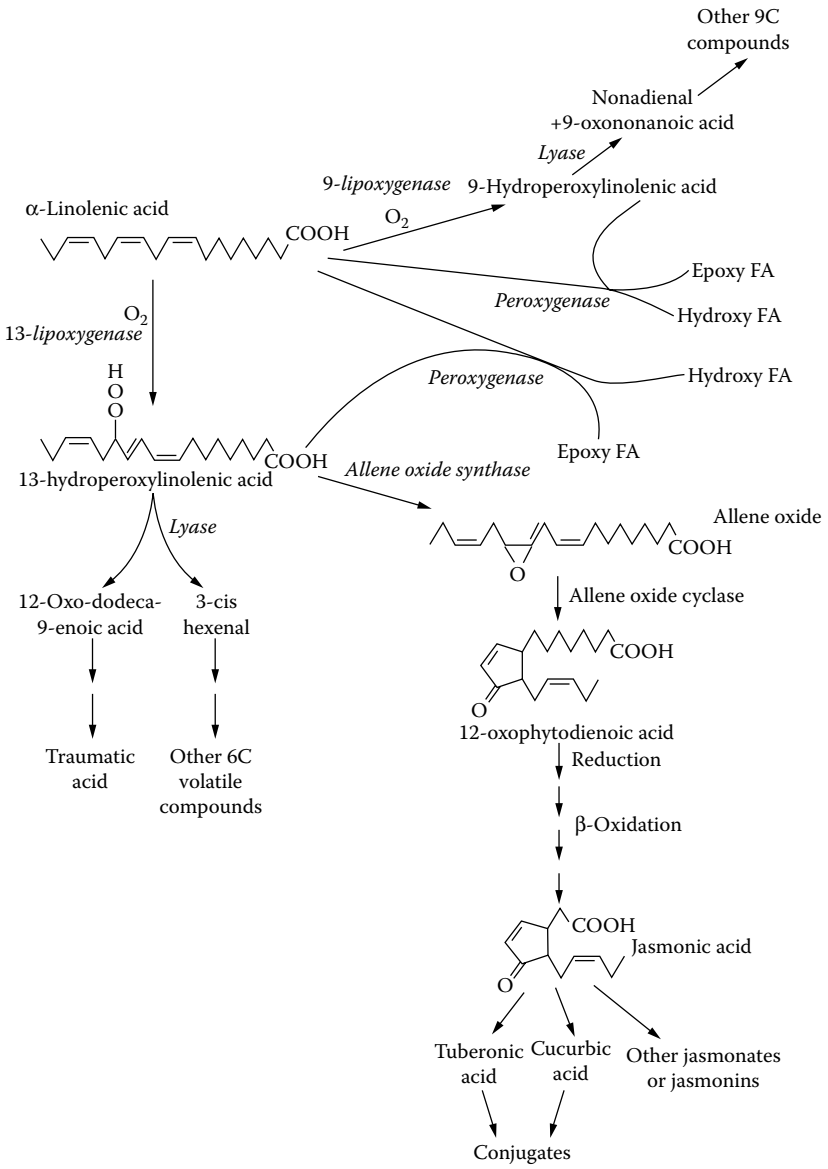


FIGURE 10.15 The α-linolenic acid cascade and oxylipin formation in plants. (From Gurr, M.I., Harwood, J.L. and Frayn, K.N. (2002). *Lipid Biochemistry*, 5th ed., Blackwell Scientific, Oxford, U.K. With permission.)

10.1.7.2 Lipid peroxidation

A constant problem with unsaturated lipids, particularly PUFAs, is the ease of their oxidation. This, in general, not only destroys the beneficial effects of the parent molecule, but often gives rise to products with harmful properties. For further discussion of this subject, see Section 11.1 and Gurr et al. (2002). Specific useful reviews are those of Morrow and Roberts (1997) on isoprostanes and by Itabe (1998) on oxidized phospholipids and atherosclerosis. Some of the products initiated by enzyme activity, such as in the lipoxygenase pathway, may also have important pathophysiological effects. For example, there is much interest in 4-hydroxy-2-nonenal as a product and mediator of oxidation stress. This compound originates from the reaction of hydroperoxide lyase with 9-hydroperoxylinoleic acid. Other aldehydes or conjugated derivatives can also have significant activity (Uchida, 2003).

10.1.8 Production and function of the eicosanoids

Eicosanoids are oxygenated 20-carbon fatty acids. Because the major precursor of these compounds in animals is arachidonic acid, the pathways leading to the eicosanoids are also often known as the "arachidonate cascade." There are three reactions involved in the initial metabolism of arachidonic acid, which itself must be released from membrane lipids through the activity of phospholipases. The enzymes involved are cyclooxygenase, 5-, 12-, or 15-lipoxygenases and various cytochrome P_{450}

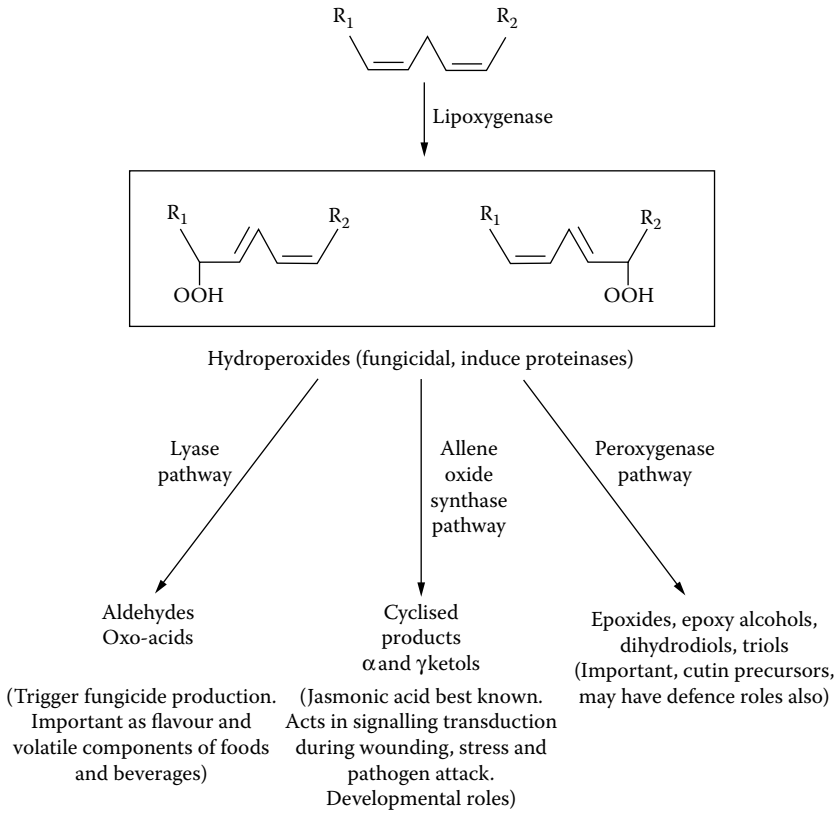


FIGURE 10.16 Lipoxygenase products and physiological roles in plants. (From Gurr, M.I., Harwood, J.L. and Frayn, K.N. (2002). *Lipid Biochemistry*, 5th ed., Blackwell Scientific, Oxford, U.K. With permission.)

TABLE 10.7 Some physiological roles for lipoxygenase-derived products in plants

Phenomenon	Notes	Compounds involved
Plant resistance to pathogens	Activation of several defence systems in "hypersensitive response". Lipoxygenase expression rapidly induced following infection	Jasmonic acid; phytoalexins (e.g., hexenal); eicosanoids from pathogen arachidonate
Mobilisation of storage lipids	13-Lipoxygenase induced, translocates to lipid body and dioxygenates storage lipids, which are then hydrolysed and the oxygenated fatty acid catabolised	13-Hydroperoxygenated lipids
Drought stress	Widespread phenomenon in plants is the drought-induced accumulation of 9-hydroperoxy derivatives of membrane lipids; function unknown	9-Hydroperoxygenated lipids
Senescence	Lipoxygenase may be involved directly in photosystem inactivation and chlorophyll oxidation; jasmonate as senescence-promoting substance, by inducing production of certain proteins; has complementary effects with ABA (abscisic acid) and may influence ethylene production	Jasmonins
Fruit ripening	Differential effects of jasmonates on ethylene formation; several fruit-specific lipoxygenase genes identified	Jasmonins
Tuber induction	Stimulate a number of associated phenomena, such as cell expansion, cytoskeleton structure and carbohydrate accumulation	Tuberonic acid and other jasmonins

Source: From Gurr, M.I., Harwood, J.L. and Frayn, K.N. (2002). *Lipid Biochemistry,* 5th Blackwell Scientific, Oxford, U.K. With permission.

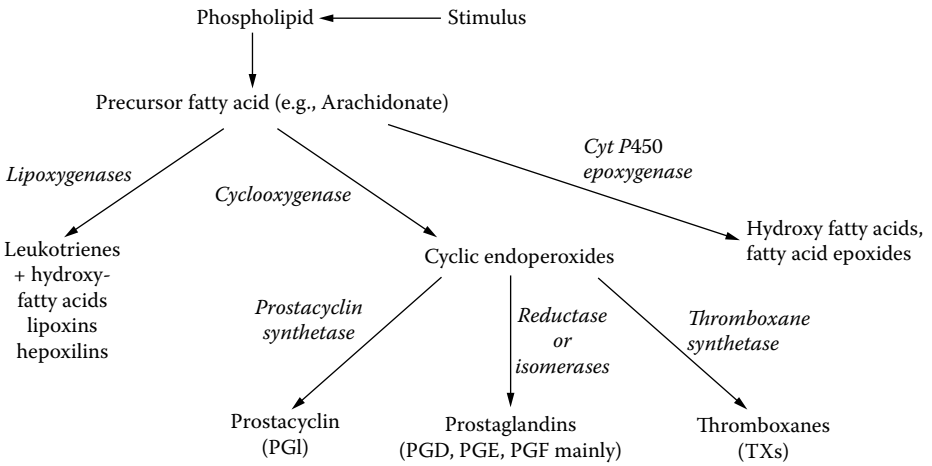


FIGURE 10.17 Overall pathway for conversion of essential fatty acids into eicosanoids. (From Gurr, M.I., Harwood, J.L. and Frayn, K.N. (2002). *Lipid Biochemistry*, 5th ed., Blackwell Scientific, Oxford, U.K. With permission.)

epoxygenases (Figure 10.17). For prostanoid structures, see Smith and Murphy (2002) and, for a general discussion, Gurr et al.(2002).

Eicosanoid synthesis is initiated following the interaction of a stimulus with the cell's plasma membrane. Interaction of the agonist with its receptor leads to the activation of one or more phospholipase. The mobilisation of arachidonate could be caused, in theory, by a number of lipid degradative enzymes. In practice, various combinations of cytosolic or secretory phospholipase A_2 enzymes appear to be used, depending on the circumstance (Smith and Murphy, 2002). Activation of the phospholipase(s) requires or is accompanied by a significant rise in intracellular Ca^{2+}. The release of arachidonate is selective (Dennis, 1987).

Once arachidonic acid (or an equivalent PUFA, such as EPA, 20:5*n*-3) is released, it can be metabolised by prostaglandin endoperoxide (PGH) synthase, also known as cyclooxygenase (COX) or prostaglandin H synthase. This enzyme has two catalytic activities: a cyclooxygenase, which catalyses the formation of PGG_2, and a peroxidase, which converts this intermediate to PGH_2 (Figure 10.18). Subsequent metabolism of PGH_2 to one of the major prostanoids then takes place via a cell-specific pathway. Prostanoids with a "2" subscript are derived from arachidonic acid, those with a "1" subscript from 8,10,14-eicosatrienoic acid and those with a "3" subscript from 5,8,10,14,17-eicosapentaenoic acid. The structure and catalytic properties of prostaglandin endoperoxide synthase (Smith and Marnett, 1991) and regulation of its expression (De Witt, 1991) have been reviewed. For a recent summary, see Kulmacz et al. (2003).

Vertebrates, from humans to fish, have two main isoforms of prostaglandin H synthase, termed PGHS-1 and -2(COX-1, COX-2). These two isoforms are structurally very similar, but have very different physiological roles and are regulated very differently (Kulmacz et al., 2003). The nonstearoidal anti-inflammatory drugs, such as aspirin or ibuprofen, act against the cyclooxygenase binding site. Since PGHS-2 (rather than PGHS-1) is implicated in chronic inflammatory complaints, there has been much interest in developing specific inhibitors against this isoform (Gurr et al., 2002). However, unexpected side-reactions have led to withdrawal of the first (specific) PGHS-2 (COX-2) inhibitors (Drazen, 2005).

Once PGH_2 has been formed by PGH synthase, further metabolism is cell-specific. For example, platelets form mainly TxA_2, endothelial cells form PGI_2 as their major prostanoid, while PGH_2 is the major product in renal collecting tubule cells. A number of these enzymes have been purified and characterised (see Smith and Murphy, 2002). PGE_2 is the best-studied prostanoid and the molecular biology and physiology of its biosynthetic pathway has been reviewed recently (Murakami and Kudo, 2004).

Prostanoids (prostaglandins, thromboxane, prostacyclin) exert a wide variety of actions in the body, which are mediated by specific receptors on plasma membranes. These receptors are classified into five basic types, termed DP, EP, FP, IP, and TP, on the basis of sensitivity to the five primary prostanoids formed from arachidonate. Details of these receptors and their physiological role will be found in Sagimoto et al. (2000).

Prostanoids are local hormones that act very near to the site of synthesis (Smith and Murphy, 2002) and which have a very short half-life (a few minutes) due to their rapid catabolism. For PGE_2, the initial reaction is oxidation to the relatively inactive 15-keto derivative. Further catabolism involves reduction of the double bond between C-13 and C-14, ω-oxidation and β-oxidation. β-Oxidation of eicosanoids is summarised by Diczfalusy (1994).

As an alternative to PGH attack, arachidonic acid can be metabolised through the action of one of three lipoxygenases. These enzymes catalyse reactions analogous to the well-known plant enzymes (Section 10.1.7). The

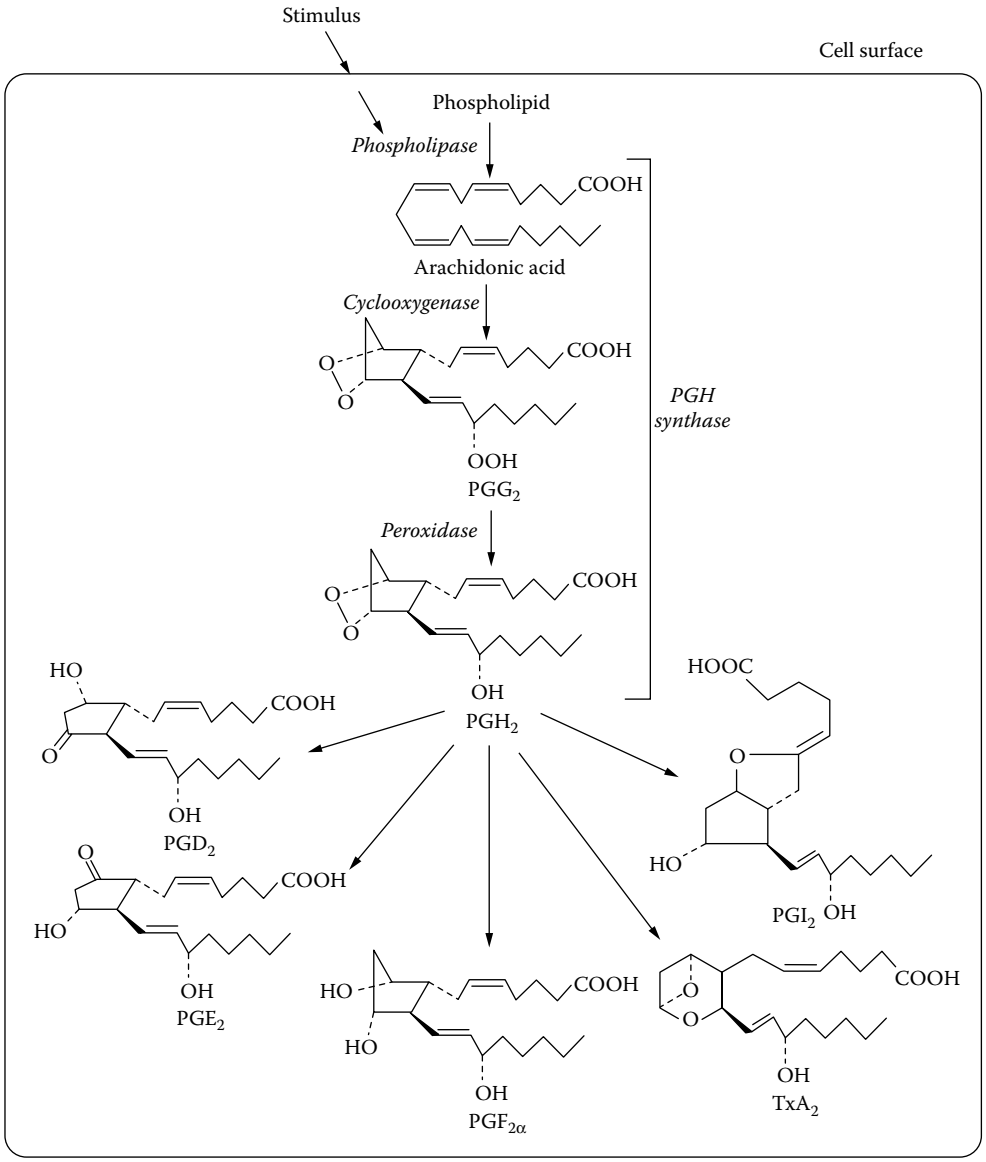


FIGURE 10.18 Structures and biosynthetic relationships among prostanoids derived from arachidonic acid. (From Smith et al. 1991a.)

lipoxygenases constitute a family of closely-related, nonhaem, iron-containing dioxygenases. The immediate products of their reaction with various eicosaenoic acids are hydroperoxy fatty acids. In the case of arachidonic acid, the products are peroxyeicosatetraenoic acids (HpETEs). Subsequent metabolism generates hydroxyeicosatetraenoic acids (HETEs) (Figure 10.19). The biochemistry and functional activity of HETEs have been reviewed by Spector et al. (1988).

The activity of the 5-lipoxygenase has been studied more thoroughly than that of the other lipoxygenases, mainly because the leukotrienes are the end products of this metabolic pathway. The production of leukotrienes LTA_4, LTB_4, and LTC_4 is shown in Figure 10.20. For summaries of the actions of leukotrienes, see Smith and Murphy (2002) and Gurr et al. (2002). Depending on the exact chemical structure concerned and the tissue tested, the leukotrienes are potent bronchoconstrictors, arterioconstrictors, vasodilators, and chemotactic agents. 5-HETE is a major product of 5-LOX activity in all cells. While 5-HETE has its own acute biological potency (Spector et al., 1988), it can also be dehydrogenated to 5-oxo-ETE. The latter compound is a strong chemoattractant and its biochemistry and functions have been reviewed (Powell and Rokach, 2005).

12-LOX is another active enzyme, giving rise initially to 12-HPETE from arachidonate (see Figure 10.19). 12-HPETE, together with its metabolite 12-HETE, has a variety of important effects on neurotransmission, white blood cells, airways, and other tissues. 12-HPETE can be converted to hepoxilins via hepoxilin synthase. Alternatively, 12-LOX can act as a lipoxin synthase in converting LTA_4

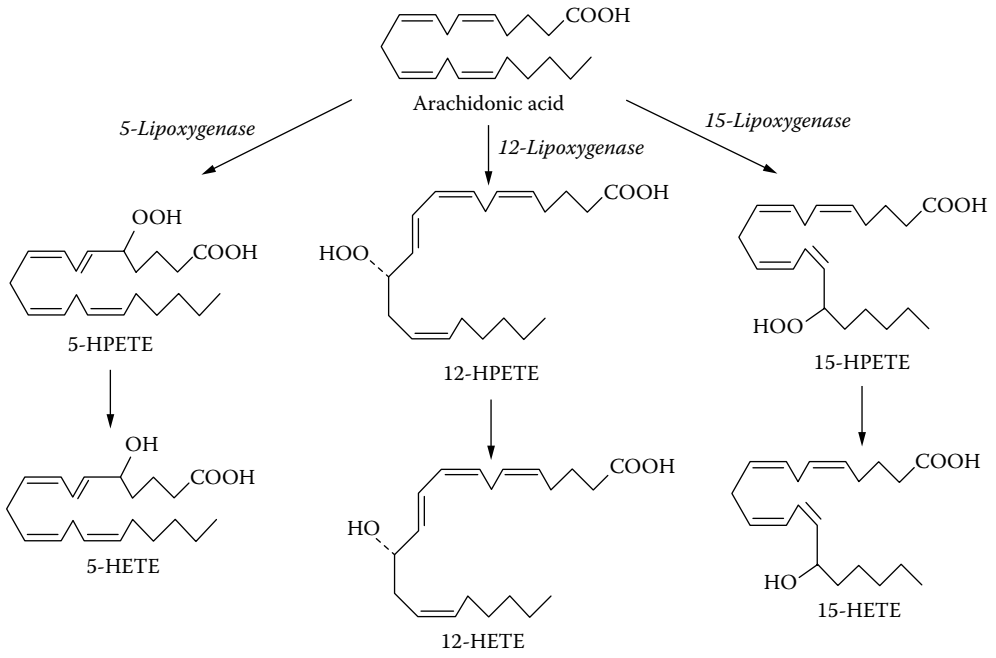


FIGURE 10.19 Lipoxygenase pathways for the synthesis of the major HETE isomers. (From Spector, et al. 1988.)

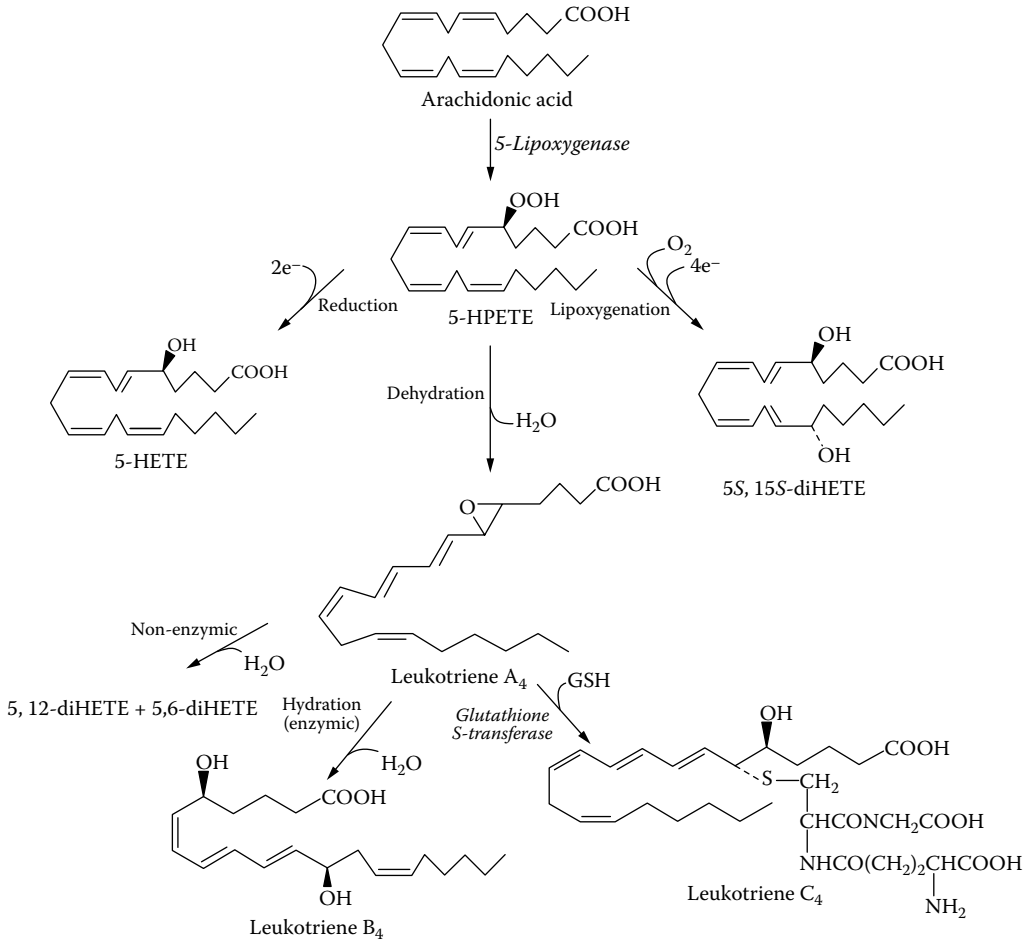


FIGURE 10.20 Formation of leukotrienes from arachidonic acid. (From Gurr and Harwood 1991.)

into lipoxin A_4. Hepoxilins are known to function in relation to the release of intracellular calcium and the opening of potassium channels, while lipoxins have roles as immunologic and hemodynamic regulators. Receptors have been identified for some of these products of 12-LOX metabolism (Yamamoto et al., 1997).

15-LOX produces 15-HPETE from arachidonate and 13-HPODE (13-hydroperoxy, 9*Z*,10*E*-octadecadienoic acid) from linoleate. These products can go on to produce 15-HETE and 13-HODE, respectively, which can then initiate various biological effects. Both 15-HETE and 13-HODE are bound to cell membrane receptors and these or related metabolites have action on erythropoiesis, the cardiovascular system, skin, respiration, and the reproductive system (Kuhn, 1996).

The third metabolic pathway for PUFAs to produce biologically active metabolites is via P_{450}-mediated reactions (see Figure 10.17). Arachidonic and linoleic acids can be oxygenated by P_{450} in four main ways: epoxidation, hydroxylation of the ω-side chain, hydroxylation of allylic or *bis*-allylic carbons, and hydroxylation with double bond migration (Oliw, 1994). Epoxyeicosatrienoic acids (EETs) with epoxy groups in the 5/6, 8/9, 11/12, or 14/15 positions (Figure 10.21), which are produced from arachidonate, have important functions in vascular smooth muscle, endothelium, myocardium, and other tissues (Spector et al., 2004). EETs are rapidly metabolised including being β-oxidised. The hydroxy products have been less well studied than the EETs but varieties of physiological functions have been noted (Oliw, 1994).

For a good general summary of the eicosanoids, refer to Smith and Murphy (2002).

10.1.9 Other conversions

Fatty acids can be modified in a number of other ways. For example, cyclopropane fatty acids are formed by the addition of a methylene group from *S*-adenosylmethionine across the double bond of a monounsaturated fatty acid. The latter is esterified to a phospholipid, so that the actual substrate is a membrane lipid. The aldehyde residues in plasmalogens and the alcohol residues in alkyl ether lipids, such as those in *Clostridium butyricum* also act as acceptors for the methyl group — in this case, forming the corresponding cyclopropane aldehydes and alcohols (Goldfine and Panos, 1971). The control of cyclopropane fatty acid synthase in bacteria, such as *E. coli,* has been examined in some detail (see Harwood and Russell, 1984). Cyclopropane fatty acids in plants seem to be made by the same mechanism as for bacteria (Mangold and Spener, 1980). These authors have also reviewed work on the synthesis of cyclopentenyl fatty acids, such as chaulmoogric (13-(2-cyclopentenyl) tridecanoic) acid.

Branched, cyclic, and unsaturated hydrocarbons in higher plants are formed from appropriate fatty acids by decarboxylation (see Kolattukudy, 1980, 1987). The process

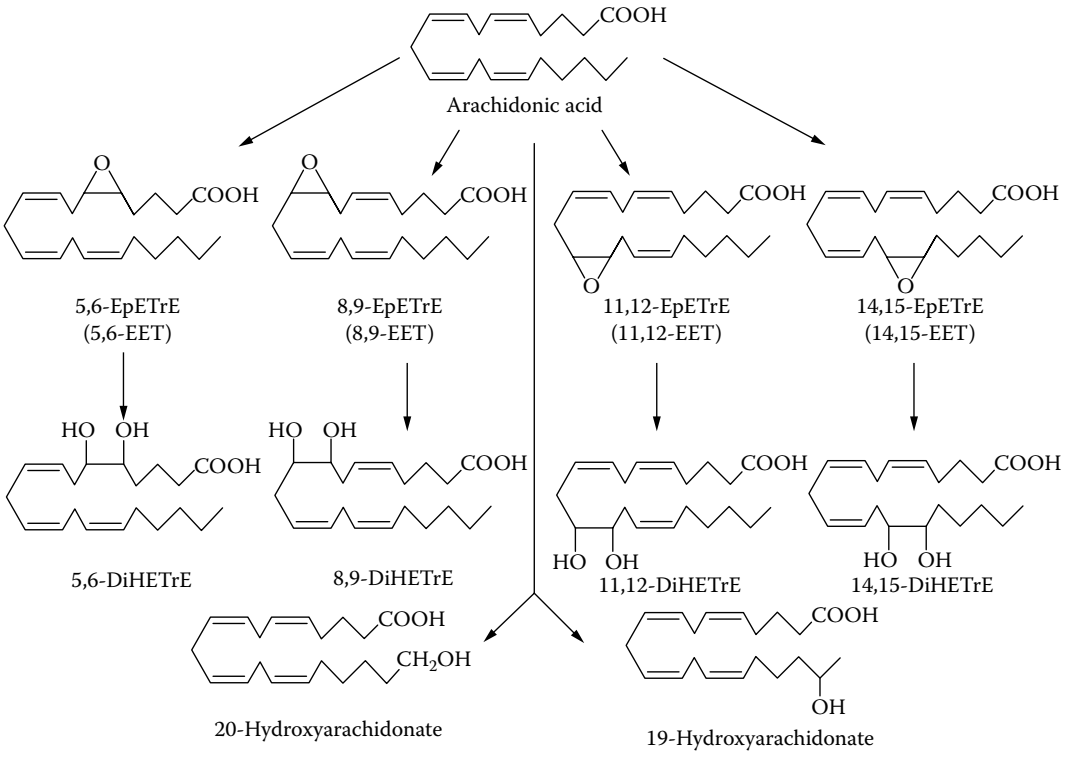


FIGURE 10.21 Structures of products of epoxygenase pathways of the arachidonate cascade. (From Smith et al. 1991a.)

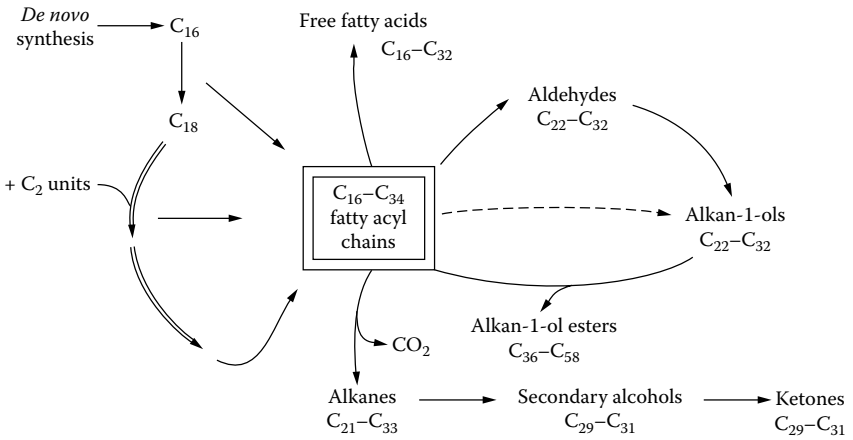


FIGURE 10.22 Conversion of fatty acids to other wax components. (From von Wettstein-Knowles, P. (1979). In *Advances in the Biochemistry and Physiology of Plant Lipids* (Eds. L-A Appelqvist and C. Liljenberg) Elsevier, Amsterdam, pp. 126. With permission.)

has also been studied in cyanobacteria, insects, and other species (see Kolattukudy, 1976). However, the exact reaction mechanism is ill defined. Plants use long-chain and very long-chain fatty acids as sources of hydrocarbon and β-diketones. These and other conversions involved in the generation of plant wax constituents are reviewed by von Wettstein-Knowles (1995), while Nelson and Blomquist (1995) discuss the formation of insect waxes. The relationship of fatty acids to the other plant wax components is shown in Figure 10.22. A recent update on the formation of the plant cuticle's components is that by Kunst et al. (2005).

References

Abulnaja, K.O. and Harwood, J.L. (1991). Interaction of thiocarbamate herbicides with fatty acid synthesis in germinating peas and their microsomal fractions *Phytochemistry.* **30**, 2883–2887.

Alberts, A. and Vagelos, P.R. (1972). Acyl-CoA carboxylase. In *The Enzymes,* vol. 6, Ed. P.D. Boyer, Academic Press, New York, pp. 37–82.

Allred, J.B. and Roman-Lopez, C.A. (1988). Enzymatically inactive forms of acetyl-CoA carboxylase in rat liver mitochondria. *Biochem. J.,* **251**, 881–885.

Amy, C.M. et al. (1989). Molecular cloning and sequencing of cDNAs encoding the entire rat fatty acid synthase. *Proc. Natl. Acad. Sci. USA,* **86**, 3114–3118.

Amy, C.M. et al. (1990). Molecular cloning of the mammalian fatty acid synthase gene and identification of the promoter region. *Biochem. J.,* **271**, 675–679.

Amy, C.M. et al. (1992). Intron-exon organization of the gene for the multifunctional animal fatty acid synthase. *Proc. Natl. Acad. Sci.,* **89**, 1005–1008.

Ansell, G.B. and Spanner, S. (1982). Phosphatidylserine, phosphatidylethanolamine and phosphatidylcholine. In *Phospholipids,* Eds. J.N. Hawthorne and G.B. Ansell, Elsevier, Amsterdam, pp. 1–49.

Asturias, F.J. et al. (2005). Structural and molecular organisation of mammalian fatty acid synthase. *Nature Struc. Mol. Biol.* **12**, 225–232.

Avery, S.V. et al. (1995) Temperature-independent changes in plasma membrane lipid order and the phagocytotic activity of the amoeba *Acanthamoeba castellanii* are closely related. *Biochem. J.* **312**, 810–816.

Barry III, C.E. et al. (1988) Mycolic acids: structure, biosynthesis and physiological functions. *Prog. Lipid. Res.* **37**, 143–179.

Beevers, H. (1980). The role of glyoxylate cycle. In *The Biochemistry of Plants, vol.* 4, Eds. P.K. Stumpf and E.E. Conn, Academic Press, New York, pp. 117–130.

Bernlohr, D.A. et al. (1997) Intracellular lipid-binding proteins and their genes. *Annu. Rev. Nutri.* **17**, 277–303.

Black, P.N. and DiRusso, C.C. (1994). Molecular and biochemical analyses of fatty acid transport, metabolism, and gene regulation in *Escherichia coli*. *Biochim. Biophys. Acta.* **1210**, 123–145.

Blée, E. (1988). Phytooxylipins and plant defense reactions. *Prog. Lipid Res.* **37**, 33–72.

Bloch, K. (1977). Control mechanisms for fatty acid synthesis in *Mycobacterium smegmatis. Adv. Enzymol.,* **45**, 1–84.

Bourre, J.M. et al. (1975). Biosynthesis of lignoceric acid from behenyl-CoA in mouse brain microsomes. Comparison between normal and Quaking mutant. *Biochem. Biophys. Res. Commun.,* **63**, 1027–1034.

Bowen, D.M. et al. (1974). The dynamic role of lipids in the nervous system. *MTP Int. Rev. Sci.,* **4**, 141–179.

Brash, A.R. (1999) Lipoxygenases: occurrence, functions, catalysis and acquisition of substrate. *J. Biol. Chem.* **274**, 23679–23682.

Bressler, R. (1970). Physiological-chemical aspects of fatty acid oxidation. In *Lipid Metabolism*, Ed. S.J. Wakil, Academic Press, New York, pp. 49–75.

Browse, J. and Somerville, C. (1991). Glycerolipid synthesis — biochemistry and regulation. *Annu. Rev. Plant Physiol.,* **42**, 467–506.

Cahoon, E.B. et al. (1999). Biosynthetic origin of double bonds: production of fatty acid components of high-value drying oils in transgenic soybean embryos. *Proc. Natl. Acad. Sci.* U.S.A. **96**, 12935–12940.

Chan, H.W.S. (1973). Soya-bean lipoxygenase: an iron-containing dioxygenase. *Biochim. Biophys. Acta,* **327**, 32–35.

Chuman, L. and Brody, S. (1989). Acyl carrier protein is present in the mitochondria of plants and eucaryotic micro-organisms. *Eur. J. Biochem.,* **184**, 643–649.

Cook, H.W. (1991). Fatty acid desaturation and chain elongation in eukaryotes. In *Biochemistry of Lipids, Lipoproteins and Membranes,* 2nd ed., Eds. D.E. Vance and J. Vance, Elsevier, Amsterdam, pp. 141–169.

Cook, H.W. and McMaster, C.R., (2002). Fatty acid desaturation and elongation in eukaryotes. In *Biochemistry of Lipids, Lipoproteins and Membranes*, Eds. D.E. Vance and J.E. Vance, 4th ed., Elsevier, Amsterdam, pp. 181–204.

Coon, M.J. et al. (1972). Functional components and mechanism of action of the resolved liver microsomal enzyme system catalyzing fatty acid, hydrocarbon and drug hydroxylation. In *Biological Hydroxylation Mechanisms*, Eds. G.S. Boyd and M.S. Smellie, Academic Press, New York, pp. 45–54.

Cripps, C. et al. (1990). The delta 12-desaturase from the house cricket, *Acheta domesticus* (Orthoptera: Gryllidae): characterization and form of the substrate. *Arch. Biochem. Biophys.,* **278**, 46–51.

Cronan, J.E. and Waldrop, G.L. (2002) Multi-subunit acetyl-CoA carboxylases. *Prog. Lipid Res.* **41**, 407–435.

Dailey, H.A. and Strittmatter, P. (1979). Modification and identification of cytochrome b_5 carboxyl groups involved in protein-protein interaction with cytochrome b_5 reductase. *J. Biol. Chem.* **254**, 5388–5396.

Dailey, H.A. and Strittmatter, P. (1980). Characterization of the interaction of amphipathic cytochrome b_5 with stearyl coenzyme A desaturase and NADPH:cytochrome P_{450} reductase. *J. Biol. Chem.* **255**, 5184–5189.

de Renobales, M. et al. (1987). Biosynthesis of linoleic-acid in insects. *Trends Biochem. Sci.* **12**, 364–366.

De Titta, G.T. et al. (1980). Carboxybiotin translocation mechanisms suggested by diffraction studies of biotin and its vitamers. *Proc. Natl Acad. Sci. USA,* **77**, 333–337.

De Witt, D.L. (1991). Prostaglandin endoperoxide synthase: regulation of enzyme expression. *Biochim. Biophys. Acta,* **1083**, 121–134.

Dennis, E.A. (1987). Regulation of eicosanoid production — role of phospholipases and inhibitors *Biotechnology,* **5**, 1294–1300.

Diczfalusy, U. (1994). β-Oxidation of eicosanoids. *Prog. Lipid Res.* **33**, 403–428.

Dittrich, F. et al. (1988). Fatty acid elongation in yeast — biochemical characteristics of the enzyme system and isolation of elongation-deficient mutants. *Eur. J. Biochem.* **252**, 477–485.

Drazen, J.M. (2005). A lesson in unexpected problems. *New Eng. J. Med.* **352,** 1031–1032.

Eaton, S. (2002). Control of mitochondrial β-oxidation flux. *Prog. Lipid Res.* **41**, 197–239.

Egmond, M.R. et al. (1977). On the mechanism of action of soybean lipoxygenase-1. A stopped-flow kinetic study of the formation and conversion of yellow and purple enzyme species. *Eur. J. Biochem.,* **76,** 469–479.

El Hussein, S.A. et al. (1988). Plant holo-(acyl carrier protein) synthase. *Biochem. J.,* **252**, 39–45.

Ernst-Fonberg, M.L. and Bloch, K. (1971). A chloroplast-associated fatty acid synthetase system in Euglena. *Arch. Biochem. Biophys.,* **143,** 392–400.

Eskin, N.A. et al. (1977). Biochemistry of lipoxygenase in relation to food quality. *Crit. Rev. Food Sci. Nutr.* **9**, 1–40.

Esselman, W.J. and Clagett, C.O. (1974). Products of linoleic hydroperoxide-decomposing enzyme of alfalfa seed. *J. Lipid Res.,* **15,** 173–178.

Fehling, E. et al. (1992). Solubilization and partial purification of constituents of acyl-CoA elongase from *Lunaria annua*. *Biochim. Biophys. Acta,* **1126**, 88–94.

Feussner, I. and Wasternack, C. (2002). The lipoxygenase pathway. *Annu Rev. Plant Biol.* **53**, 275–297.

Finnerty, W.R. (1989). Microbial lipid metabolism. In *Microbial Lipids,* vol. 2, Eds. C. Ratledge and S.G. Wilkinson, Academic Press, London, pp. 525–558.

Fulco, A.J. (1974). Metabolic alterations of fatty acids. *Annu. Rev. Biochem.,* **43**, 215–241.

Gaffney, B.J. (1996). Lipoxygenase: structural principles and spectroscopy. *Annu. Rev. Biochem,* **25**, 431–459.

Galliard, T. (1974). Unusual fatty acids in plants. *Recent Adv. Phytochem.,* **8,** 209–241.

Galliard, T. (1980). Degradation of acyl lipids: hydrolytic and oxidative enzymes. In *The Biochemistry of Plants,* vol. 4, Eds. P.K. Stumpf and E.E. Conn, Academic Press, New York, pp. 85–116.

Gaillard, T. and Chan, H.W.S. (1980). Lipoxygenases. In *The Biochemistry of Plants,* vol. 4, Eds. P.K. Stumpf and E.E. Conn, Academic Press, New York, pp. 131–161.

Galliard, T. and Matthew, J.W. (1975). Enzymic reactions of fatty acid hydroperoxides in extracts of potato tuber. I. Comparison 9D- and 13L-hydroperoxy-octadecadienoic acids as substrates for the formation of a divinyl ether derivative. *Biochim. Biophys. Acta.* **389**, 1–9.

Galliard, T. et al. (1974). The enzymic and non-enzymic degradation of colneleic acid, an unsaturated fatty acid ether intermediate in the lipoxygenase pathway of linoleic acid oxidation in potato (*Solanum tuberosum*) tubers. *Biochem. J.* **138**, 23–31.

Gardner, H.W. (1975). Decomposition of linoleic acid hydroperoxides. Enzymic reactions compared with nonenzymic. *Agric. Food Chem.* **23**, 129–136.

Gardner, H.W. (1991). Recent investigations into the lipoxygenase pathway of plants. *Biochim. Biophys, Acta.* **1084**, 221–239.

Garton, G.A. (1977). Fatty acid metabolism in ruminants. In *Biochemistry of Lipids II MTP Int. Rev. Sci.,* **14**, 337–370.

Gerhardt, B. (1992). Fatty acid degradation in plants. *Prog. Lipid Res.* **31**, 417–446.

Glatz, J.F.C. and van der Vusse, G.J. (1996) Cellular fatty acid-binding proteins: their function and physiological significance. *Prog. Lipid Res.* **35**, 243–282.

Goldberg, I. et al. (1973). Fatty acyl-coenzyme A elongation in brain of normal and quaking mice. *Science,* **182**, 497–499.

Goldfine, H. and Panos, C. (1971). Phospholipids of *Clostridium butyricum*. IV. Analysis of the positional isomers of monounsaturated and cyclopropane fatty acids and alk-1'-enyl ethers by capillary column chromatography. *J. Lipid Res.* **12**, 214–220.

Goodridge, A.G. (1991). Fatty acid synthesis in eukaryotes. In *Biochemistry of Lipids, Lipoproteins and Membranes,*

2nd ed., Eds. D.E. Vance and J. Vance, Elsevier, Amsterdam, pp. 111–139.

Graham, I.A. and Eastmond, P.J. (2002) Pathways of straight and branched chain fatty acid catabolism in higher plants. *Prog. Lipid. Res.* **41**, 156–181.

Grechkin, A (1998). Recent developments in biochemistry of the plant lipoxygenase pathway. *Prog. Lipid Res.* **37**, 317–352.

Greville, G.B. and Tubbs, P.K. (1968). The. catabolism of long chain fatty acids in mammalian tissues. *Essays Biochem.* **4**, 155–212.

Grosch, W. et al. (1977). Co-oxidation of beta-carotene and canthaxanthine by purified lipoxygenases from soya beans. *Z. Lebensm. Unters. Forsch.* **163**, 77–81.

Gunsalus, I.C. et al. (1975). Oxygenase-catalyzed biological hydroxylations. *Annu. Rev. Biochem.* **44**, 377–407.

Gurr, M.I. (1974). Biosynthesis of unsaturated fatty acids. In *Biochemistry of Lipids,* Ed. T.W. Goodwin, Butterworths, London, pp. 181–236.

Gurr, M.I. and Harwood, J.L. (1991) *Lipid Biochemistry,* 4th ed., Chapman & Hall, London.

Gurr, M.I. et al. (2002) *Lipid Biochemistry,* 5th ed., Blackwell Science, Oxford.

Hamberg, M. et al. (1974). Oxygenases in fatty acid and steroid metabolism. In *Molecular Mechanisms of Oxygen Activation,* Ed. O. Hayaishi, Academic Press, New York, pp. 29–85.

Hamberg, M. et al. (1999). Alpha-oxidation of fatty acids in higher plants. Identification of a pathogen-inducible oxygenase (piox) as an alpha-dioxygenase and biosynthesis of 2-hydroperoxylinolenic acid. *J. Biol. Chem.* **274**, 24503–24513.

Harwood, J.L. (1988). Fatty acid metabolism. *Annu. Rev. Plant Physiol.* ***39,*** 101–138.

Harwood, J.L. (1990). Lipid synthesis. In *Target Sites for Herbicide Action,* Ed. R.C. Kirkwood, Plenum, New York, pp. 57–94.

Harwood, J.L. (1996). Recent advances in the biosynthesis of plant fatty acids. *Biochim. Biophys. Acta.* **1301,** 7–56.

Harwood, J.L. (2005). Fatty acid biosynthesis. In *Plant Lipids: Biology, Utilisation and Manipulation*, Ed. D.J. Murphy, Blackwell Publishing, Oxford, pp. 27–66.

Harwood, J.L. and Aparicio. R. Eds. (2000) *Handbook of Olive Oil.* Aspen Publishing, Gaithersburg, MD.

Harwood, J.L. and Russell, N.J. (1984) *Lipids in Plants and Microbes,* Allen and Unwin, London.

Harwood, J.L. et al. (1990). Enzymes of fatty acid synthesis. In *Methods in Plant Biochemistry,* vol. 3 (Ed. P.J. Lea), Academic Press, London, pp. 193–217.

Hashimoto, T. (1990). Purification properties and biosynthesis of peroxisomal beta oxidation enzymes. In *Fatty Acid Oxidation: Clinical, Biochemical and Molecular Aspects,* Eds. K. Tanaka and P.M. Costes, Alan R. Liss, New York, pp. 137–152.

Heath, R.J. et al. (2001). Lipid biosynthesis as a target for antibacterial agents. *Prog. Lipid Res.* **40**, 467–497.

Heath, R.J. et al. (2002). Fatty acid and phospholipid metabolism in prokaryotes. In *Biochemistry of Lipids, Lipoproteins and Membranes*, Eds. D.E. Vance and J.E. Vance 4th ed., Elsevier, Amsterdam, pp. 55–92.

Hildebrand, D.F. et al. (2005). Fatty acid manipulation. In *Plant Lipids: Biology, Utilisation and Manipulation* (Ed. D.J. Murphy) Blackwell Publishing, Oxford, pp. 67–102.

Holman, R.T. et al. (1969). Substrate specificity of soybean lipoxidase. *J. Biol. Chem.* **244**, 1149–1151.

Itabe, H. (1998). Oxidised phospholipids as a new landmark in atherosclerosis. *Prog. Lipid Res.* **37**, 181–207.

Iwabuchi, M. et al. (2003). Delta 12-oleate desaturase-related enzymes associated with formation of conjugated trans-delta II, *cis*-delta 13 double bonds. *J. Biol. Chem.* **278**, 4603–4610.

Jackowski, S. et al. (1987). Acetoacetyl-acyl carrier protein synthase, a potential regulator of fatty acid biosynthesis in bacteria. *J. Biol. Chem.* **262**, 7927–7931.

Jackowski, S. et al. (1991). Lipid metabolism in prokaryotes. In *Biochemistry of Lipids, Lipoproteins and Membranes*, 2nd ed., Eds. D.E. Vance and J. Vance, Elsevier, Amsterdam, pp. 43–85.

Jaworski, J.G. (1987). Biosynthesis of monoenoic and polyenoic fatty acids. In *The Biochemistry of Plants, vol.* 9, Eds. P.K. Stumpf and E.E. Conn, Academic Press, New York, pp. 159–174.

Jaworski, J.G. et al. (1989). A cerulenin insensitive short chain 3-ketoacyl-acyl carrier protein synthase in *Spinacia oleracea* leaves. *Plant Physiol.* **90***,* 4144.

Johns, R.B. and Perry, G.J. (1977). Lipids of marine bacterium *Flexibacter-polymorphus. Arch. Microbiol.* **104**, 267–271.

Jones, A.V.M. and Harwood, J.L. (1980). Desaturation of linoleic acid from exogenous lipids by isolated chloroplasts. *Biochem. J.* **190**, 851–854.

Jones, A.L. et al. (1992). Induction of delta 12-desaturase activity during temperature adaptation in *Acanthamoeba castellanii*. *Biochem. Soc. Trans.* **20**, 170S.

Jones, A.L. et al. (1993). Temperature induced membrane–lipid adaptation in *Acanthamoeba castellanii. Biochem. J.* **290**, 273–278.

Kaneda, T. (1977). Fatty acids of the genus Bacillus: an example of branched-chain preference. *Bact. Rev.* **41**, 391–418.

Kearns, E.V. et al. (1991). The role of cytochrome b_5 in delta 12 desaturation of oleic acid by microsomes of safflower (*Carthamus tinctorius* L.). *Arch. Biochem. Biophys.* **284**, 431–436.

Kim, K-H (1997). Regulation of mammalian acetyl-CoA carboxylase. *Annu. Rev. Nutri.* **17,** 77–99.

Kindl, H. (1987). β-oxidation of fatty acids by specific organelles. In *The Biochemistry of Plants,* vol. 9, Eds. P.K. Stumpf and E.E. Conn, Academic Press, New York, pp. 31–52.

Klein, K. et al. (1971). Fatty acid degradation in *Escherichia coli*. An inducible system for the uptake of fatty acids and further characterization of old mutants. *Eur. J. Biochem.* **19**, 442–450.

Knowles, J.R. (1989). The mechanism of biotin-dependent enzymes. *Annu. Rev. Biochem.* **58**, 195–221.

Knutzon, D.S. et al. (1991). Nucleotide sequence of a complementary DNA clone encoding stearoyl-acyl carrier protein desaturase from castor bean. *Plant Physiol.* **96**, 344–345.

Kolattukudy, P.E. Ed. (1976) *Chemistry and Biochemistry of Natural Waxes,* Elsevier, New York.

Kolattukudy, P.E. (1980). Cutin, suberin, and waxes. In *The Biochemistry of Plants,* vol. 4, Eds. P.K. Stumpf and E.E. Conn, Academic Press, New York, pp. 571–645.

Kolattukudy, P.E. (1987). Lipid-derived defensive polymers and waxes and their role in plant-microbe interaction. In *The Biochemistry of Plants,* vol. 9, Eds. P.K. Stumpf and E.E. Conn, Academic Press, New York, pp. 291–314.

Krakower, G.R. and Kim, K.H. (1981). Purification and properties of acetyl-CoA carboxylase phosphatase. *J. Biol. Chem.* **256**, 2408–2413.

Kridl, J.C., Knutzon, D.S., Johnson, W.B., Thompson, G.A., Radke, S.E., Turner, J.C. and Knauf, V.C. (1991) *Int. Soc. for Plant Mol. Biol., 3rd Int. Congr., Tucson, AZ,* October, Poster 723.

Kuhn, H. (1996). Biosynthesis, metabolization and biological importance of the primary 15-lipoxygenase metabolites 15-hydro(pero)xy-5Z, 8Z, 10Z, 13E-eicosatetraenoic acid and 13-hydro(epro)xy-9Z, 10E-octadecadienoic acid. *Prog. Lipid Res.* **35**, 203–226.

Kulmacz, R.J. et al. (2003). Comparison of the properties of prostaglandin H synthase-1 and -2. *Prog. Lipid Res.* **42**, 377–404.

Kunau, W.H. et al. (1995) β-Oxidation of fatty acids in mitochondria, peroxisomes and bacteria: a century of continued progress. *Prog. Lipid. Res.* **34**, 267–342.

Kunst, L. et al. (2005). The plant cuticle: formation and structure of epidermal surfaces. In *Plant Lipids: Biology, Utilisation and Manipulation*, Ed. D.J. Murphy, Blackwell Publishing, Oxford, pp. 270–302.

Kusunose, M. et al. (1964). Enzymatic omega-oxidation of fatty acids. II. Substrate specificity and other properties of the enzyme system. *J. Biol. Chem.* **239**, 2135–2139.

Lazarow, P.B. and Moser, H.W. (1989). Disorders of peroxisome biogenesis. In *The Metabolic Basis of Inherited Disease,* 6th ed., Eds. C.R. Scriver, A.L. Beaudet, W.S. Sly and D. Valle, McGraw-Hill, New York, pp. 1479–1509.

Lekakis, N.J. (1977). α-Oxidation of fatty-acids in *Escherichia-coli*. *FEMS Microbiol. Lett.* **1**, 289–292.

Lenk, W. (1972). Biochemical acyl hydroxylations. *Prog. Drug Res.***16**, 229–308.

Lent, B. and Kim, K.H. (1980). Purification and properties of a kinase which phosphorylates and inactivates acetyl-CoA carboxylase**.** *J. Biol. Chem.* **257**, 1897–1901.

Leonard, A.E. et al. (2004) Elongation of long-chain fatty acids. *Prog. Lipid Res.* **43**, 36–54.

Leonardi, R. et al. (2005) Coenzyme A: back in action. *Prog. Lipid Res.* **44**, 125–153.

Lessire, R. et al. (1989). Involvement of a beta-ketoacyl-CoA intermediate in acyl-CoA elongation by an acyl-CoA elongase purified from leek epidermal cells. *Biochim. Biophys. Acta.* **1006**, 35–40.

Li, S.-J. and Cronan, J.E. (1992). The gene encoding the biotin carboxylase subunit of *Escherichia coli* acetyl-CoA carboxylase. *J. Biol. Chem.* **267***,* 855–863.

Libertini, L.J. and Smith, S. (1978). Purification and properties of a thioesterase from lactating rat mammary gland which modifies the product specificity of fatty acid synthetase. *J. Biol. Chem.* **253**, 1393–1401.

Lynen, F.O. (1967). The role of biotin-dependent carboxylations in biosynthetic reactions. *Biochem. J.,* **102**, 381–400.

Maloy, S.R. et al. (1980). Elevated levels of glyoxylate shunt enzymes in *Escherichia coli* strains constitutive for fatty acid degradation. *J. Bacteriol.* **143**, 720–725.

Mangold, H.K. and Spener, F. (1980). Biosynthesis of cyclic fatty acids. In *The Biochemistry of Plants,* vol. 4, Eds. P.K. Stumpf and E.E. Conn, Academic Press, New York, pp. 647–663.

Matsuda, S. et al. (1993). Decreased messenger RNA of arachidonate 12-lipoxygenase in platelets of patients with myeloproliferative disorders. *Biochim. Biophys. Acta.* **1080**, 243–249.

McGarry, J.D. and Foster, D.W. (1980). Regulation of hepatic fatty acid oxidation and ketone body production. *Annu. Rev. Biochem.* **49**, 395–420.

McKeon, T.A. and Stumpf, P.K. (1982). Purification and characterization of the stearoyl-acyl carrier protein desaturase and the acyl-acyl carrier protein thioesterase from maturing seeds of safflower. *J. Biol. Chem.* **257***,* 12141–12147.

Mcllwain, H. (1966) *Biochemistry and the Central Nervous System,* Churchill, London.

Metz, J.G. et al. (2001). Production of polyunsaturated fatty acids by polyketide synthases in both prokaryotes and eukaryotes. *Science* **293**, 290–293.

Mikami, K. and Murata, N. (2003). Membrane fluidity and the perception of environmental signals in cyanobacteria and plants. *Prog. Lipid Res.* **42**, 527–543.

Mikolajczyk, S. and Brody, S. (1990). De novo fatty acid synthesis mediated by acyl-carrier protein in *Neurospora crassa* mitochondria. *Eur. J. Biochem.* **187**, 431–437.

Moreau, R.A. and Stumpf, P.K. (1981). Recent studies of the enzymic synthesis of ricinoleic acid by developing castor beans. *Plant Physiol.* **67**, 672–676.

Morris, L.J. (1970). Mechanisms and stereochemistry in fatty acid metabolism. *Biochem. J.* **108**, 681–693.

Morrow, J. D. and Roberts, L.J. (1997). The isoprostanes: unique bioactive products of lipid peroxidation. *Prog. Lipid Res.* **36**, 1–21.

Moss, J. and Lane, M.D. (1971). The biotin-dependent enzymes. *Adv. Enzymol.* **35***,* 321–442.

Mukherji, M. et al. (2003). The chemical biology of branched-chain lipid metabolism. *Prog. Lipid. Res.* **42,** 359–376.

Murakami, M. and Kudo, I. (2004). Recent advances in the molecular biology and physiology of the prostaglandin E_2-biosynthetic pathway. *Prog. Lipid Res.* **43**, 1–35.

Murata, N. and Wada, H. (1995). Acyl lipid desaturases and their importance in the tolerance and acclimatization to cold of cyanobacteria. *Biochem. J.* **308**, 1–8.

Naggert. J. et al. (1988). Molecular cloning and sequencing of a cDNA encoding the thioesterase domain of the rat fatty acid synthetase. *J. Biol. Chem.* **263**, 1146–1150.

Naggert, J. et al. (1991). Expression in *Escherichia coli*, purification and characterization of two mammalian thioesterases involved in fatty acid synthesis**.** *Biochem. J.* **273**, 787–790.

Nelson, D.R. and Blomquist, G. (1995) Insect waxes. In *Waxes: Chemistry, Molecular Biology and Functions*, Ed. R.J. Hamilton, The Oily Press, Dundee, U.K., pp. 1–90.

Nichols, B.W. et al. (1967). Interrelationships between fatty acid biosynthesis and acyl-lipid synthesis in *Chlorella vulgaris. Biochem., J.* **104**, 486–496.

Nikawa, J. et al. (1979). Inhibitory effects of long-chain acyl coenzyme A analogues on rat liver acetyl coenzyme A carboxylase. *FEBS Lett.,* **102**, 223–226.

Nunn, W.D. (1986). A molecular view of fatty acid catabolism in *Escherichia coli*. *Microbiol. Rev.* **50**, 179–192.

Ohlrogge, J.B. (1987). Biochemistry of plant acyl carrier proteins. In *The Biochemistry of Plants,* vol. 9, Eds. P.K. Stumpf and E.E. Conn, Academic Press, New York, pp. 137–157.

Ohlrogge, J. B. and Jaworski, J.G. (1997) Regulation of fatty acid synthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **48**, 109–136.

Ohlrogge, J. et al. (1991). The genetics of plant lipids. *Biochim. Biophys. Acta.,* **1082**, 1–26.

Oliw, E.H. (1994). Oxygenation of polyunsaturated fatty acids by cytochrome P_{450} monoxygenases. *Prog. Lipid Res.* **33**, 329–354.

Osmundsen, H. et al. (1991). Metabolic aspects of peroxisomal beta-oxidation. *Biochim. Biophys. Acta,* **1085,** 141–158.

Ozasa, H. et al. (1984). Biosynthesis of enzymes of rat-liver mitochondrial beta-oxidation. *Eur. J. Biochem.,* **144,** 453–458.

Piazza, G. Ed. (1996). *Lipoxygenase and Lipoxygenase Pathway Enzymes.* Am. Oil Chem. Soc., Champaign, IL.

Polakis, S.E. et al. (1974). Acetyl- coenzyme A carboxylase system of *Escherichia coli*. Studies on the mechanisms of the biotin carboxylase- and carboxyltransferase-catalyzed reactions. *J. Biol. Chem.,* **249**, 6657–6667.

Pollard, M.R. and Stumpf, P.K. (1980). Biosynthesis of C20 and C22 fatty acids by developing seeds of *Limnanthes alba*. *Plant Physiol.* **66,** 649–655.

Pollard, M.R. et al. (1991). A specific acyl-ACP thioesterase implicated in medium-chain fatty acid production in immature cotyledons of *Umbellularia californica*. *Arch. Biochem. Biophys.* **284**, 306–312.

Post-Beittenmiller, D. et al. (1991). *In vivo* pools of free and acylated acyl carrier proteins in spinach. Evidence for sites of regulation of fatty acid biosynthesis. *J. Biol. Chem.,* **266**, 1858–1865.

Poulose, A.J. et al. (1981). Primary structure of a chymotryptic peptide containing the "active serine" of the thioesterase domain of fatty acid synthase. *Biochem. Biophys. Res. Commun.* **103**, 377–382.

Powell, W.S. and Rokach, J. (2005). Biochemistry, biology and chemistry of the 5-lipoxygenase product 5-oxo-ETE. *Prog. Lipid Res.* **44**, 154–183.

Pugh, E.L. and Kates, M. (1975). Characterization of a membrane-bound phospholipid desaturase system of *Candida lipolytica*. *Biochim. Biophys. Acta,* **380,** 442–453.

Rainwater, D.L. and Kolattukudy, P.E. (1982). Purification and characterization of acyl-CoA carboxylase from the goose uropygial gland, which produces multimethyl-branched acids and evidence for its identity with avian acetyl-CoA carboxylase. *Arch. Biochem. Biophys.* **213**, 372–383.

Rangan, V.S. and Smith, S. (2002). Fatty acid synthesis in eukaryotes. In *Biochemistry of Lipids, Lipoproteins and Membranes*, Eds. D.E. Vance and J.E. Vance, 4th ed., Elsevier, Amsterdam, pp. 151–179.

Roessler, P.G. (1990). Purification and characterization of acetyl-CoA carboxylase from the diatom *Cyclotella cryptica*. *Plant Physiol.* **92**, 73–78.

Rosahl, S. and Feussner, I. (2005). Oxylipins. In *Plant Lipids: Biology, Utilisation and Manipulation*, Ed. D.J. Murphy, Blackwell Publishing, Oxford, pp. 329–354.

Rutter, A.J. et al. (2002) Oxygen induction of a novel fatty acid n-6 desaturase in the soil protozoon, *Acanthamoeba castellanii. Biochem. J.* **368**, 57–67.

Samols, D. et al. (1988). Evolutionary conservation among biotin enzymes. *J. Biol. Chem.* **263**, 6461–6464.

Sanchez, J. and Salas, J.J. (2000). Biogenesis of olive oil aroma. In *Handbook of Olive Oil*, Eds. J.L. Harwood and R. Aparicio, Aspen Publishing, Gaithersburg, MD, pp. 79–99.

Schewe, T. and Kuhn, H. (1991). Do 15-lipoxygenases have a common biological role? *Trends Biochem. Sci.* **16,** 369–373.

Schmid, K.M. and Ohlrogge, J.B. (2002). Lipid metabolism in plants. In *Biochemistry of Lipids, Lipoproteins and Membranes*, Eds. D.E. Vance and J.E. Vance, 4th ed., Elsevier, Amsterdam, pp. 93–126.

Schmidt, H. and Heinz, E. (1990). Involvement of ferredoxin in desaturation of lipid-bound oleate in chloroplasts. *Plant Physiol.* **94**, 241–220.

Schroepfer, G.J. and Bloch, K. (1965). The stereospecific conversion of stearic acid to oleic acid. *J. Biol. Chem.* **240**, 54–63.

Schulz, H. (1991a). Oxidation of fatty acids. In *Biochemistry of Lipids, Lipoproteins and Membranes*, 2nd ed., Eds. D.E. Vance and J. Vance, Elsevier, Amsterdam, pp. 87–110.

Schulz, H. (1991b). Beta oxidation of fatty acids. *Biochim. Biophys. Acta.* **1081**, 109–120.

Schweizer, E. (1984). Genetics of fatty acid biosynthesis in yeast. In *Fatty Acid Metabolism and its Regulation*, Ed. S.Numa, Elsevier, Amsterdam, pp. 59–83.

Schweizer, E. (1989). Biosynthesis of fatty acids and related compounds. In *Microbial Lipids,* vol. 2, Eds. C. Ratledge and S.G. Wilkinson, Academic Press, London, pp. 3–51.

Schweizer, E. et al. (1973). Pantetheine-free mutants of the yeast fatty-acid-synthetase complex. *Eur. J. Biochem.* **39**, 353–362.

Sekiya, J. and Hatanaka, A. (1977). *Cis*-3-hexenal and normal-hexanal formation from linolenic and linoleic acids in alfalfa cells cultured in vitro. *Plant Sci. Lett.* **10**, 165–169.

Siedow, J.N. (1991). Plant lipoxygenase — structure and function *Annu. Rev. Plant Physiol.* **42**, 145–188.

Sigal, E. (1991). The molecular biology of mammalian arachidonic acid metabolism. *Am. J. Physiol.* **260**, L13–28.

Slabas, A.R. and Fawcett, T. (1992). The biochemistry and molecular biology of plant lipid biosynthesis. *Plant Mol. Biol.* **19**, 169–191.

Smith, M.A. et al. (1990). Electron-transport components of the 1-acyl-2-oleoyl-sn-glycero-3-phosphocholine delta 12-desaturase (delta 12-desaturase) in microsomal preparations from developing safflower (*Carthamus tinctorius* L.) cotyledons. *Biochem. J.* **272**, 23–29.

Smith, M.A. et al. (1992). Evidence for cytochrome b_5 as an electron donor in ricinoleic acid biosynthesis in microsomal preparations from developing castor bean (*Ricinus communis* L.). *Biochem. J.* **287**, 141–144.

Smith, S. et al. (2003) Structural and functional organization of the animal fatty acid synthase. *Prog. Lipid Res.* **42,** 289–317.

Smith, W.L. (1989). The eicosanoids and their biochemical mechanisms of action. *Biochem. J.* **259**, 315–324.

Smith, W.L. and Marnett, L.J. (1991). Prostaglandin endoperoxide synthase: structure and catalysis. *Biochim. Biophys. Acta.* **1083**, 1–17.

Smith, W.L. and Murphy, R.C. (2002). The eicosanoids: cyclooxygenase, lipoxygenase and epoxygenase pathways. In *Biochemistry of Lipids, Lipoproteins and Membranes*, Eds. D.E. Vance and J.E. Vance, 4th ed., Elsevier, Amsterdam, pp. 341–371.

Smith, W.L. et al. (1991a). The eicosanoids: cyclooxygenase, lipoxygenase, and epoxygenase pathways. In *Biochemistry*

of Lipids, Lipoproteins and Membranes, 2nd ed., Eds. D.E. Vance and J. Vance, Elsevier, Amsterdam, pp. 297–325.

Smith, W.L. et al. (1991b). Prostaglandin and thromboxane biosynthesis. *Pharm. Ther.* **49**, 153–179.

Soling, H.D. and Seufert, C.D. Eds. (1978). *Biochemical and Clinical Aspects of Ketone body Metabolism,* Georg Thiem, Stuttgart.

Spaapen, L.J.M., et al. (1977). On the properties of a pea lipoxygenase. *Biochim. Biophys. Acta*, **488**, 517–520.

Spector, A.A. et al. (2004). Epoxyeicosatrienoic acids (EETs): metabolism and biochemical function. *Prog. Lipid Res.* **43,** 55–90.

Spector, A.A. et al. (1988). Hydroxyeicosatetraenoic acids (HETEs). *Prog. Lipid Res.* **27**, 271–323.

Stern, J.R. (1961). Crotonase. In *The Enzymes,* 2nd ed,, vol. 5, Eds. P.D. Boyer, H. Lardy and K. Myrback, Academic Press, New York, pp. 511–529.

Streckert, G. and Stan, H.J. (1975). Conversion of linoleic acid hydroperoxide by soybean lipoxygenase in the presence of guaiacol: identification of the reaction products. *Lipids,* **10**, 847–854.

Strittmatter, P. et al. (1974). Purification and properties of rat liver microsomal stearyl coenzyme A desaturase. *Proc. Natl Acad. Sci. USA,* **71**, 4565–4569.

Strittmatter, P. et al. (1988). Bacterial synthesis of active rat stearyl-CoA desaturase lacking the 26-residue amino-terminal amino acid sequence. *J. Biol. Chem.* **263,** 2532–2535.

Stumpf, P.K. (1970). Fatty acid metabolism in plants. In *Lipid Metabolism*, Ed. S.J. Wakil, Academic Press, New York, pp. 79–106.

Stumpf, P.K. (1987). The biosynthesis of saturated fatty acids. In *The Biochemistry of Plants,* vol. 9, Eds. P.K. Stumpf and E. E. Conn, Academic Press, New York, pp. 121–136.

Sugimoto, Y. et al. (2000). Distribution and function of prostanoid receptors: studies from knockout mice. *Prog. Lipid Res.* **39**, 289–392.

Sumper, M. and Riepertinger, C. (1972). Structural relationship of biotin-containing enzymes. Acetyl-CoA carboxylase and pyruvate carboxylase from yeast. *Eur. J. Biochem.,* **29**, 237–248.

Takai, T. et al. (1987). Primary structure of the biotin-binding site of chicken liver acetyl-CoA carboxylase. *FEBS Lett.* **212**, 98–102.

Takai, T. et al. (1988). Primary structure of chicken liver acetyl-CoA carboxylase deduced from cDNA sequence. *J. Biol. Chem.* **263**, 2651–2657.

Theorell, H. et al. (1947). Crystalline lipoxidase. *Acta Chem. Scand.,* **1**, 571–576.

Thiede, M.A. and Strittmatter, P. (1985). The induction and characterization of rat liver stearyl-CoA desaturase mRNA. *J. Biol. Chem.* **260**, 14459–14463.

Thiede, M.A. et al. (1986). Construction and sequence of cDNA for rat liver stearyl coenzyme A desaturase. *J. Biol. Chem.* **261**, 13230–13235.

Thomas, K. et al. (1998). Oxygen induces fatty acid (n-6) desaturation independently of temperature. *FEBS Lett.* **425,** 171–174.

Thompson, G.A. et al. (1991). Primary structures of the precursor and mature forms of stearoyl-acyl carrier protein desaturase from safflower embryos and requirement of ferredoxin for enzyme activity. *Proc. Natl Acad. Sci. USA,* **88**, 2578–2582.

Tocher, D.R. et al. (1998). Recent advances in the biochemistry and molecular biology of fatty acyl desaturases. *Prog. Lipid Res.* **37**, 73–107.

Trotter, P.J. (2001). The genetics of fatty acid metabolism in *Saccharomyces cerevisiae. Annu. Rev. Nutr.* **21**, 97–109.

Uchida, K. (2003). 4-Hydroxy-2-nonenal: a product and mediator of oxidative stress. *Prog. Lipid Res.* **42**, 318–343.

Umeka, S. and Nozawa, Y. (1984). Effects of transient anaerobic stress on fatty acid desaturation and electron-transport system. *Biochim. Biophys. Acta.* **792**, 25–32.

Valentine, R.C. and Valentine, D.L. (2004). *Omega*-3 fatty acids in cellular membranes: a unified concept. *Prog. Lipid Res.* **43,** 383–402.

Vance, D.E. and Vance, J.E. Eds. (2002). *Biochemistry of Lipids, Lipoproteins and Membranes* 4th ed., Elsevier, Amsterdam.

Vanhanen, S. et al. (2000). A consensus sequence for long-chain fatty acid alcohol oxidases from *Candida* identifies a family of genes involved in lipid omega-oxidation in yeast with homologues in plants and bacteria. *J. Biol. Chem.* **275,** 4445–4452.

Veerkamp, J.H. and Maatman, R.G.H.J. (1995) Cytoplasmic fatty acid-binding proteins: their structure and genes. *Prog. Lipid Res.* **34**, 17–52.

Veldink, G.A. et al. (1977). Plant lipoxygenases. *Prog. Chem. Fats Other Lipids.* **15**, 131–66.

Verhoeven, N.M. and Jakobs, C. (2001) Human metabolism of phytanic acid and pristanic acid. *Prog. Lipid. Res.* **40**, 453–466.

Verhue, W. H. and Francke, A. (1972). Heterogeneity of soybean lipoxygenase. *Biochim. Biophys. Acta.* **284**, 4353.

Voelker, T.A. et al. (1992). Fatty acid biosynthesis redirected to medium chains in transgenic oilseed plants. *Science,* **257**, 72–74.

Volpe, J.J. and Vagelos, P.R. (1973). Saturated fatty acid biosynthesis and its regulation. *Annu. Rev. Biochem.* **42**, 21–60.

Volpe, J.J. and Vagelos, P.R. (1976). Mechanisms and regulation of biosynthesis of saturated fatty acids. *Physiol. Rev.* **56**, 339–417.

von Wettstein-Knowles, P. (1979). Genetics and biosynthesis of plant epicuticular waxes. In *Advances in the Biochemistry and Physiology of Plant Lipids*, Eds. L-A Appelqvist and C. Liljenberg, Elsevier, Amsterdam, pp. 1–26.

von Wettstein-Knowles, P. (1982). Biosynthesis of epicuticular lipids as analysed with the aid of gene mutations in barley. In *Biochemistry and Metabolism of Plant Lipids*, Eds. J.F.G.M. Wintermans and P.J.C. Kuiper, Elsevier, Amsterdam, pp. 69–78.

von Wettstein-Knowles, P. (1995). Biosynthesis and genetics of waxes. In *Waxes: Chemistry, Molecular Biology and Functions*, Ed. R.J. Hamilton, The Oily Press, Dundee, U.K., pp. 91–130.

Wada, H. and Murata, N. (1989). *Synechocystis* PCC6803 mutants defective in desaturation of fatty acids. *Plant Cell Physiol.* **30**, 971–978.

Wada, H. et al. (1990). Enhancement of chilling tolerance of a cyanobacterium by genetic manipulation of fatty acid desaturation. *Nature,* **347**, 200–203.

Wada, K. and Tanabe, T. (1983). Dephosphorylation and activation of chicken liver acetyl-coenzyme-A carboxylase. *Eur. J. Biochem.* **135**, 17–23.

Wakil, S.J. (1970). Fatty acid metabolism. In *Lipid Metabolism*, Ed. S.J. Wakil, Academic Press, New York, pp. 1–48.
Wakil, S.J. et al. (1983). Fatty acid synthesis and its regulation. *Annu. Rev. Biochem.* **52**, 537–579.
Walker, K.A. and Harwood, J.L. (1986). Evidence for separate elongation enzymes for very-long-chain-fatty-acid synthesis in potato (*Solanum tuberosum*). *Biochem. J.* **237**, 41–46.
Walsh, M.C. et al. (1990). The short chain condensing enzyme has a widespread occurrence in the fatty acid synthetases from higher-plants. *Phytochemistry.* **29**, 3797–3799.
Wardale, D.A. et al. (1978). Localization of fatty-acid hydroperoxide cleavage activity in membranes of cucumber fruit. *Phytochemistry.* **17**, 205–212.
Watkins, P.A. (1997) Fatty acid activation. *Prog. Lipid Res.* **36**, 55–83.
Webber, R.J. and Edmond, J. (1979). The in vivo utilization of acetoacetate, D-(-)-3-hydroxybutyrate, and glucose for lipid synthesis in brain in the 18-day-old rat. Evidence for an acetyl-CoA bypass for sterol synthesis. *J. Biol. Chem.* **254**, 3912–3920.
Weber, F. and Grosch, W. (1976). Co-oxidation of a carotenoid by the enzyme lipoxygenase: influence on the formation of linoleic acid hydroperoxides. *Z. Lebensm, Unters. Forsch.* **161**, 223–230.
White, S.W. et al. (2005). The structural biology of Type II fatty acid biosynthesis. *Ann. Rev. Biochem.* **74**, 791–831.
Witkowski, A. et al. (1987). Molecular cloning and sequencing of a cDNA encoding the acyl carrier protein and its flanking domains in the mammalian fatty acid synthetase. *Eur. J. Biochem.* **165**, 601–606.
Witkowski, A. et al. (1991). Structural organization of the multifunctional animal fatty acid synthase. *Eur. J. Biochem.* **198**, 571–579.
Worsham, L.M.S. et al. (1988). Chemical cross-linking and its effect on fatty acid synthetase activity in intact chloroplasts from *Euglena gracilis. Biochim. Biophys. Acta.* **963**, 423–438.
Wurtele, E.S. and Nickolau, B.J. (1990). Plants contain multiple biotin enzymes: discovery of 3-methylcrotonyl-CoA carboxylase, propionyl-CoA carboxylase and pyruvate carboxylase in the plant kingdom. *Arch. Biochem. Biophys.* **278**, 179–186.
Yamamoto, S. (1989). Mammalian lipoxygenases: molecular and catalytic properties. *Prostaglandins, Leukotrienes Essent. Fatty Acids* **35**, 219–229.
Yamamoto, S. (1991). "Enzymatic" lipid peroxidation: reactions of mammalian lipoxygenases. *Free Radical Biol. Med.* **10**, 149–159.
Yamamoto, S. (1992). Mammalian lipoxygenases: molecular structures and functions. *Biochim. Biophys. Acta.* **1128**, 117–131.
Yamamoto, S. et al. (1997). Arachidonate 12-lipoxygenases. *Prog. Lipid Res,* **36**, 23–41.
Zimmerman, D.L. and Coudron, C.A. (1979). Identification of traumatin, a wound hormone, as 12-oxo-trans-10-dodecanoid acid. *Plant Physiol.* **63**, 536–541.

10.2 Glycerophospholipids

10.2.1 Biosynthesis

The first reactions in glycerophospholipid synthesis can be regarded as the stepwise acylation of glycerol 3-phosphate. The product of these acylations, phosphatidic acid, is a key intermediate in glycerolipid metabolism as illustrated in Figure 10.23. In addition to providing the backbone of the glycerophospholipids, phosphatidate (via diacylglycerol) also acts as a precursor of triacylglycerols (Section 10.3) and the glycosylglycerides (Section 10.4).

The biosynthesis of phosphatidate in animals begins with the activation of fatty acids to their acyl-CoAs by one of several chain-length-dependent acyl-CoA ligases (synthetases) (Bloch and Vance, 1977; Groot et al., 1976; Brindley, 1991). The most important locations of the long-chain acyl-CoA ligases are the endoplasmic reticulum and mitochondria (Brindley, 1991). Some ligases show preferences towards saturated or unsaturated fatty acids. Of course, some of the enzymes play a role more in degradation (by providing acyl-CoAs for β-oxidation) than in biosynthesis. The peroxisomal acyl-CoA synthetases come into this category (Section 10.1.6). A review of the biochemistry of acyl-CoAs is that by Waku (1992).

The main acceptor for acyl-CoA in most tissues is *sn*-glycerol 3-phosphate, derived from glycolysis. Glycerol 3-phosphate acyltransferase is divided equally between mitochondrial and endoplasmic reticulum in mammalian liver, but in other tissues the endoplasmic reticulum is the main site. The latter enzyme is on the cytoplasmic face. The mitochondrial and endoplasmic reticulum enzymes can be distinguished by their relative sensitivities to heat, proteolytic enzymes, and SH-reagents (Brindley, 1991). The substrate selectivities of different enzymes are discussed by Waku (1992). In general, a variety of saturated and unsaturated acyl-CoAs are used. The second acyltransferase (lysophosphatidate acyl transferase) has a strong preference for unsaturated fatty acids in animals. In most subcellular fractions (and most animal tissues), the second acyltransferase has much higher activity than the first acyltransferase, so that lysophosphatidate does not accumulate. The substrate specificities of the two enzymes are such that the glycerolipids of animals show a preferential location of saturated fatty acids at the *sn*-1 position and unsaturated fatty acids at the *sn*-2 position. Some properties of the lysophosphatidate acyltransferase are covered in Brindley (1991). See also Coleman et al. (2002) for details of the above two acyltransferases.

The acylation of dihydroxyacetone phosphate and the subsequent reduction of acyldihydroxyacetone phosphate to monoacylphosphatidic acid provide an alternative route for phosphatidic acid synthesis (Brindley, 1991). Acyltransferases that utilize dihydroxyacetone phosphate have been detected in several tissues (Bell and Coleman, 1980) and purified from guinea pig liver peroxisomes (Webber and Hajra, 1992).

Another possible alternative pathway for phosphatidic acid synthesis (involving diacylglycerol kinase) has been demonstrated in rat liver (see Bell and Coleman, 1980). However, this enzyme is probably not very important quantitatively for phosphatidic acid formation. See also

Brindley and Sturton (1982) for a review of phosphatidic acid formation.

Once phosphatidate has been formed, it can be converted to diacylglycerol through the action of phosphatidate phosphohydrolase. This is a key enzyme in glycerolipid metabolism, controlling, on the one hand, the supply of carbon for the major membrane phospholipids, phosphatidylcholine and phosphatidylethanolamine, and the major storage lipid, triacylglycerol, and, on the other, controlling the relative production of anionic phosphoglycerides by a competing cytidylyltransferase (see Figure 10.23 and below). Phosphatidate phosphohydrolase has been well reviewed (Brindley, 1988; see also Brindley, 1991; Coleman et al., 2000). Phosphatidate phosphohydrolase in animal tissues seems to be subject to control by translocation between the endoplasmic reticulum and cytosolic compartments (Brindley, 1991). In rat liver it may catalyse the rate-limiting step in glycerolipid synthesis because it has the lowest *in vitro* activity of all the enzymes in the pathway, varies directly with dietary or hormonal regimes that affect triacylglycerol synthesis and decreases with some drugs that inhibit nonpolar lipid formation (Bell and Coleman, 1980).

As shown in Figure 10.23, phosphatidylcholine (the major animal phosphoglyceride) and phosphatidylethanolamine are both synthesized by a (cytidine 5'-diphosphate) CDP-base pathway. This is their major route of formation (Bell and Coleman, 1980; Ansell and Spanner, 1982). Three enzyme steps are required.

First, the base is phosphorylated by a kinase enzyme. Choline kinase (EC 1.7.1.32) and ethanolamine kinase (EC 2.7.1.82) are soluble enzymes and have been purified from several tissues. The activities may reside in the same protein (Ulane, et al., 1977), although separate enzymes have been purified from rat liver (Brophy and Vance, 1976). The purified choline kinase is a dimer of mass 42 kDa (Vance, 1991).

The second enzyme of the pathway is the cytidylyltransferase (EC 2.7.7.15), and this enzyme is partly soluble and partly associated with the endoplasmic reticulum (see Bell

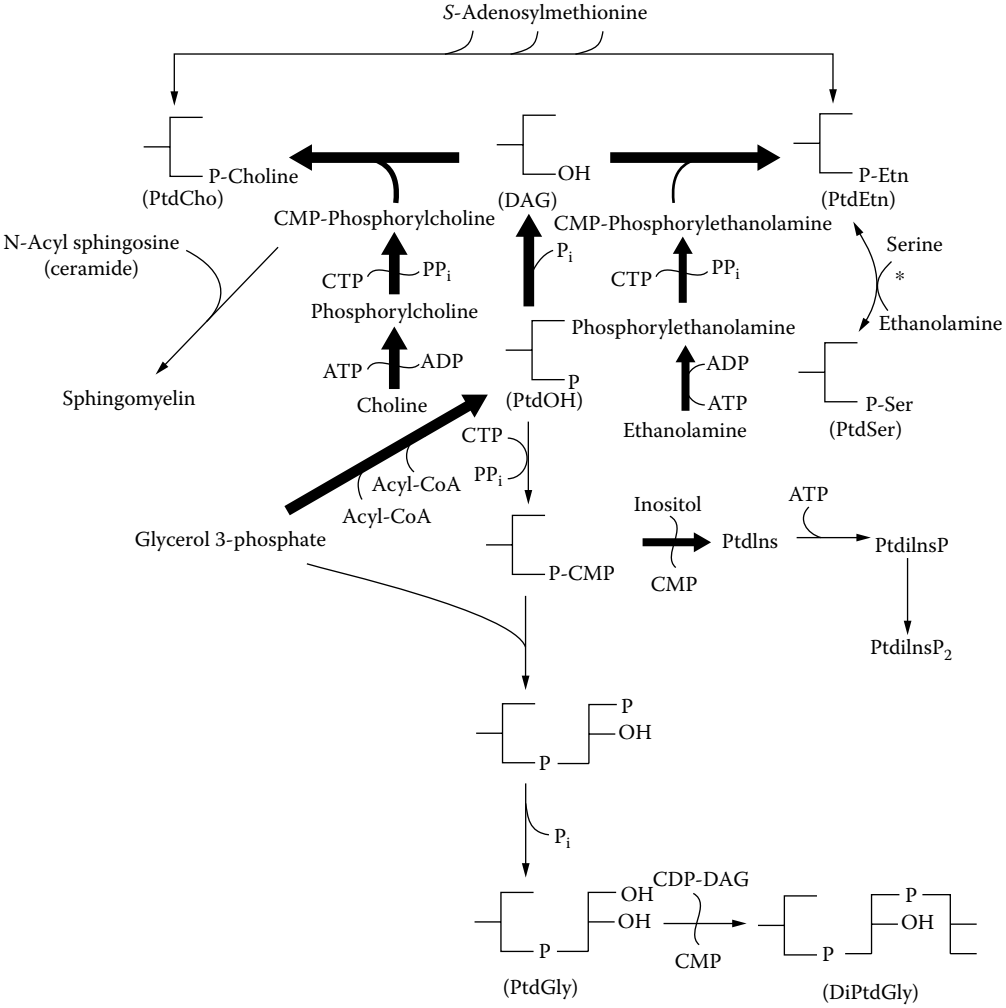


FIGURE 10.23 Phospholipid metabolism in animals. The relative thickness of lines shows the approximate carbon flow down each pathway. The asterisk (*) shows that other phospholipids can act as acceptors for serine in base exchange.

and Coleman, 1980). The soluble protein in animals can be aggregated under various conditions and its activity raised by association with membranes (see Vance, 1991). The enzyme seems to be the rate-limiting step in the CDP-choline pathway in animals (Vance and Choy, 1979). The enzyme has been purified (Feldman and Weinhold, 1987), and the cDNA coding for its protein identified and expressed (Vance, 1990). The equivalent enzyme, CDP-ethanalominephosphate cytidylyltransferase (EC 2.7.7.14), has been partly purified from rat liver (see Vance, 1991). CDP-choline cytidylyltransferase is normally thought to be the most important enzyme for regulation of phosphatidylcholine formation (Vance, 2002).

Choline phosphotransferase (EC 2.7.8.2) and ethanolamine phosphotransferase (EC 2.7.8.1) are located in the endoplasmic reticulum and catalyse the final step in the CDP-base pathway. Their complete purification has yet to be achieved from animals. The two enzymes seem to have somewhat different properties and appear to be distinct proteins (see Vance, 1991).

Phosphatidylcholine can also be synthesized by the stepwise methylation of phosphatidylethanolamine (Figure 10.23). Methyl groups are transferred from *S*-adenosyl-L-methionine, and this pathway is a minor one in animals (20% of liver phosphatidylcholine synthesis, but undetectable in other tissues; Bell and Coleman, 1980). In liver, the three transmethylation reactions are catalysed by a single enzyme with a molecular mass of about 18 kDa. The rate of conversion of phosphatidylethanolamine to phosphatidylcholine appears to be regulated by substrate supply (Vance, 1991). A review of phosphatidylethanolamine methylation has been published (Vance and Ridgway, 1988) and Vance (2002) has updated information and speculated on the role of the methylation pathway in forming phosphatidylcholine.

Phosphatidylserine accounts for 5 to 15% of the total phospholipids in animal cells. It is made by a base exchange reaction (see Figure 10.23) in which the head group of a preexisting phospholipid is exchanged for serine. The enzyme has been purified, and its base exchange activity is not due to phospholipase D activity (see Vance, 1991). Studies in CHO cells showed that there were two phosphatidylserine synthases, one using phosphatidylcholine and a second, phosphatidylethanolamine (Kuge and Nishijima, 1997).

Instead of being dephosphorylated, phosphatidate can be converted to CDP-diacylglycerol. This reaction, catalysed by phosphatidate:CDP-diacylglycerol cytidylyltransferase (EC 2.7.7.41), is important for the synthesis of negatively charged phospholipids, such as phosphatidylglycerol, diphosphatidylglycerol, and the inositol phospholipids (see Figure 10.23). The cytidylyltransferase has been purified from animals (Bell and Coleman, 1980) and cDNAs identified for two isoforms (Vance, 2002).

Phosphatidylglycerol can be made by mitochondrial and microsomal fractions from most animal tissues. However, the mitochondrial enzymes are probably more physiologically relevant (Bell and Coleman, 1980), since most of the phosphatidylglycerol (except in lungs; Section 11.7) is used for diphosphatidylglycerol formation. The phosphatidylglycerol phosphate synthetase has been localised in the inner mitochondrial membrane, but the phosphatase (see Figure 10.23) is soluble. Together with CDP-diacylglycerol, phosphatidylglycerol is a co-substrate for diphosphatidylglycerol synthesis in animals (see Figure 10.23). This contrasts with the bacterial pathway (see Figure 10.28). Diphosphatidylglycerol (cardiolipin) synthesis in different organisms has been discussed in detail by Schlame et al. (2000) who also discuss the lipid's function in mitochondria and relevance to a number of human diseases. A third polyglycerophospholipid, *bis*(monoacylglycerol) phosphate, comprises less than 1% of the phospholipids of most animal tissues except for alveolar (lung) macrophages, where it represents 14 to 18% of the phospholipids. Its pathway of formation was proposed following experiments with macrophage-like cells. Phosphatidylglycerol was hydrolysed by phospholipase A_2 and the lyso product acylated using another phospholipid. This yields *bis*(monoacylglycero)phosphate (BMP). After reorientation of the glycerol backbone, a second acylation (again using a phospholipid as donor) forms *sn*-1: *sn*-1'-BMP. Spontaneous rearrangement causes the acyl residues to move to the *sn*-3 positions (Amidon et al., 1995).

Phosphatidylinositol is also formed from CDP-diacylglycerol, though in this case only one enzyme step is involved (see Figure 10.23). Further phosphorylation to phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate are catalysed by kinase reactions. The first kinase appears to be present in isoforms and these are integral membrane proteins. One enzyme with a low K_m for ATP (20-70 μM) and a molecular mass of 55 kDa has been purified to homogeneity (Downes and MacPhee, 1990). Phosphatidylinositol 4-phosphate kinase can be isolated from cytosolic and membrane sources. It appears to be a peripheral membrane protein (Vance, 1991). The above two kinases are important in the "phosphatidylinositol cycle" (Figure 10.24), which is connected with the important function of phosphatidylinositol 4,5-bisphosphate as a precursor of second messages that activate many regulatory processes in animal cells (see Section 10.2.2 and Section 10.6.3; also McPhee, 2002). Over recent years, a large number of inositol phospholipids have been identified, particularly those with phosphates at the 3-position and which have important signaling functions (Section 10.6) and Rameh and Cantley, 1997). Interconversions of inositol phospholipids through the action of various kinases and phosphatases are shown in Figure 10.25. See Freeman et al. (1998) for a review on phosphoinositide kinases and Vanhaesebroeck et al. (2001) for one on the synthesis and function of the 3-phosphorylated inositol lipids.

Phospholipids cannot only be remodeled by base exchange reactions (Taki et al., 1978), but also by reacylation

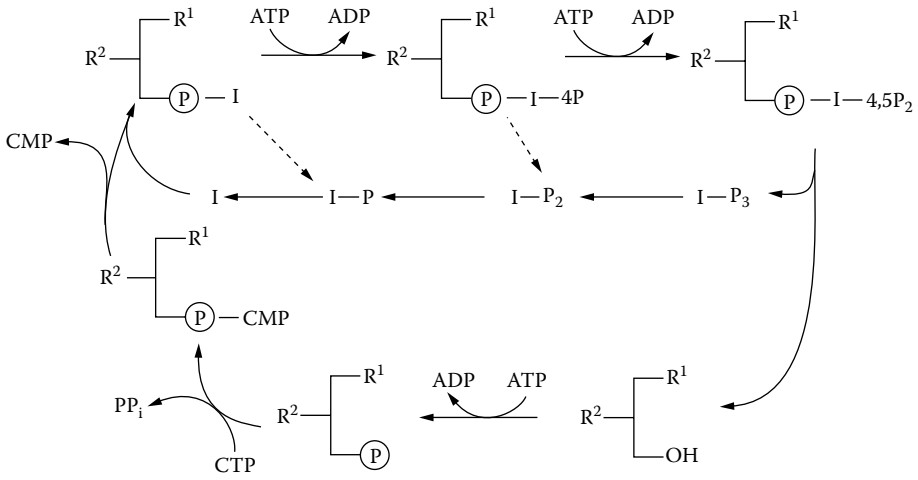


FIGURE 10.24 The phosphatidylinositol cycle. I = inositol. (From Vance 1991.)

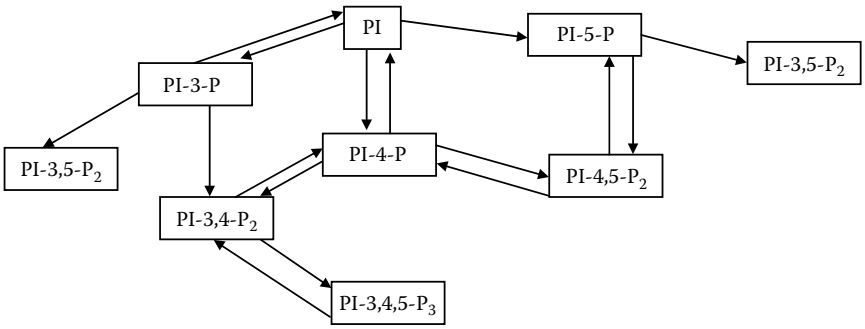


FIGURE 10.25 Interconversion of phosphoinositides. PI=phosphatidylinositol. The connecting recactions are catalysed by 3-, 4-, or 5- kinases and 3-, 4-, and 5- phosphatases.

reactions. The latter are extremely important in determining the final molecular species of lipid that are synthesised as well as allowing the participation of specific fatty acids in particular metabolic processes (e.g., release of arachidonate for prostaglandin formation (see van den Bosch, 1982). The entire subject of reacylation and molecular species turnover has been well reviewed by one of the major research workers in this field, Bill Lands (Hills and Lands, 1970). More recent discoveries are covered by Bell and Coleman (1980) and by Hawthorne and Ansell (1982) (see also MacDonald and Sprecher, 1991). Remodelling can occur on either the *sn*-1 or *sn*-2 positions (Vance, 2002).

The above discussion has centred on the formation of diacylglycerophospholipids, but many phospholipids in nature have *O*-alkyl bonds. These include such important molecules as the plasmalogens and platelet-activating factor (PAF). For reviews on the metabolism, regulation and function of ether-linked glycerolipids, refer to Synder et al. (1985, 2002). Enzymes of the acyl dihydroxyacetone phosphate pathway for glycerolipid synthesis are located in peroxisomes as well as the endoplasmic reticulum. Their physiological function in peroxisomes as well as their role in the production of both acyl and alkyl glycerolipids has been reviewed (Hajra, 1995). The coregulation of sterol and phospholipid metabolism is covered by Ridgeway et al. (1991).

The overall pathways for phosphoglyceride synthesis in plants have been reviewed (Mudd, 1980; Moore, 1982; Harwood, 1979, 1989). For a recent update, see Dormann (2005). In general, the formation of these lipids is similar to that in animals, but there are some points of difference (Figure 10.26). First, there are two systems for the initial acylation of glycerol 3-phosphate. In the chloroplast-located pathway, acyl-ACPs are used and the substrate specificity of the enzymes results in the preferential formation of *sn*-1-oleoyl, *sn*-2-palmitoyl molecular species. Subsequent utilisation of such phosphatidate for lipid synthesis retains this (*sn*-1 C18, *sn*-2 C16) distribution of fatty acids and gives rise to what have been called "prokaryotic" molecular species by analogy with cyanobacterial lipid metabolism (see Roughan and Slack, 1984; Harwood, 1989; Joyard and Douce, 1987). A second glycerol 3-phosphate acylation system is located in the endoplasmic reticulum, uses acyl-CoAs, and has a substrate specificity such that more saturated fatty acyl groups

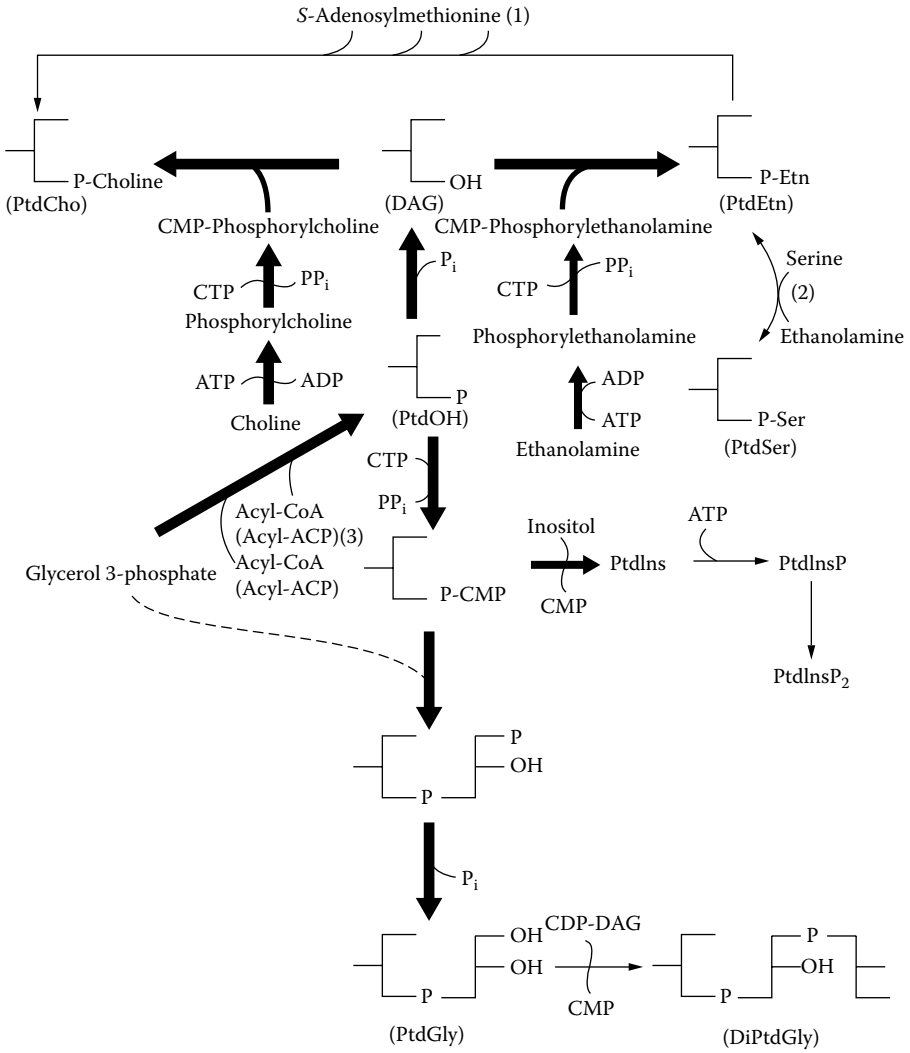


FIGURE 10.26 Phospholipid metabolism in plants. The relative thickness of arrows indicates the carbon flux along individual reactions. *Notes:* (1) Other pathways for incorporating ethanolamine into PtdCho exist (see text); (2) other phospholipids can serve for base exchange; (3) acyl-ACPs are used by chloroplasts, but acyl-CoAs by the endoplasmic reticulum.

are usually located at the *sn*-1 position, just as in animals (see Harwood, 1989). The endoplasmic reticulum acylations are responsible for extrachloroplastic phospholipid synthesis and triacylglycerol formation (Harwood and Page, 1993), as well as some chloroplast lipid synthesis (see Roughan and Slack, 1984; Browse and Somerville, 1991).

The major plant extrachloroplastic lipid, phosphatidylcholine, is mainly made by the CDP-base pathway (Harwood, 1979; Moore, 1982). Separate choline and ethanolamine kinases have been purified from soybean (Harwood, 1979), and the cytidylyltransferase step appears to be rate-limiting (Price-Jones and Harwood, 1986). Separate base transferases for choline and for ethanolaminephosphate appear to be present in soybean (see Harwood, 1979).

Although evidence has been produced that plants can convert phosphatidylethanolamine to phosphatidylcholine by stepwise methylation (Mudd, 1980; Moore, 1982), some recent experiments suggest that methylation occurs on various water-soluble intermediates, such as ethanolaminephosphate, which are then incorporated into phosphatidylcholine via phosphatidyldimethyl- (or methyl-) ethanolamine (see Williams and Harwood, 1994).

Phosphatidylserine, phosphatidylglycerol, and phosphatidylinositol seem to be synthesized in plants by pathways similar to those discussed above for animals (Mudd, 1980; Moore, 1982; and see Figure 10.23 and Figure 10.26). Phosphatidylglycerol synthesis has been demonstrated in chloroplasts (see Harwood, 1989) in keeping with the major role of this lipid in thylakoid membranes. The metabolism of inositol lipids and their function in cellular regulation in plants has been reviewed recently (Drobak, 2005).

N-Acylphosphatidylethanolamine is a minor phospholipid in plants (Dormann, 2005), but there is some interest in it as a source of signalling metabolites (see Chapman, 2004).

Pathways for the biosynthesis of phospholipids in yeast are generally similar to those demonstrated in other eukaryotes, except that phosphatidylserine is made exclusively from CDP-diacylglycerol (Figure 10.27) and not by base exchange. Phosphatidylserine is also converted to phosphatidylethanolamine by decarboxylation. Work with yeast has been particularly useful as a model system for other eukaryotes because of a large knowledge base in classical genetics and the ability to elucidate and manipulate individual genes coding for enzymes in the pathways. Purification and properties of CDP-diacylglycerol synthetase, phosphatidylserine synthetase, phosphatidylinositol synthetase, and phosphatidylinositol kinase are described by Carman and Henry (1989). Carman and Henry (1999) reviewed the pathways of synthesis in *S.cerevisiae* in detail with information on the genes encoding individual enzymes. Particularly informative sections deal with the genetic and biochemical regulation of phospholipid synthesis and its interaction with other metabolic pathways.

Phospholipid biosynthesis in microorganisms has been well reviewed by Pieringer (1989). Major pathways for *Escherichia coli* are shown in Figure 10.28. Several points are of note. First, the acylation of glycerol 3-phosphate uses acyl-ACPs and, thus, phospholipid synthesis is coupled directly to fatty acid formation (Jackowski et al., 1991). Secondly, as in yeasts (see Figure 10.27), phosphatidylserine is made from CDP-diacylglycerol and can be decarboxylated to phosphatidylethanolamine. Thirdly, the

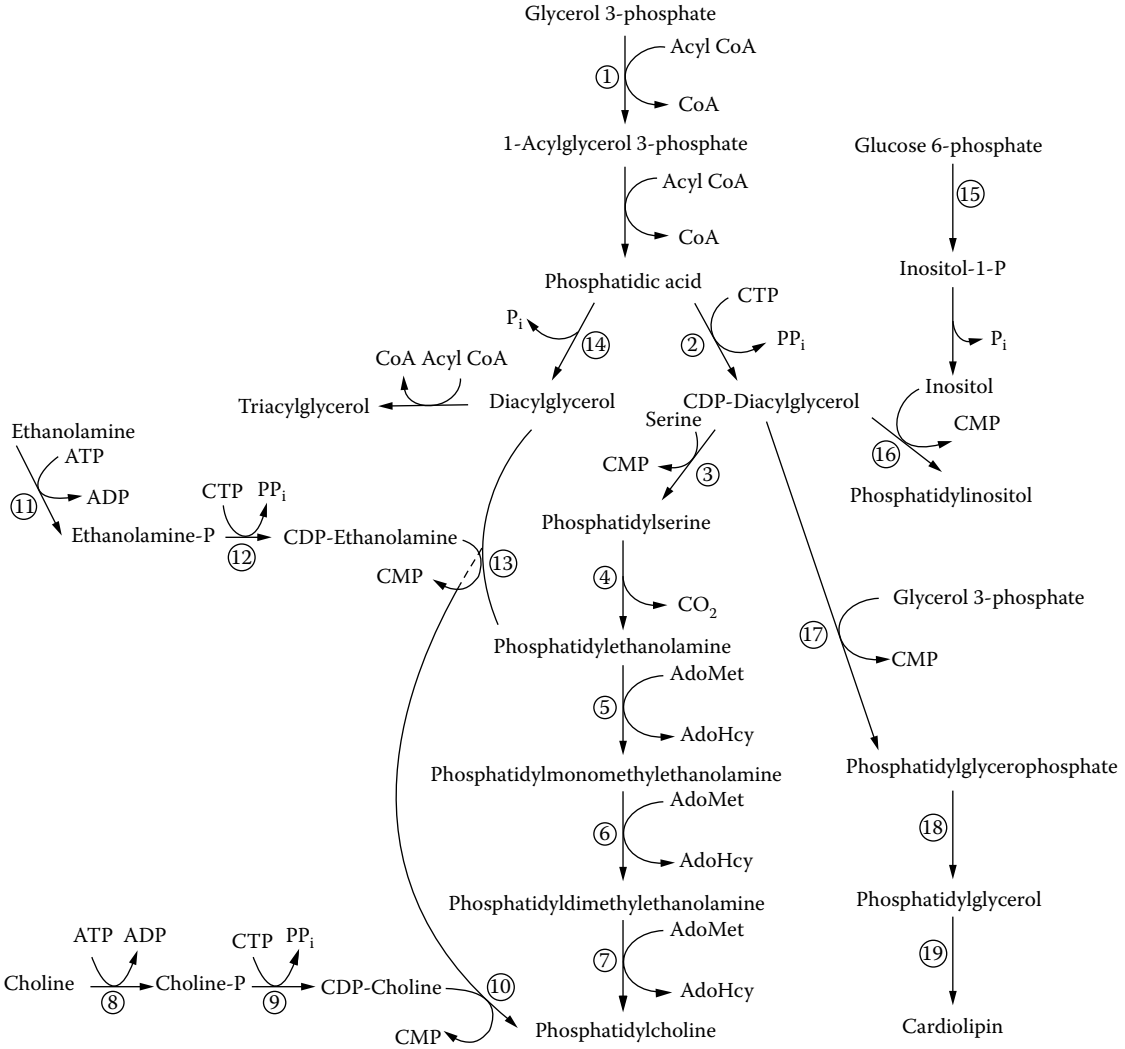


FIGURE 10.27 Phospholipid biosynthetic pathways in *Saccharomyces cerevisiae.* The indicated reactions are catalysed by the following enzymes: (1) glycerol 3-phosphate acyltransferase; (2) CDP-DG synthetase; (3) PS synthetase; (4) PS decarboxylase; (5) PE methyltransferase; (6) and (7) phospholipid methyltransferase; (8) choline kinase; (9) cholinephosphate cytidylyltransferase; (10) cholinephosphotransferase; (11) ethanolamine kinase; (12) ethanolaminephosphate cytidylyltransferase; (13) ethanolaminephosphotransferase; (14) PA phosphatase; (15) I-1-P synthetase; (16) PI synthetase; (17) PGP synthetase; (18) PGP phosphatase; (19) CL synthetase. (From Carman and Henry (1989).)

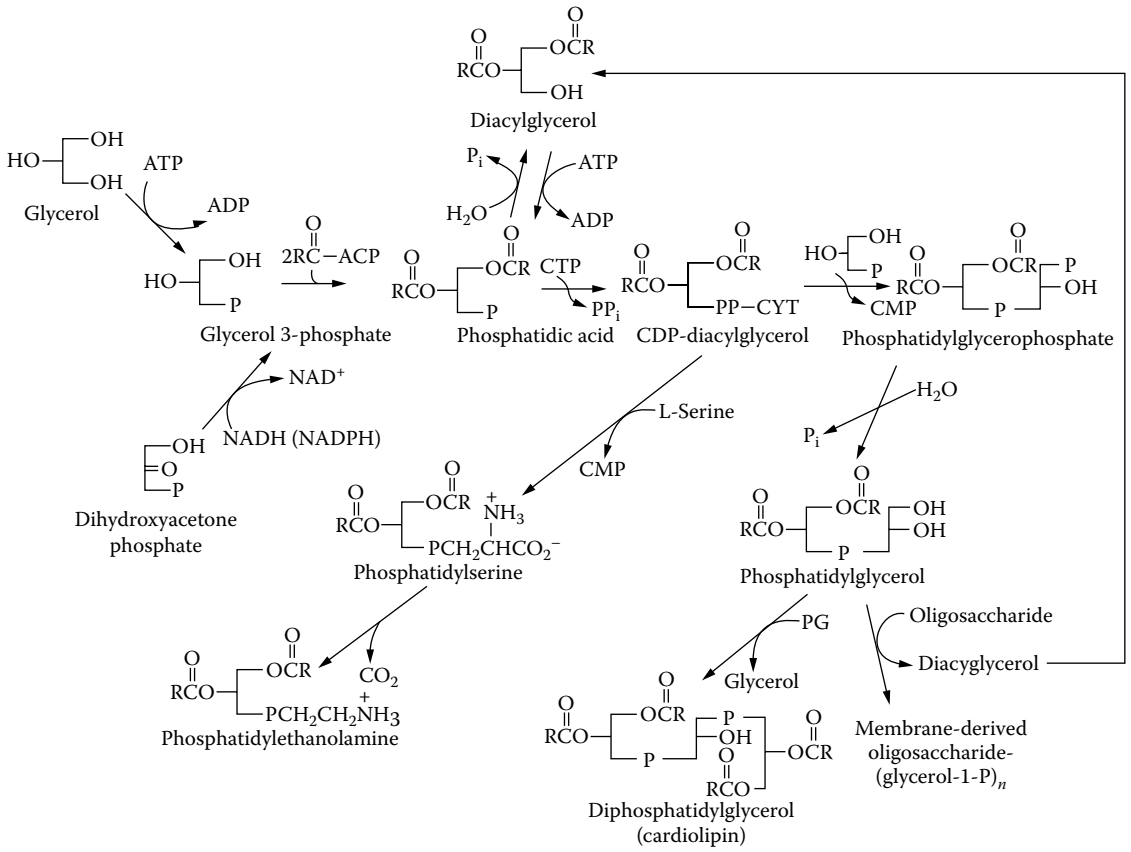


FIGURE 10.28 Phospholipid metabolism in *Escherichia coli*. (From Pieringer (1989).)

CDP-base pathway for phosphatidylethanolamine is not used. And, finally, diphosphatidylglycerol is made by a reaction involving two phosphatidylglycerol molecules (Figure 10.28). See also Gurr et al. (2002) and Heath et al. (2002).

Most research in bacteria has concentrated on *E.coli*, a Gram-negative bacterium with a rather restricted habitat. It cannot be emphasized too strongly that pathways and enzymes studied in *E.coli* may not be typical (or even present) in all bacteria (Harwood and Russell, 1984). The overall pathways for phospholipid synthesis in a Gram-positive bacterium, *Streptococcus falcium*, are shown in Figure 10.29. There has been recent interest in the biosynthesis and occurrence of phosphatidylcholine in bacteria. The occurrence of this lipid in bacteria is thought to be underestimated and, indeed, in *Acetobacter aceti* represents 73% of the phospholipids. Although it was always thought to be made by methylation of phosphatidylethanolamine, many bacteria use a phosphatidylcholine synthase, which is a member of the CDP-alcohol phosphatidyltransferase group of enzymes. Moreover, the CDP-choline pathway *may* be present in some bacteria (Sohlenkamp et al., 2003).

Individual features of lipid metabolism in bacteria and other prokaryotes or microbes are covered by Pieringer (1989), Jackowski et al. (1991), and Cronan (2003). Smith (1993) has reviewed phospholipid synthesis in protozoa.

Methods for the isolation and study of numerous enzymes of phospholipid metabolism are given in Dennis and Vance (1992). A summary of phospholipid biosynthesis in different organisms is found in Gurr et al. (2002) and in various chapters of Vance and Vance (2002).

10.2.2 Breakdown

The breakdown of glycerophospholipids is catalysed by a series of phospholipases designated A, B, C and D depending on their positions of attack (Figure 10.30). In addition, phosphatidic acid phosphatase is important, but this has already been dealt with (Section 10.2.1). Two phospholipase A specificities are recognized, and these are named A_1 and A_2, depending on the position of the ester hydrolysed in the diacylphosphoglyceride. Phospholipase B is the name often used for an enzyme that is a monoacylphosphoglyceride acylhydrolase and can be active at either position. However, they are active on diacylphosphoglycerides, whereas lysophospholipases remove the remaining acyl group from a monoacyl phospholipid (see below for further discussion). For reviews of the basic features of phospholipase action, the reader is referred to Hill and Lands (1970), Dawson (1973), van den Bosch (1982), Dennis (1983), Waite (1987), and Wilton and Waite (2002).

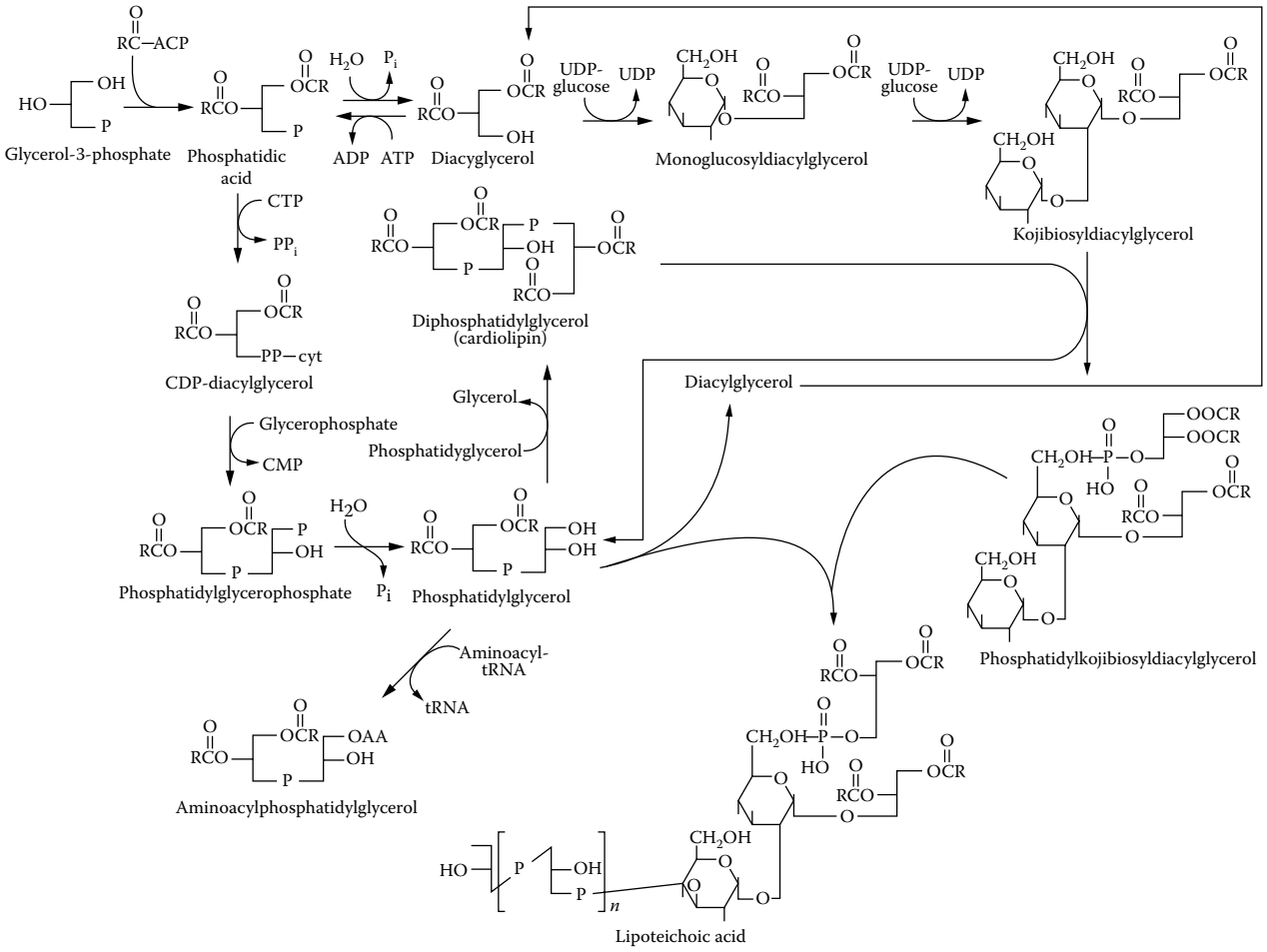


FIGURE 10.29 Lipid metabolism in *Streptococcus faecium* (= *faecalis*) ATCC9790. For simplicity not all hydroxyl groups on glucose are shown. (From Pieringer (1989).)

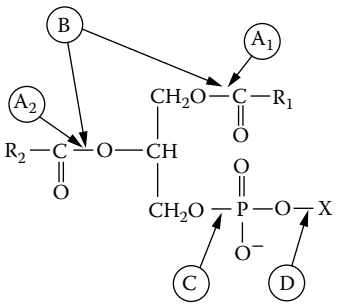


FIGURE 10.30 Position of phosphoglyceride hydrolysis for different phospholipases.

In common with many other lipid-metabolising enzymes, the assay of phospholipases deserves careful consideration. Principally, the potential difficulties are due to the water-insoluble nature of the substrate, the effects of various incubation additions (e.g., solvents, detergents), which can make a big difference to activity, and the problems in kinetic interpretation. Various assay methods have been discussed (Waite, 1987) and an important volume of *Methods in Enzymology* (Dennis, 1991) has been published. The interpretation of the kinetics of phospholipase inhibition has been the subject of considerable attention (see Harwood and John, 1990) and suicide-inhibitory bifunctionally-linked substrates have been developed as phospholipase A_2 inhibitors (Washburn and Dennis, 1991).

Phospholipase A enzymes — particularly those from snake venoms or digestive secretions — have been widely studied. Phospholipase A_1 is found in microsomal and liposomal fractions (cf. Newkirk and Waite, 1971; Gatt, 1968). It specifically deacylates phosphatidylcholine or phosphatidylethanolamine at the 1-position. Both of these substrates are hydrolysed at the same rate by the adrenal medulla lysosomal enzyme, but that from brain prefers phosphatidylcholine as substrate. Detergents will increase phosphatidylethanolamine hydrolysis by the brain enzyme. A phospholipase A_1 (which is relatively specific for phosphatidylglycerol) has been reported from the spores of some bacteria (Raybin et al., 1972), but most bacterial enzymes are unspecific for either the 1- or the 2-positions.

Examples of phospholipase A (unspecific) enzymes from bacteria include the so-called detergent-resistant

enzyme from the outer membrane of *E.* coli and a cytoplasmic enzyme from the same bacterium (Nakagawa et al., 1991). These enzymes differ considerably in their properties (Harwood and Russell, 1984). A number of Gram-negative bacteria have phospholipase A activity in their outer membranes, but it is often not determined whether they are phospholipase A_1 or A_2. Several other lipases have been noted to have phospholipase (usually phospholipase A_1) activity. These include rat hepatic lipase (Waite et al., 1991) and milk lipoprotein lipase (Bengtsson-Olivecrona and Olivecrona, 1991).

The specificity of phospholipase A_2 enzymes varies considerably even among those from snake venoms (see Dawson, 1973). The enzymes remove a fatty acyl group on the glycerol carbon adjacent to the phosphoryl substituent — i.e., position 2 in a natural phosphoglyceride. If the phosphoryl substituent is at the 2-position, then only a fatty acid with the correct steric configuration is hydrolysed. Substrate specificities also vary markedly with the chain length and degree of unsaturation. For the snake venom enzyme hydrolysing phosphatidylcholine, the rate of hydrolysis is in the order: (1-unsat., 2-sat.) > (1-unsat., 2-unsat.) > (1-sat., 2-polyunsat.) > (1-sat., 2-mono-unsat.) > (1-sat., 2-sat.). In contrast, the pancreatic enzyme exhibits no preference for chain length or degree of unsaturation. The hydrolysis of certain phospholipids can be increased in different ways for the various phospholipase A_2 enzymes, e.g., by detergent or diethyl ether addition. This aspect and comments on the underlying physicochemical mechanisms are discussed by Dawson (1973), Slotboom et al. (1982), and Waite (1987), where further references will be found. Specific details of purification and characterization of individual phospholipases are given in Dennis (1991). Receptors for phospholipase A_2 enzymes of various types have been discovered and studied. They are thought to play crucial roles in the physiological actions of phospholipases A_2 (Ohera et al., 1995). Based on sequence data, phospholipase A_2 enzymes from animals are classified into 10 groups, which can be simplified into three major types, based on their physiological properties and function. These are (1) the secretory, low-molecular weight PLA_2; (2) cytosolic Ca^{2+}-dependent PLA_2; and (3) the intracellular Ca^{2+}-independent PLA_2 (Balsinde et al., 1991). Groups (1) and (3) are also reported from plants (Wang, 2001). Enzymes belonging to (1), secretory phospholipase A_2 enzymes from plants and their regulatory and catayltic properties, have been reviewed by Lee et al., (2005).

Because of the ready availability of the pancreatic phospholipase A_2, this enzyme has been thoroughly examined by enzymologists. The phospholipase is secreted as a zymogen and activated by cleavage of a peptide of three to seven amino acids. The amino acid sequence of the active enzyme has been determined for a number of different preparations (pig, ox, horse, and human). The different preparations show a high degree of homology, e.g., the human phospholipase differs from the others in only a single amino acid residue out of 123 to 125 in each case (Verheij et al., 1983). Further details are given in Waite (1987). A gene encoding the cobra venom phospholipase A_2 has been expressed in *E. coli* and the recombinant enzyme shown to have the expected activity characteristics (Kelley et al., 1992).

Lysophospholipases are probably distributed as widely as the phospholipases A (Waite, 1987). A true definition of lysophospholipases is difficult, as discussed by van den Bosch (1982). Indeed, a number of enzymes in this group also have activity against diacyl phospholipids and, therefore, are more properly referred to as phospholipase-B or -A_1 enzymes (van den Bosch et al., 1974). Recent examples are a human phosphatidylserine — specific phospholipase A_1 that also has lysophosphatidylserine lysophospholipase activity (Nagai et al., 1999) and two phospholipase B lysophospholipases from *S. cerevisiae* (Merkel et al., 1999). Also, there are several lysophospholipases that show esterase activity and whose classification is difficult. For more discussion, see Waite (1987) and specific chapters on individual lysophospholipases or the so-called phospholipase B from *Penicillium notatum* in Dennis (1991).

Phospholipase C enzymes are secreted by several bacteria, particularly pathogens, such as *Clostridium* spp. These proteins are zinc metalloenzymes and some also require Ca^{2+} for activity. The phospholipase C enzymes vary widely in substrate specificity (van den Bosch, 1982). The enzyme from *C. perfringens* attacks choline-containing lipids most readily. Such hydrolysis requires a positive zeta-potential brought about by long-chain cations or by the addition of Ca^{2+} or Mg^{2+}. Phosphatidylcholine breakdown in the presence of Ca^{2+} is greatly increased by the addition of specific concentrations of sodium deoxycholate. The phospholipase C from *Bacilus cereus* attacks a large number of phospholipids (e.g., PG, DPG), which are poorly hydrolysed by the *C. perfringens* enzyme. It will also hydrolyse short-chain phosphoglycerides, which are poor substrates for the latter enzyme (Dawson, 1973). The use of phospholipase C enzymes in the preparation of diacylglycerols for molecular species examination is discussed by Christie (1982).

Mammalian phospholipase C enzymes are now known to be central in regulatory processes (Waite, 1987). Most, if not all, of these enzymes are specific for phosphatidylinositol or its phosphorylated derivatives. Specific enzymes are discussed in Dennis (1991) and their role in transmembrane signaling in various organisms (Berridge and Irvine, 1989) and in higher plants (Hetherington and Drobak, 1992) has been reviewed (see also Section 10.6). Specific examples of important functional aspects of such phospholipase C activity are given by Turk et al. (1987) and by Cockcroft (1992) in relation to insulin secretion by pancreatic islets and in neutrophils, respectively.

Plant phospholipase C enzymes can be divided into three groups: (1) enzymes acting on the phosphoinositides,

(2) nonspecific phospholipase Cs that act on phosphatidylcholine and some other phospholipids, and (3) a phospholipase C that hydrolyses glycosylphosphatidylinositol anchors on proteins. The first type of phospholipase C has many important regulatory functions, including responses to stimuli, in plants (Wang, 2001).

There are few examples of phospholipase D (EC 3.1.4.4) in bacteria (Harwood and Russell, 1984; Cockcroft, 1996), but many plant tissues exhibit high activity. Plant phospholipase D is also a transferase enzyme that catalyses transphosphatidylation to an acceptor molecule. If phospholipase D enzymes are not fully inactivated, then this reaction will lead to the artifactual formation of phosphatidylmethanol in plant tissues extracted with methanolic solutions (Harwood, 1980). A variety of water-soluble primary alcohols can be used as acceptors (e.g., propanol, ethanol, ethylene glycol, glycerol). Most enzymes are stimulated in the presence of linear aliphatic ethers (Dawson, 1973; Galliard, 1980). A possible function in plants for phospholipase D in synthesizing phospholipids has now been largely discounted because the phosphatidylglycerol thus made is a racemic mixture, unlike the naturally occurring phospholipid (Yang et al., 1967). However, Batrakov et al. (1975) found evidence for stereospecificity in the transfer. Roughan and Slack (1976) have claimed that phospholipase D is a structural protein that, under certain nonphysiological conditions, possesses enzymic activity.

In plants, phospholipase D (PLD) enzymes can be divided into three groups, based on their properties: (1) a conventional PLD that is most active at 20 to 100 mM Ca^{++}, (2) a polyphosphoinositide-dependent PLD that is active at micromolar Ca^{++}, and (3) a phosphatidylinositol-specific PLD that is independent of Ca^{++}. For *Arabidopsis*, five groups based on gene sequence were identified. PLDs in plants present interesting properties for activation and regulation and appear to play physiological roles in a wide range of stress conditions. These include freezing, drought, wounding, pathogen infection, nutrient deficiency and air pollution (Wang, 2001).

There has been some recent interest in the possible role of mammalian phospholipase D in signal transduction, in that a wide variety of agents (including hormones, neurotransmitters and growth factors) have been shown to activate a phospholipase D to hydrolyse phosphatidylcholine (see e.g., Hurst et al., 1990; Xie and Dubyak, 1991). Various GTPases are known to activate the enzyme in mammals and its physiological relevance has been discussed (Cockcroft, 1996). This article also provides information on the enzymology of phospholipase Ds from different organisms and earlier reviews on the subject. Yamane et al. (1989) discuss the commercial use of phospholipase D enzymes for transphosphatidylation purposes.

Phosphatidate phosphohydrolase, although a phospholipid degradative enzyme, is mainly involved in the biosynthesis of lipids (Gurr and Harwood, 1991). In mammalian tissues the enzyme may control the overall rate of lipid (triacylglycerol) deposition. A comprehensive review of the enzyme in different organisms and tissues has been published by Brindley (1988) (see also Dennis, 1991).

Many plant tissues possess high activities of nonspecific lipid acyl hydrolases, which can also give rise to problems with the solvent extraction of plants. Acyl hydrolases will hydrolyse fatty acids from a large number of lipids including phosphoglycerides (cf. Galliard, 1980) The enzymes have been purified from several tissues and their substrate specificities and other properties determined. Potato tubers and leaves from *Phaseolus* spp. are particularly rich sources (cf. Galliard, 1980) and the position of bond cleavage has been determined for the latter enzyme to be on the fatty acid side of the oxygen ester bond (Burns et al., 1980). An important example of a plant acyl hydrolase is patatin, which is a storage protein from potato tubers. Patatin-like proteins are produced in a number of plants in response to stresses, such as virus or fungal infection (Meijer and Munnik, 2003).

For an overall review of the functions of phospholipases in such areas as the phosphatidylinositol cycle, the archidonate cascade, in the digestion of dietary fat in lipoprotein metabolism and in snake venoms, see Waite (1987) and also Vance (1991). Phospholipase A_2 has also been invoked in the protection of membranes from lipid peroxidation damage (van Kuijk et al., 1987).

References

Amidon, B. et al. (1995). Biosynthetic conversion of phosphatidylglycerol to *sn*-1:*sn*-1 bis(monoacylglycero) phosphate in a macrophage-like cell line. *Biochemistry.* **34**, 5554–5560.

Ansell, G.B. and Spanner, S. (1982). Phosphatidylserine, phosphatidylethanolamine and phosphatidylcholine. In *Phospholipids*, Eds. J.N. Hawthorne and G.B. Ansell, Elsevier, Amsterdam, pp. 1–49.

Balsinde, J. et al. (1999). Regulation and inhibition of phospholipase A_2. *Annu. Rev. Pharmacol. Toxicol.* **39**, 175–189.

Batrakov, S.G. et al. (1975). Steric analysis of glycerophospholipids by circular dichroism. Stereospecificity of phospholipase D catalyzed transesterification. *Biochem. Biophys. Res. Commun.,* **66,** 755–762.

Bell, R.M. and Coleman, R.A. (1980). Enzymes of glycerolipid synthesis in eukaryotes. *Annu. Rev. Biochem.* **49**, 459–487.

Bengtsson-Olivecrona, G. and Olivecrona, T. (1991). Phospholipase activity of milk lipoprotein lipase. In *Methods in Enzymology,* vol. 197, Ed. E.A. Dennis, Academic Press, New York, pp. 345–356.

Berridge, M.J. and Irvine, R.F. (1984). Inositol triphosphate, a novel second messenger in cellular signal transduction. *Nature,* **312**, 315–321.

Berridge, M.J. and Irvine, R.F. (1989). Inositol phosphates and cell signalling. *Nature,* **341**, 197–205.

Bloch, K. and Vance, D. (1977). Control mechanisms in the synthesis of saturated fatty acids *Annu. Rev. Biochem.* **46**, 263–298.

Brindley, D.N. Ed. (1988) *Phosphatidate Phosphohydrolase,* vols. I and II, CRC Press, Boca Raton, FL.

Brindley, D.N. (1991). Metabolism of triacylglycerols. In *Biochemistry of Lipids, Lipoprotein and Membranes*, Eds. D.E. Vance and J. Vance, Elsevier, Amsterdam, pp. 171–203.

Brindley, D.N. and Sturton, R.G. (1982). Phosphatidate metabolism and its relation to triglycerol biosynthesis. In *Phospholipids*, Eds. J.N. Hawthorne and G.B. Ansell, Elsevier, Amsterdam, pp. 179–213.

Brophy, P.J. and Vance, D.E. (1976). Copurification of choline kinase and ethanolamine kinase from rat liver by affinity chromatography. *FEBS Lett.,* **62**, 123–125.

Browse, J. and Somerville, C.R. (1991). Glycerolipid synthesis: biochemistry and regulation. *Annu. Rev. Plant Physiol.,* **42**, 467–506.

Burns, D.D. et al. (1980). Properties of acyl hydrolase enzymes from *Phaseolus multiflorus* leaves. *Phytochemisty.* **19**, 2281–2285.

Carman, G.M. and Henry, S.A. (1989). Phospholipid biosynthesis in yeast. *Annu. Rev. Biochem.,* **58**, 635–669.

Carman, G.M. and Henry, S.A (1999). Phospholipid biosynthesis in the yeast, *Saccharomyces cerevisiae* and interrelationship with other metabolic processes. *Prog. Lipid Res.* **38**, 361–399.

Chapman, K.D. (2004). Occurrence, metabolism and prospective functions of *N*-acylethanolamines in plants. *Prog. Lipid Res.* **43**, 302–327.

Christie, W.W. (1982) *Lipid Analysis,* 2nd ed., Pergamon, Oxford.

Cockcroft, S. (1992). G-protein-regulated phospholipases C, D and A_2-mediated signalling in neutrophils. *Biochim. Biophys. Acta,* **1113**, 135–160.

Cockcroft, S. (1996) Phospholipase D: regulation by GTPases and protein kinase C and physiological relevance. *Prog. Lipid Res.* **35**, 345–370.

Cronan, J.E. (2003). Bacterial membrane lipids: where do we stand? *Ann. Rev. Microbiol.* **57**, 203–224.

Dawson, R.M.C. (1973). Specificity of enzymes involved in the metabolism of phospholipids. In *Form and Function of Phospholipids*, Eds. G.B. Ansell, R.M.C. Dawson and J.N. Hawthorne, Elsevier, Amsterdam, pp. 97–116.

Dennis, E.A. (1983). Phospholipases. In *The Enzymes,* vol. XVI, Ed. P. Boyer, Academic Press, New York, pp. 307–353.

Dennis, E.A. Ed. (1991) *Methods in Enzymology,* vol. 197, Academic Press, New York.

Dennis, E.A. and Vance, D.E. Eds. (1992) *Methods in Enzymology,* vol. 209, Academic Press, New York.

Dormann, P. (2005). Membrane lipids. In *Plant Lipids: biology Utilisation and Manipulation*, Ed. D.J. Murphy, Blackwell Publishing, Oxford, U.K., pp. 123–161.

Downes, C.P and MacPhee, C.H. (1990). Myo-inositol metabolites as cellular signals. *Eur. J. Biochem.* **193**, 1–18.

Drobak, B.K. (2005) Inositol-containing lipids: roles in cellular signalling. In *Plant Lipids: Biology, Utilisation and Manipulation*, Ed. D.J. Murphy, Blackwell Publishing, Oxford, U.K.. pp. 303–328.

Feldman, D.A. and Weinhold, P.A. (1987). CTP: phosphorylcholine cytidylyltransferase from rat liver. Isolation and characterization of the catalytic subunit. *J. Biol.Chem.* **262**, 9075–9081.

Fruman, D.A. et al. (1998). Phosphoinositide kinases. *Annu. Rev. Biochem.* **67**, 481–507.

Galliard, T. (1980). Degradation of acyl lipids: hydrolytic and oxidative enzymes. In *Biochemistry of Plants,* vol. 4, Eds. P.K. Stumpf and E.E. Conn, Academic Press, New York, pp. 85–116.

Gatt, S. (1968). Purification and properties of phospholipase A-1 from rat and calf brain. *Biochim. Biophys. Acta.* **159**, 304–316.

Groot, P.H.E. et al. (1976). Fatty acid activation: specificity, localization, and function. *Adv. Lipid Res.* **14**, 75–126.

Gurr, M.I. and Harwood, J.L. (1991). *Lipid Biochemistry,* 4th ed., Chapman & Hall, London.

Gurr, M.I. et al. (2002). *Lipid Biochemistry*, 5th ed., Blackwell Scientific, Oxford, U.K.

Hajra, A.K. (1995). Glycerolipid biosynthesis in peroxisomes (microbodies). *Prog. Lipid Res.* **34**, 343–364.

Harwood, J.L. (1979). The synthesis of acyl lipids in plant tissues. *Prog. Lipid Res.* **18**, 55–86.

Harwood, J.L. (1980). Plant acyl lipids: structure, distribution and analysis. In *Biochemistry of Plants,* vol. 4, Eds. P.K. Stumpf and E.E. Conn, Academic Press, New York, pp. 1–55.

Harwood, J.L. (1989). Lipid metabolism in plants. *Crit. Rev. Plant Sci.* **8**, 1–43.

Harwood, J.L. and John, R.A. (1990). Evaluation of inhibitors of lipolytic enzymes. *Trends Biochem. Sci.* **15**, 409–410.

Harwood, J.L. and Page, R. (1993). Biochemistry of oil synthesis. In *Designer Oilseed Crops*, Ed. D.J. Murphy, VCH Press, Weinheim, Germany, pp. 165–194.

Harwood, J.L., and Russell, N.J. (1984) *Lipids in Plants and Microbes,* Allen and Unwin, Hemel Hempstead, U.K.

Hawthorne, J.N. and Ansell, G.B. Eds. (1982) *Phospholipids,* Elsevier, Amsterdam.

Heath, R.J. et al. (2002). Fatty acid and phospholipid synthesis in prokaryotes. In *Biochemistry of Lipids, Lipoproteins and Membranes*, Eds. D.E. Vance and J.E. Vance, 4th ed., Elsevier, Amsterdam, pp. 55–92.

Heller, M. (1978). Phospholipase D. *Adv. Lipid Res.,* **16**, 267–326.

Hetherington, A.M. and Drobak, B.K. (1992). Inositol-containing lipids in higher plants *Prog. Lipid Res.* **31**, 53–63.

Hill, E.E. and Lands, W.E.M. (1970). Phospholipid metabolism. In *Lipid Metabolism*, Ed. S.J. Wakil, Academic Press, New York, pp. 185–275.

Huang, K.-S., Li, S. and Low, M.G. (1991). Glycosylphosphatidylinositol-specific phospholipase D. In *Methods in Enzymology,* vol. 197, Ed. E.A. Dennis, Academic Press, New York, pp. 567–575.

Hurst, K.M. et al. (1990). The roles of phospholipase D and a GTP-binding protein in guanosine 5'-[gamma-thio]triphosphate-stimulated hydrolysis of phosphatidylcholine in rat liver plasma membranes. *Biochem. J.* **272**, 749–753.

Jackowski, S. et al. (1991). Lipid metabolism in prokaryotes. In *Biochemistry of Lipids, Lipoproteins and Membranes*, Eds. D.E. Vance and J. Vance, Elsevier, Amsterdam, pp. 43–85.

Jackson, R.L. and McLean, L.R. (1991). Human postheparin plasma lipoprotein lipase and hepatic triglyceride lipase. In *Methods in Enzymology,* vol. 197, Ed. E.A. Dennis, Academic Press, New York, pp. 339–345.

Joyard, J. and Douce, R. (1987). Galactolipid synthesis. In *The Biochemistry of Plants,* vol. 9, Eds. P.K. Stumpf and E.E. Conn, Academic Press, New York, pp. 215–274.

Kelley, M.J. et al. (1992). Renaturation of cobra venom phospholipase A2 expressed from a synthetic gene in *Escherichia coli*. *Biochim. Biophys. Acta.* **1118**, 107–115.

Kent, C. (1995). Eukaryotic phospholipid biosynthesis. *Annu. Rev. Biochem.* **64**, 315–343.

Kobayashi, M. and Kanfer, J.N. (1991). Solubilization and purification of rat tissue phospholipase D. In *Methods in Enzymology*, vol. 197, Ed. E.A. Dennis, Academic Press, New York, pp. 575–583.

Kuge, O. and Nishijima, M. (1997). Phosphatidylserine synthases I and II of mammalian cells. *Biochim. Biophys. Acta.* **1348**, 151–156.

Lee, H.Y. et al. (2005). Multiple forms of secretory phospholipase A_2 in plants. *Prog. Lipid Res.* **44**, 52–67.

MacDonald, J.I.S. and Sprecher, H. (1991). Phospholipid fatty acid remodeling in mammalian cells. *Biochim. Biophys. Acta.* **1084**, 105–121.

Martin, T.F.J. (1998). Phosphoinositide lipids as signalling molecules: common themes for signal transduction, cytoskeletal regulation and membrane trafficking. *Annu. Rev. Cell Dev. Biol.***14**, 231–264.

McPhail, L.C. (2005). Glycerolipids in signal transduction. In *Biochemistry of Lipids, Lipoproteins and Membranes*, Ed. D.E. Vance and J.E. Vance, 4th ed., Elsevier, Amsterdam, pp. 315–340.

Meijer, H.J.G. and Munnik, T. (2003). Phospholipid-based signalling in plants. *Annu. Rev. Plant Biol.* **54**, 265–306.

Merkel. O. et al. (1999). Characterisation and function *in vivo* of two novel phospholipases B lysophospholipases from *Sacharomyces cerevisiae*. *J. Biol. Chem.* **274**, 28121–28127.

Moore, T.M. (1982). Phospholipid biosynthesis. *Annu. Rev. Plant Physiol.* **33**, 235–259.

Mudd, J.B. (1980). Phospholipid biosynthesis. In *Biochemistry of Plants,* vol. 4, Eds. P.K. Stumpf and E.E. Conn, Academic Press, New York, pp. 249–282.

Mudd, S.H. and Datko, A.H. (1986). Phosphoethanolamine bases as intermediates in phosphatidylcholine synthesis by *Lemna. Plant Physiol.* **82**, 126–135.

Munnik, T. et al. (1998). Phospholipid signalling in plants. *Biochim. Biophys. Acta.* **1389**, 222–272.

Nagai, Y. et al. (1999). An alternative splicing form of phosphatidylserine-specific phospholipase A1 that exhibits lysophosphatidylserine-specific lysophospholipase activity in humans. *J. Biol. Chem.* **274**, 11053–59.

Nakagawa, Y. et al. (1991). Detergent-resistant phospholipase A_1 from *Escherichia coli* membranes. In *Methods in Enzymology,* vol. 197, Ed. E.A. Dennis, Academic Press, New York, pp. 309–315.

Newkirk, J.D. and Waite, M. (1971). Identification of a phospholipase A1 in plasma membranes of rat liver. *Biochim. Biophys. Acta.* **225**, 224–233.

O'Doherty, P.J.A. (1978). Metabolic studies with natural and synthetic fatty acids and enantiomeric acylglycerols. In *Handbook of Lipid Research,* vol. 1, Ed. A. Kuksis, Plenum, New York, pp. 289–339.

Ohara, O. et al. (1995). Structure and function of phospholipase A_2 receptor. *Prog. Lipid Res.* **34**, 117–138.

Pieringer, R.A. (1989). Biosynthesis of non-terpenoid lipids. In *Microbial Lipids,* vol. 2, Eds. C. Ratledge and S.G. Wilkinson, Academic Press, London, pp. 51–114.

Price-Jones, M.J. and Harwood, J.L. (1986). The control of CTP:choline phosphate cytidylyltransferase activity in pea (*Pisum sativum* L.). *Biochem. J.* **240**, 837–842.

Rameh, L.E. and Cantely, L.C. (1999). The role of phosphoinositide 3-kinase lipid products in cell function. *J. Biol. Chem.* **274**, 8347–8350.

Raybin, D.M. et al. (1972). A phospholipase in *Bacillus megaterium* unique to spores and sporangia. *Biochemistry.* **11**, 1754–1760.

Ridgeway, N.D. et al. (1999). Integration of phospholipid and sterol metabolism in mammalian cells. *Prog. Lipid. Res.*, **38**, 337–360.

Roughan, P.G. and Slack, C.R. (1976). Is phospholipase D really an enzyme? A comparison of *in situ* and *in vitro* activities. *Biochim. Biophys. Acta.* **431**, 86–95.

Roughan, P.G. and Slack, C.R. (1984). Glycerolipid synthesis in leaves. *Trends Biochem. Sci.* **9**, 383–386.

Sambanthamurthi, R. et al. (2002). Chemistry and biochemistry of palm oil. *Prog. Lipid Res.,* **39**, 507–558.

Schlame, M. et al. (2000). The biosynthesis and functional role of cardiolipin. *Prog. Lipid Res.* **39**, 257–288.

Slotboom, A.J. et al. (1982). On the mechanism of phospholipases A_2. In *Phospholipids*, Eds. J.N. Hawthorne and G.B. Ansell, Elsevier, Amsterdam, pp. 359–434.

Smith, J.D. (1993). Phospholipid synthesis in protozoa. *Prog. Lipid Res.,* **32**, 47–60.

Snyder, F. (1991). Metabolism, regulation, and function of ether-linked glycerolipids and their bioactive species. In *Biochemistry of Lipids, Lipoproteins and Membranes*, Eds. D.E. Vance and J. Vance, Elsevier, Amsterdam, pp. 241–267.

Snyder, F., et al. (1985). Ether-linked glycerolipids and their bioactive species: enzymes and metabolic regulation. In *The Enzymes of Biological Membranes*, Ed. A.N. Martinosi, Plenum, New York, pp. 1–58.

Snyder, F. et al. (2002). Ether-linked lipids and their bioactive species. In *Biochemistry of Lipids, Lipoproteins and Membranes*, Eds. D.E. Vance and J.E. Vance, 4th ed., Elsevier, Amsterdam, pp. 233–262.

Sohlenkamp, C. et al. (2003). Biosynthesis of phosphatidylcholine in bacteria. *Prog. Lipid Res.*, **42**, 115–162.

Taki, T. et al. (1978). The "base-exchange" reaction: the serine enzyme. *Adv. Exp. Med. Biol.* **101**, 301–318.

Turk, J. et al. (1987). The role of phospholipid-derived mediators including arachidonic acid, its metabolites, and inositoltrisphosphate and of intracellular Ca^{2+} in glucose-induced insulin secretion by pancreatic islets. *Prog. Lipid Res.* **26**, 125–181.

Ulane, R.E. et al. (1977). A rapid accurate assay for choline kinase. *Anal. Biochem.* **79**, 526–534.

van den Bosch, H. (1982). Phospholipases. In *Phospholipids*, Eds. J.N. Hawthorne and G.B. Ansell, Elsevier, Amsterdam, pp. 313–357.

van den Bosch, H. et al. (1974). Isolation and properties of a phospholipase A_1 activity from beef pancreas. *Biochim. Biophys. Acta.* **348**, 197–209.

van Kuijk, F.J.G.M. et al. (1987). A new role for phospholipase-A_2 — protection of membranes from lipid-peroxidation damage. *Trends Biochem. Sci.* **12**, 31–34.

Vance, D.E. (1990). Phosphatidylcholine metabolism: masochistic enzymology, metabolic regulation and lipoprotein assembly. *Biochem. Cell Biol.* **68**, 1151–1165.

Vance, D.E. (1991). Phospholipid metabolism and cell signalling in eukaryotes. In *Biochemistry of Lipids, Lipoproteins and Membranes*, Eds. D.E. Vance and J. Vance, Elsevier, Amsterdam, pp. 205–240.

Vance, D.E. (2005). Phospholipid biosynthesis in eukaryotes. In *Biochemistry of Lipids, Lipoproteins and Membranes*, Eds. D.E. Vance and J.E. Vance, 4th ed., Elsevier, Amsterdam, pp. 205–232.

Vance, D.E. and Choy, I.C. (1979). How is phosphatidylcholine biosynthesis regulated? *Trends Biochem.Sci.* **4**,145–148.

Vance, D.E. and Ridgway, N.D. (1988). The methylation of phosphatidylethanolamine. *Prog. Lipid Res.* **27**, 61–79.

Vance, D.E. and Vance, J.E. Eds. (2002). *Biochemistry of Lipids, Lipoproteins and Membranes.* Elsevier, Amsterdam.

Vance, J.E. (1998). Eukaryotic lipid biosynthetic enzymes: the same but not the same. *Trends Biochem. Sci.* **23**, 423–428.

Vanhaesebroeck, B. et al. (2001). Synthesis and function of 3-phosphorylated inositol lipids. *Annu. Rev. Biochem.***70**, 535–602.

Verheij, H.M. et al. (1983). The complete primary structure of phospholipase A_2 from human pancreas. *Biochim. Biophys. Acta.* **747**, 93–99.

von Wettstein-Knowles, P. (1979). Genetics and biosynthesis of plant epicuticular waxes. In *Advances in the Biochemistry and Physiology of Plant Lipids*, Eds. L-A Appelqvist and C. Liljenberg, Elsevier, Amsterdam, pp. 1–26.

Waite, M. (1987). The phospholipases. In *Handbook of Lipid Research,* vol. 5, Ed. D.J. Hanahan, Plenum, New York, pp. 111–133.

Waite, M. et al. (1991). Purification and substrate specificity of rat hepatic lipase. In *Methods in Enzymology,* vol. 197, Ed. E.A. Dennis, Academic Press, New York, pp. 331–339.

Waku, (1992). Origins and fates of fatty acyl-CoA esters. *Biochim. Biophys. Acta.* **1124**, 101–111.

Wang, X. (2001). Plant phospholipases. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **52**, 211–231.

Washburn, W.N. and Dennis, E.A. (1991). Suicide-inhibitory bifunctionally linked substrates (SIBLINKS) as phospholipase A2 inhibitors. Mechanistic implications. *J. Biol. Chem.* **266**, 5042–5048.

Webber, K.O. and Hajra, A.K. (1992). Dihydroxyacetone phosphate acyltransferase. In *Methods in Enzymology,* vol. 209, Eds. E.A. Dennis and D.E. Vance, Academic Press, New York, pp. 92–98.

Williams, M. and Harwood, J.L. (1994). Alternative pathways of phosphatidylcholine synthesis in olive (*Olea europeea* L.) callus cultures. *Biochem. J.* **304**, 463–468.

Wilton, D.C. and Waite, M. (2002). Phospholipases. In *Biochemistry of Lipids, Lipoproteins and Membranes*, Eds. D.E. Vance and J.E. Vance, 4th ed., Elsevier, Amsterdam, pp. 291–314.

Xie, M. and Dubyak, G.R. (1991). Guanine-nucleotide- and adenine-nucleotide-dependent regulation of phospholipase D in electropermeabilized HL-60 granulocytes. *Biochem. J.* **278**, 81–89.

Yamane, T. et al. (1989). Conversion of phospholipids by phospholipase D. In *Fats for the Future* (Ed. R.C. Cambie), Ellis Horwood, Chichester, U.K., pp. 128–138.

Yang, S.F. et al. (1967). Transphosphatidylation by phospholipase D. *J. Biol. Chem.* **242**, 477–484.

10.3 Glyceride metabolism

10.3.1 Triacylglycerol synthesis

The lipid stores of animals and almost all plants are triacylglycerols. These compounds, therefore, are of very great importance in physiology as well as to the food industry. One difference between animals and plants is in the relative amounts of *de novo* synthesized acyl chains in the triacylglycerols. Plants from necessity must make all of the glyceride molecules from simple starting materials (ultimately from photosynthetically fixed CO_2). In contrast, animals make use of dietary fatty acids, which to a large degree determine the fatty acid composition of the triacylglycerol stores (e.g., copepods consuming phytoplankton; Bauermeister and Sargent, 1979).

In oil seeds the triacylglycerol stores are located in oil bodies. These intracellular structures are surrounded by a half-unit phospholipid membrane containing unique proteins called oleosins. The latter have been isolated and sequenced from a number of plants and probably serve both to stabilize the oil bodies as well as to provide binding sites for lipases to function during germination (for reviews, see Murphy, 1990; Huang, 1992).

In animals most triacylglycerol is stored in adipose tissue, though, in times of metabolic stress, significant amounts may build up in liver, heart and skeletal muscle. Also, it should be noted that the variable distribution of adipose tissue stores and the metabolic properties of such tissue in different compartments of the body has important implications for health (Abate and Garg, 1995). The formation of triacylglycerols removes the potentially harmful effects of fatty acids or fatty acyl-CoAs (Brindley, 1991). A major function of animal triacylglycerols is to allow the transport of acyl moieties about the body, in the form of the serum lipoproteins. Two major classes of lipoprotein are relevant. Chylomicrons carry absorbed dietary fat from the intestine to other organs, while very low density lipoproteins carry triacylglycerol from the liver to other tissues (Section 7.5 and Section 11.2).

There has been considerable recent interest in the use of oleaginous microorganisms as sources of triacylglycerols, particularly those with unusual fatty acids. In general, yeasts and moulds offer the best possibilities for industrial exploitation, and few bacteria accumulate appreciable amounts of triacylglycerols (actinomycetes are the most noticeable exceptions). The whole topic of the biotechnology of oils and fats in microorganisms is reviewed by Ratledge (1989) and Cohen and Ratledge (2005). See also Davies and Holdsworth (1992).

The major pathways for triacylglycerol synthesis are shown in Figure 10.31. The formation of phosphatidic acid by the glycerol phosphate pathway or the dihydroxyacetone phosphate pathway has already been discussed (Section 10.2.1). In addition, see reviews by Gurr (1980) and O'Doherty (1978). Phosphatidic acid can also be formed from monoacylglycerol or diacylglycerol, but, as will be seen, this is not of relevance for triacylglycerol synthesis. Attempts to estimate the relative contribution of different pathways for phosphatidic acid formation (cf. Hill and Lands, 1970; O'Doherty, 1978) indicate that about 50% of lipid glycerol enters by the dihydroxyacetone pathway (e.g., Manning and Brindley, 1972; Pollock et al., 1975). In some tissues (e.g., Ehrlich ascites tumour cells), the acyldihydroxyacetone pathway is the major route of synthesis (Synder, 1972).

The enzymes responsible for the acylation of glycerol phosphate have been purified and studied many times. For reviews of their purification and properties, see Lennarz (1970), Hill and Lands (1970), O'Doherty (1978), Gurr (1980), and Stymne and Stobart (1987). The glycerol 3-phosphate acyltransferase and monoacylglycerol 3-phosphate acyltransferase enzymes in both animals and plants differ in their substrate specificities. Typically, the glycerol 3-phosphate acyltransferase uses more saturated acyl-CoAs than are subsequently attached to the *sn*-1 position. The major site of both enzymes is the endoplasmic reticulum, though animal mitochondria (particularly those of liver) also contain a second glycerol 3-phosphate acyltransferase (Brindley, 1991). In plant chloroplasts, glycerol 3-phosphate acylating enzymes (which use acyl-ACP substrates and have different substrate specificities to their endoplasmic reticulum counterparts) are present. Although triacylglycerol synthesis can take place in chloroplasts (particularly under adverse environmental conditions; see Sakaki et al., 1985), the chloroplast enzymes are mainly important for the generation of thylakoid acyl lipids (Ohlrogge et al., 1991). For the dihydroxyacetone pathway (see Figure 10.31), acyltransferases have been found in both the endoplasmic reticulum and in peroxisomes (Brindley, 1991).

As indicated in Figure 10.31, diacylglycerol can originate from two sources. Either it is formed by phosphatidate phosphohydrolase or it is synthesised from monoacylglycerol. The first demonstration of an enzyme system capable of converting monoacylglycerols to triacylglycerols was the work of Clark and Hübscher (1960) with preparations from rabbit intestine. Indeed, the reactions have been studied most thoroughly in intestine, although activity has been detected in a large number of mammalian tissues including kidney, pancreas, adipose tissue, arterial walls, ascites tumour cells and mammary glands (cf. O'Doherty, 1978; Brindley, 1991). The first enzyme in the pathway is monoacylglycerol acyltransferase (EC 2.3.1.22). The enzyme is particulate and is found in microsomal fractions — probably arising from the endoplasmic reticulum. Evidence for the monoacylglycerol pathway in plants is poor, and it seems probable that diacylglycerol arises from phosphatidate phosphatase in these phyla (Gurr, 1980). For comments on the extensive literature concerning the substrate specificities and other properties of mammalian monoacylglycerol acyltransferases, refer to O'Doherty (1978) and Dircks and Sul (1999).

Phosphatidate phosphohydrolase has already been briefly discussed in relation to glycerophospholipid synthesis (Section 10.2.1). The enzyme was first discovered in plants (Kates, 1955) and, subsequently, identified in animal tissues. It has been purified from a large number of mammalian tissues including liver, kidney, intestinal mucosa, adipose tissue, erythrocyte membranes, and avian salt glands (cf. O'Doherty, 1978). The intracellular distribution of mammalian phosphatidate phosphohydrolase is complicated. It has been found in several particulate fractions — mitochondrial, lysosomal, and microsomal (cf. Sedgwick and Hübscher, 1967). However, quantitatively, the most important of these fractions is the endoplasmic reticulum. In addition, there is significant cytosolic activity in animals. The cytosolic phosphatidate phosphohydrolase is able to translocate to the endoplasmic reticulum and mitochondria. The response of such a Mg^{2+}-dependent enzyme to hormones and metabolites and variations in its intracellular distribution between soluble and particulate compartments, are compatible with an important role in controlling triacylglycerol synthesis. For a full discussion of this function, see Brindley (1988, 1991).

In plants, phosphatidate phosphohydrolase has a less obvious role in controlling the rate of triacylglycerol synthesis, since the latter occurs usually at a specific developmental period when it is not in competition with other metabolic pathways, such as membrane lipid synthesis (Harwood and Griffiths, 1992). The enzyme has been detected in several subcellular compartments (see Harwood and Price-Jones, 1988), and, moreover, its activity in chloroplast envelopes appears to be important in controlling the flux of lipid carbon between the extrachloroplastic and the chloroplast compartments. This is important in giving rise to different molecular species of lipids in various plants (see Heinz and Roughan, 1987; Ohlrogge et al., 1991). There have been some suggestions that the activity of phosphatidate phosphohydrolase may be important in triacylglycerol accumulation in seeds (Ichihara et al., 1990) even though the enzyme, unlike in animals, is not under acute (hormonal) control.

Synthesis of triacylglycerols requires the enzyme diacylglycerol acyltransferase (EC 2.3.1.30). The enzyme is located in the endoplasmic reticulum (Brindley, 1991) and its activity is especially high in lypogenic tissues like adipose and liver. Its properties and those of other acyltransferases involved in the glycerol phosphate pathway for triacylglycerol formation were reviewed by Dircks and Sul (1991). Diacylglycerol acyltransferase is unique to

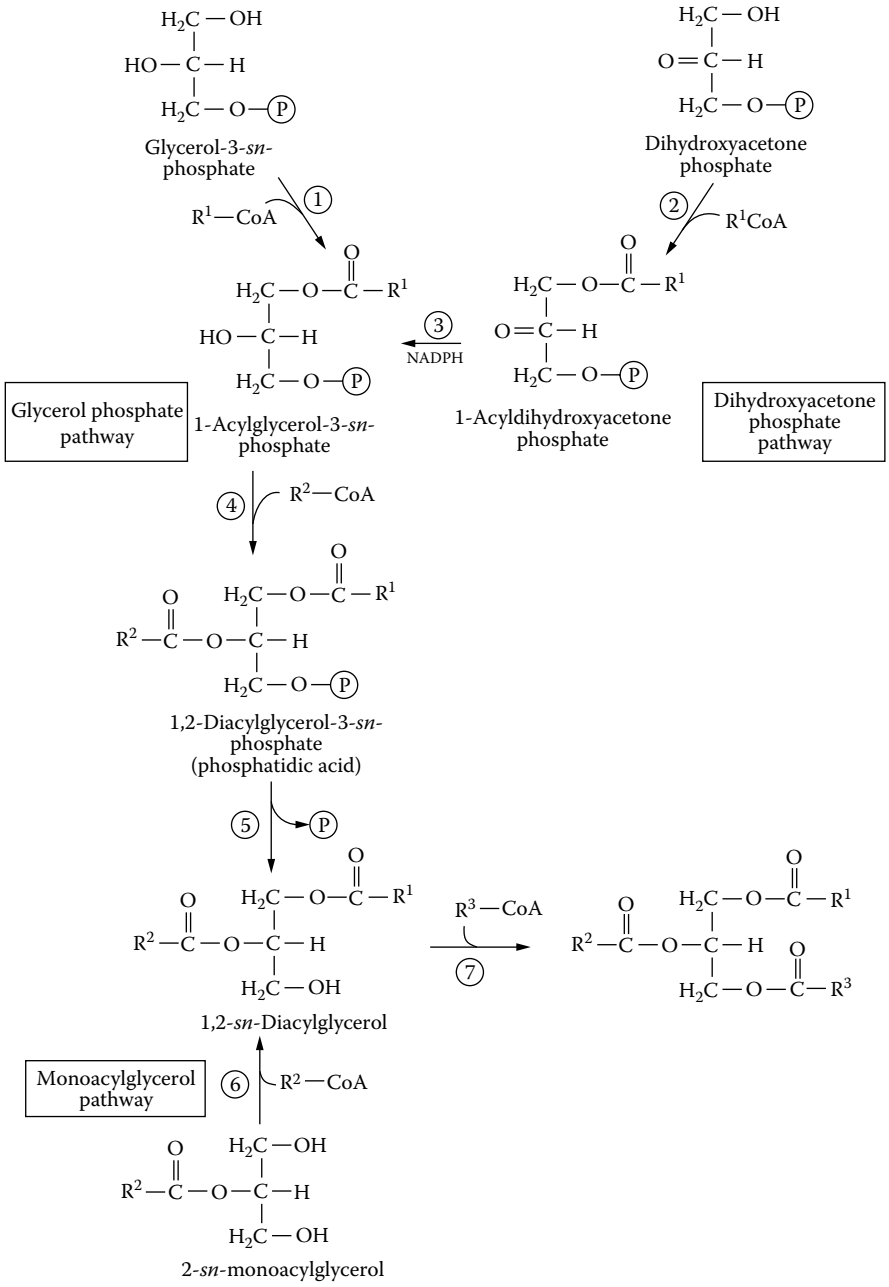


FIGURE 10.31 Major pathways of triacylglycerol synthesis. Reactions (1), (4), (5), and (7), glycerol phosphate pathway; reactions (2), (3), (4), (5), and (7), dihydroxyacetone phosphate pathway; reactions (6) and (7), monoacylglycerol pathway. Enzymes: (1) glycerol phosphate 1-acyltransferase; (2) dihydroxyacetone phosphate acyltransferase; (3) acyldihydroxyacetone phosphate reductase; (4) 1-acylglycerol phosphate 2-acyltransferase; (5) phosphatidate phosphohydrolase; (6) monoacylglycerol acyltransferase; (7) diacylglycerol acyltransferase.

triacylglycerol synthesis and, as such, might be expected to be important for regulation, at least under certain conditions (Mayorek et al., 1989). So far as can be ascertained, it competes with the choline and ethanolamine phosphotransferases (that form zwitteronic phospholipids; Section 10.2.1) for a common pool of diacylglycerol (Brindley, 1991). Diacylglycerol acyltransferase can use a wide range of saturated and unsaturated acyl-CoAs (Dircks and Sul, 1991), but has considerably better activity with its natural 1,2-diacylglycerol substrate than the 1,3-isomer (Hill et al., 1968).

The diacylglycerol acyltransferase of plants has been studied in several tissues, though not yet purified. In many plants, it seems less specific for its acyl-CoA substrate than are the other acytransferases (Stymne and Stobart, 1987). It is located in endoplasmic reticulum and has low activity when measured *in vitro* (e.g., Berneth and Frentzen, 1990). The latter fact, together with the significant buildup of

diacylglycerol during triacylglycerol formation *in vivo* (Perry and Harwood, 1990) and *in vitro* (Perry and Harwood, 1991), has led to suggestions that diacylglycerol acyltransferase may exert significant flux control over oil accumulation in some crops (see, e.g., Griffiths and Harwood, 1990).

Apart from diacylglycerol acyltransferase being used to form triacylglycerol, evidence for acyl-CoA independent pathways has been obtained in animal tissues and oilseeds. Thus, in mammals, a diacylglycerol transacylase has been reported (Lehner and Kuksi, 1996). In oilseeds, the same enzyme activity is also present, but, in addition, phospholipid: diacylglycerol acyltransferase is found (see Weselake, 2005), although the quantitative importance of the latter in many tissues seems to be slight (see Ramli et al., 2005).

Mammalian triacylglycerol synthesis is affected by a large number of factors. These include nutritional, hormonal, and pharmacological effects (cf. O'Doherty, 1978, for review). A discussion of the control of triacylglycerol synthesis in animals is given by Brindley (1991).

General accounts of triacylglycerol synthesis in plants are given by Gurr (1980), Stymne and Stobart (1987), Harwood and Page (1993), and Weselake (2005). Specialist accounts of lipid synthesis in the two important oil crops, olive and palm, are given in Salas et al. (2000) and Sambanthamurthi et al. (2000), respectively. Weselake and Taylor (1999) detail the use of microspore-derived cultures to examine triacylglycerol biosynthesis in oilseed rape.

Another subject worthy of mention are the efforts by breeders to increase oil yields. These have been quite successful (e.g., maize varieties have been changed from an average 4 to 5% oil content to give IHO lines (20%) or ILO (0.5%) lines). However, the complexity of the phenotype precludes elucidation of the exact genetic basis of these changes (Ohlrogge et al., 1991). On the other hand, a 20% increase in seed fatty acid content (per unit seed weight) has been induced in *Arabidopsis* by a single gene variation (James and Dooner, 1990).

10.3.2 Triacylglycerol breakdown

The breakdown of triacylglycerol is catalysed by lipases. A large number of such enzymes have been purified from animals, plants, and microbes (cf. Brockerhoff and Jensen, 1974). It should be noted that the term "lipase" is frequently misused. A true lipase is one that attacks triacylglycerols and acts only at an oil–water interface. This definition, therefore, excludes enzymes acting on water-soluble esters (esterases) or those preferentially hydrolysing other lipids (acyl hydrolases).

Since triacylglycerols are important dietary constituents (Section 11.1), there is interest in digestive lipases. Although pharyngeal lipases have been found and studied, their importance during overall digestion remains to be established (cf. O'Doherty, 1978). In contrast, pancreatic lipase is the best known and most investigated of all lipolytic enzymes. It acts on mono-, di-, and triacylglycerols, although the reaction rate is slower with partial glycerides (Brockerhoff and Jensen, 1974). It probably lacks stereospecificity. For a thorough review of pancreatic lipase, see Jensen (1971).

Metabolism of triacylglycerols in animals requires the interaction of lipoprotein lipase (involved in uptake of acyl chains from plasma) and hormone-sensitive lipase (involved in release of fatty acids from lipid stores). Some aspects of lipoprotein lipase action are discussed in Section 11.3, and the reader is also referred to Brockerhoff and Jensen (1974), Jensen (1971), and Frayn et al. (1995). The enzyme is also known as clearing factor lipase, requires apo-C_{II} for activity, and may be bound via heparin sulfate proteoglycan at the endothelial surface *in vivo* (Williams et al., 1983). Considerable work has been carried out on intracellular processing of the enzyme in active tissues (Cryer, 1981) and on the action of hormones in controlling the adipose and heart tissue enzymes (Ashby and Robinson, 1980; de Gasquet et al. 1975). There is now considerable sequence information of lipoprotein lipase. The sequence is extraordinarily conserved (87 to 94% with different mammalian enzymes). Comparisons with other lipases have also been made (see Wang et al., 1992). For further information on the structural and functional domains of lipoprotein lipase, the structure and function of apo-C_{II}, and the reaction kinetics of the enzyme, see Wang et al., (1992).

Hormone-sensitive lipase tissue activity is stimulated by adrenaline (epinephrine, glucagons, ACTH (corticotrophin), TSH (thyrotropin) and serotonin (Jensen, 1971). These hormones are presumed to exert their effects on adipose tissue by stimulating adenylate cyclase. Certainly, an increased formation of cAMP (cyclic AMP) is brought about by lipolytic hormones and cAMP has been shown to stimulate lipase activity in cell-free preparations (cf. O'Doherty, 1978). Phosphorylation/dephosphorylation has been implicated (Belfarage et al., 1983). The cDNA for rat hormone-sensitive lipase has been reported (Holm et al., 1988) and increased expression of the mRNA for this enzyme found in the adipose tissue of cancer patients (Thompson et al., 1993). A good review is that of Langin et al. (1996) and Bernlohr et al. (2002), also summarises this topic. One typical feature of the mobilisation of fatty acids from adipose tissue, which has not yet been fully explained, is that their release is selective for both chain-length and unsaturation. Part of the reason is due to selectivity properties of hormone-sensitive lipase, but other factors appear important (Raclot, 2003).

Other mammalian lipases that have been studied are milk lipase (cf. Jensen, 1971) and monoacylglycerol lipase. The latter enzyme occurs in several tissues including intestine, liver, and adipose tissue. It has high activity with monoacylglycerols when compared to diacylglycerols or triacylglycerols (see O'Doherty, 1978).

Lipase activity has been found in a wide range of plant materials with most work on oil seeds and cereals. One of the best characterized enzymes is the castor bean acid lipase. Ory and coworkers (Ory, 1969) have characterized the enzyme, which is associated with the spherosome membrane. Huang and Moreau (1978) studied lipolytic activity in a range of germinating oil seeds. The enzyme from peanuts seems to be mainly associated with microsomal fractions (Theimer and Rosnitschek, 1978) and is clearly distinguished from the glyoxysomal monoacylglycerol lipase (Huang and Moreau, 1978). Several lipases have been purified from cereal seeds, such as wheat, oat, and rice. Galliard (1980) has reviewed this work (cf. also Jensen, 1971) and has discussed the role of plant growth regulators in controlling activity. For a more recent review, see Huang (1987).

Although bacteria do not store energy as triacylglycerol, a number of bacterial lipases have been discovered and studied. An extensive review of microbial lipases (and esterases) has been made by Lawrence (1967). It should be emphasized also that many of the microbial "lipases" (e.g., the widely studied *Rhizopus arrhizus* enzyme) are, in fact, acyl hydrolases. More recent information is available in Finnerty (1989).

The features of lipases useful for industry in lipid modifications have been discussed by Eigtved (1992), and the monograph by Alberghina et al. (1991) contains many specific examples of individual lipases, their characteristics and potential (or actual) industrial use.

References

Abate, N. and Garg, A (1995). Heterogeneity in adipose tissue metabolism: causes, implications and management of regional adiposity. *Prog. Lipid Res.* **34**, 53–70.

Akesson, B. et al. (1970). Initial incorporation into rat liver glycerolipids of intraportally injected (9,10-$^3H_2$) palmitic acid. *Biochim. Biophys. Acta.* **18**, 44–56.

Alberghina, L. et al., Eds. (1991). *Lipases: Structure, Mechanism and Genetic Engineering,* VCH Press, New York.

Appelqvist, L.A. (1975). Biochemical and structural aspects of storage and membrane lipids in developing oil seeds. In *Recent Advances in Chemistry and Biochemistry of Plant Lipids*, Eds. T. Gallaird and E.I. Mercer, Academic Press, London, pp. 247–254.

Ashby, P. and Robinson, D.S. (1980). Effects of insulin, glucocorticoids and adrenaline on the activity of rat adipose-tissue lipoprotein lipids. *Biochem, J.* **188**, 185–192.

Bauermeister and Sargent, J. (1979). Wax esters: major metabolites in the marine environment. *Trends Biochem. Sci.* 4, 209–211.

Belfrage, P. et al. (1983). Hormonal regulation of adipose tissue lipolysis by reversible phosphorylation of hormone-sensitive lipase. In *Cell Function and Differentiation,* Part C, Eds. G. Akoyunoglou, et al., Alan R. Liss, New York, pp. 213–223.

Berneth, K. and Frentzen, M. (1990). Utilization of erucoyl-CoA by acyltransferases from developing seeds of *Brassica-napus* (L) involved in triacylglycerol biosynthesis. *Plant Sci.* **67**, 21–28.

Bernlohr, D.A. et al. (2002). Adipose tissue and lipid metabolism. In *Biochemistry of Lipids, Lipoproteins and Membranes*, Ed. D.E. Vance, 4th ed., Elsevier, Amstedam, p. 257–281.

Brindley, D.N. (1978). Some aspects of the physiological and pharmacological control of the synthesis of triacylglycerols and phospholipids. *Int. J. Obes.* **2**, 7–16.

Brindley, D.N. Ed. (1988). *Phosphatidate Phosphohydrolase,* vols. I and II, CRC Press, Boca Raton, FL.

Brindley, D.N. (1991). Metabolism of triacylglycerols. In *Biochemistry of Lipids, Lipoprotein and Membranes*, Eds. D.E. Vance and J. Vance, Elsevier, Amsterdam, pp. 171–203.

Brokerhoff, H. and Jensen, R.G. (1974). *Lipolytic Enzymes,* Academic Press, New York.

Clark, B. and Hübscher, G. (1960). Biosynthesis of glycerides in the mucosa of the small intestine. *Nature.* **183**, 35–37.

Cohen, Z. and Ratledge, C (2005). Single cell oils. *Am. Oil Chem. Soc.* Champaign, IL.

Coleman, R.A. et al. (2000). Physiological and nutritional regulation of enzymes of triacylglycerol synthesis. *Annu. Rev. Nutr.* **20**, 77–103.

Cryer, A. (1981). Tissue lipoprotein lipase activity and its action in lipoprotein metabolism. *Int. J. Biochem.* 13, 525–541.

Davies, R.J. and Holdsworth, J. E. (1992). Synthesis of lipids in yeasts, biochemistry, physiology and production. *Adv. Appl. Lipid Res.* 1, 119–159.

de Gasquet, P. et al. (1975). Effect of glucocorticoids on lipoprotein-lipase activity in rat-heart and adipose-tissue. *Horm. Metab. Res.* **7**, 152–157.

Dircks, L. and Sul H.S. (1999). Acyltransferases of de novo glycerolphospholipid biosynthesis. *Prog. Lipid Res.* **38**, 461–479.

Douce, R. and Joyard, J. (1980). Plant galactolipids. In *Biochemistry of Plants,* vol. 4, Eds. P.K. Stumpf and E.E. Conn, Academic Press, New York, pp. 321–362.

Eigtved, P. (1992). Enzymes and lipid modification. *Appl. Lipid Res.* **1**, 1–64.

Finnerty, W.R. (1989). Microbial lipid metabolism. In *Microbial Lipids,* vol. 2, Eds. C. Ratledge and S.G. Wilkinson, Academic Press, London, pp. 525–566.

Frayn, K.N. et al. (1995). Coordinated regulation of hormone-sensitive lipase and lipoprotein lipase in human adepose tissue *in vivo. Adv. Enz. Regul.* **35**, 163–178.

Galliard, T. (1980). Degradation of acyl lipids: hydrolytic and oxidative enzymes. In *Biochemistry of Plants,* vol. 4, Eds. P.K. Stumpf and E.E. Conn, Academic Press, New York, pp. 85–116.

Gibbons, G.F. et al. (2000). Mobilisation of triacylglycerol stores. *Biochim. Biophys. Acta.* 1483, 37–57.

Griffiths, G. and Harwood, J.L. (1990). Triacylglycerol synthesis in maturing cotyledons of cocoa. In *Plant Lipid Biochemistry, Structure and Utilization*, Eds. P.J. Quinn and J.L. Harwood, Portland, London., pp. 216–218.

Gurr, M.I. (1980). The biosynthesis of triacylglycerols. In *Biochemistry of Plants*, Eds. P.K. Stumpf and E.E. Conn, Academic Press, New York, pp. 205–248.

Harwood, J.L. and Griffiths, G. (1992). Biochemistry of plant lipids. In *Advances in Plant Cell Biochemistry and Biotechnology*, Ed. I.M. Morrison, JAI Press, London, pp. 1–52.

Harwood, J.L. and Page, R. (1994). Biochemistry of oil synthesis. In *Designer Oilseed Crops*, Ed. D.J. Murphy, VCH Press, New York, pp. 165–194.

Harwood, J.L. and Price-Jones, M.J. (1988). Phosphatidate phosphohydrolase in plants and microorganisms. In *Phosphatidate Phosphohydrolase,* vol. II, Ed. D.N. Brindley, CRC Press, Boca Raton, FL, pp. 1–37.

Heinz, E. (1977). Enzymatic reactions in galactolipid biosynthesis. In *Lipids and Lipid Polymers in Higher Plants,* Eds. M. Tevini and H.K. Lichtenhaler, Springer-Verlag, Berlin, pp. 102–120.

Heinz, E. and Roughan, P.G. (1983). Similarities and differences in lipid-metabolism of chloroplasts isolated from 18:3 and 16:3 plants. *Plant Physiol.* **72**, 273–279.

Hill, E.E. and Lands, W.E.M. (1970). Phospholipid metabolism. In *Lipid Metabolism*, Ed. S.J. Wakil, Academic Press, New York, pp. 185–275.

Hill, E.E. et al. (1968). The selective incorporation of ^{14}C-glycerol into different species of phosphatidic acid, phosphatidylethanolamine, and phosphatidylcholine. *J. Biol. Chem.* **243**, 4440–4451.

Hitchcock, C. and Nichols, B.W. (1971). *Plant Lipid Biochemistry,* Academic Press, London.

Holm, C. et al. (1988). Hormone-sensitive lipase: sequence, expression, and chromosomal localization to 19 centq13.3. *Science,* **241**, 1503–1506.

Huang, A.H.T. (1987). Lipases. In *The Biochemistry of Plants*, Eds. P.K. Stumpf and E.E. Conn, vol. 9, Academic Press, New York, pp. 91–119.

Huang, A.H.C. (1992). Oil bodies and oleosins in seeds. *Annu. Rev. Plant Physiol.* **43**, 177–200.

Huang, A.H.C. and Moreau, R.A. (1978). Lipases in storage tissues of peanut and other oil seeds during germination. *Planta.* **141**, 111–116.

Ichihara, K. et al. (1990). Intracellular translocation of phosphatidate phosphatase in maturing safflower seeds — a possible mechanism of feed forward control of triacylglycerol synthesis by fatty acids. *Biochim. Biophys. Acta.* **1043**, 227–234.

James, D.W. and Dooner, H.K. (1990). Isolation of ems-induced mutants in arabidopsis altered in seed fatty-acid composition. *Theor. Appl. Genet.* **80**, 241–245.

Jensen, R.G. (1971). Lipolytic enzymes. *Prog. Chem. Fats Other Lipids.* **11**, 347–394.

Kanoh, H. and Ohno, K. (1973). Studies on 1,2-diglycerides formed from endogenous lecithins by back-reaction of rat-liver microsomal CDP-choline-1,2-diacylglycerol cholinephosphotransferase. *Biochim. Biophys. Acta.* **326**, 17–25.

Kates, M. (1995). Hydrolysis of lecithin by plant plastid enzymes. *Can. J. Biochem. Physiol.* **33**, 575–589.

Langin, D. et al. (1996). Adipocyte hormone-sensitive lipase: a major regulator of lipid metabolism. *Proc. Nutr. Soc.* **55**, 93–109.

Lawrence, R.C. (1967). Microbial lipases and related esterases. Part II. Estimation of lipase activity, characterization of lipases — recent work concerning their effect on dairy products. *Dairy Sci. Abstr.* **29**, pp. 59–70.

Lehner, R. and Kuksis, A (1996). Biosynthesis of triacylglycerols. *Prog. Lipid Res.* **35**, 169–202.

Lennarz, W.J. (1970). Bacterial lipids. In *Lipid Metabolism*, Ed. S.J. Wakil, Academic Press, New York, pp. 155–184.

Manning, R. and Brindley, D.N. (1972). Tritium isotope effects in the measurement of the glycerol phosphate and dihydroxyacetone phosphate pathways of glycerolipid biosynthesis in rat liver. *Biochem. J.* **130**, 1003–1012.

Mayorek, N. et al. (1989). Triacylglycerol synthesis in cultured rat hepatocytes. The rate-limiting role of diacylglycerol acyltransferase. *Eur. J. Biochem.* **182**, 395–400.

Mitchell, M.P. et al. (1971). Properties of phosphatidate phosphohydrolase. *Eur. J. Biochem.* **18**, 214–220.

Moore, T.J. et al. (1973). Enzymes of phospholipid metabolism in endoplasmic-reticulum of castor bean endosperm. *Plant Physiol.* **52**, 50–53.

Murphy, D.J. (1990). Storage lipid bodies in plants and other organisms. *Prog. Lipid Res.* **29**, 299–324.

O'Doherty, P.J.A. (1978). Metabolic studies with natural and synthetic fatty acids and enantiomeric acylglycerols. In *Handbook of Lipid Research,* vol. 1, Ed. A. Kuksis, Plenum Press, New York, pp. 289–339.

O'Doherty, P.J. and Kuksis, A. (1975). Stimulation of triacylglycerol synthesis by Z protein in rat liver and intestinal mucosa. *FEBS Lett*. **60**, 256–258.

O'Doherty, P.J. et al. (1974). Effect of phosphatidylcholine on triacylglycerol synthesis in rat intestinal mucosa. *Can J Biochem.* **52**, 726–733.

Ohlrogge, J.B. et al. (1991). The genetics of plant lipids. *Biochim. Biophys. Acta.* 1082, 1–26.

Ory, R.L. (1969). Acid lipase of the castor bean. *Lipids.* **4**, 177–185.

Perry, H.J. and Harwood, J.L. (1990). Studies of lipid metabolism in developing oilseed rape. In *Plant Lipid Biochemistry, Structure and Utilization*, Eds. P.J. Quinn and J.L. Harwood, Portland, London, pp. 204–206.

Perry, H.J. and Harwood, J.L. (1991). Lipid metabolism during seed development in oilseed rape (*Brassica napus* L. cv. Shiralee). *Biochem. Soc. Trans.* **19**, 243S.

Pollock, R.J. et al. (1975). The relative utilization of the acyl dihydroxyacetone phosphate and glycerol phosphate pathways for synthesis of glycerolipids in various tumors and normal tissues. *Biochim. Biophys. Acta.* **380**, 421–435.

Quinn, P.J. and Harwood, J.L. Eds. (1990) *Plant Lipid Biochemistry, Structure and Utilization,* Portland Press, London.

Raclot, T. (2003). Selective mobilisation of fatty acids from adipose tissue triacylglycerols. *Prog. Lipid Res.* **42**, 257–288.

Ramli, U.S. et al. (2005). Metabolic control analysis reveals an important role for diacylglycerol acyltransferase in olive but not in oil palm lipid accumulation. *FEBS J.* **222**, 5764–5770.

Ratledge, C. (1989). Biotechnology of oils and fats. In *Microbial Lipids,* vol. II, Eds. C. Ratledge and S.J. Wilkinson, Academic Press, London, pp. 567–613.

Roughan, P.G. (1987). On the control of fatty acid compositions of plant glycerolipids. In *The Metabolism, Structure and Function of Plant Lipids*, Eds. P.K. Stumpf, J.B. Mudd and W.D. Nes, Plenum, New York, pp. 247–254.

Roughan, P.G. and Slack, C.R. (1982). Cellular organization of glycerolipid metabolism. *Annu. Rev. Plant Physiol.* **33**, 97–132.

Sakaki, T. et al. (1985). Polar and neutral lipid changes in spinach leaves with ozone fumigation — triacylglycerol synthesis from polar lipids. *Plant Cell Physiol.* **26**, 253–262.

Salas, J.J. et al. (2000). Biochemistry of lipid metabolism in olive and other oil fruits. *Prog. Lipid Res.* **39**, 151–180.

Sedgwick, B. and Hubscher, G. (1967). Partial purification and properties of a soluble phosphatidate phosphohydrolase from rat liver. *Biochim. Biophys. Acta.* **144,** 397–408.

Smith, M.E. et al. (1967). The role of phosphatidate phosphohydrolase in glyceride biosynthesis. *Eur. J. Biochem.* **3**, 70–77.

Snyder, F.L. (1972). *Ether Lipids: Chemistry and Biology,* Academic Press, New York.

Stymne, S. and Stobart, A.K. (1987). Triacylglycerol biosynthesis In *The Biochemistry of Plants,* vol. 9, Eds. P.K. Stumpf and E.E. Conn, Academic Press, New York, pp. 175–214.

Sul, H.S. and Wang, D. (1998). Nutritional and hormonal regulation of enzymes in fat synthesis. Studies of fatty acid synthase and mitochondrial glycerol 3-phosphate acyltransferase gene transcription. *Annu. Rev. Nutr.* **18**, 331–351.

Theimer, R.R. and Rosnitscheck, I. (1978). Development and intracellular-localization of lipase activity in rapeseed (*Brassica-napus*-L) cotyledons. *Planta.* **139**, 249–256.

Thompson, M.P. et al. (1993). Increased expression of the mRNA for hormone-sensitive lipase in adipose tissue of cancer patients. *Biochim. Biophys. Acta.***1180**, 236–242.

Wang, C.-S. et al. (1992). Structure and functional properties of lipoprotein lipase. *Biochim. Biophys. Acta.* **1123**, 1–17.

Weselake, R.J (2005). Storage lipids. In *Plant Lipids: Biology, Utilisation and Manipulation*, Ed. D.J. Murphy, Blackwell Publishing, Oxford, U.K., pp. 162–225.

Weselake, R.J. and Taylor, D.C. (1999). The study of storage lipid biosynthesis using microspore-derived cultures of oilseed rape. *Prog. Lipid Res.* **38**, 401–460.

Williams, M.P. et al. (1983). Heparan sulphate and the binding of lipoprotein lipase to porcine thoracic aorta endothelium. *Biochim. Biophys. Acta,* **756**, 83–91.

Wilson, R.F. and Rinne, R.W. (1976). Involvement of phospholipids in triglyceride biosynthesis by developing soybean cotyledons. *Plant Physiol.* **57**, 556–559.

10.4 Glycosylglycerides

10.4.1 Galactosylglycerides

The two galactosylglycerides, monogalactosyldiacylglycerol (MGDG), and digalactosyldiacylglycerol (DGDG) are the major lipid components of the photosynthetic membranes of oxygen-evolving organisms. Because of that, they are the most prevalent membrane lipids in the world. They are rare or only found in trace amounts in other organisms. Trigalactosyl and tetragalactosyl derivatives are minor components of some chloroplasts, and in marine algae and in some bacteria the sugar residue(s) may be glucose.

The pathway for galactosylglyceride synthesis is outlined in Figure 10.32. UDP-galactose is generated in the cytoplasm of plant leaf cells and is used by two galactosyltransferase enzymes, which are located in the outer half of the envelope membrane. Joyard and Douce (1976) showed that the galactosyltransferase activity was located in the plastid envelope and that any activity in microsomal

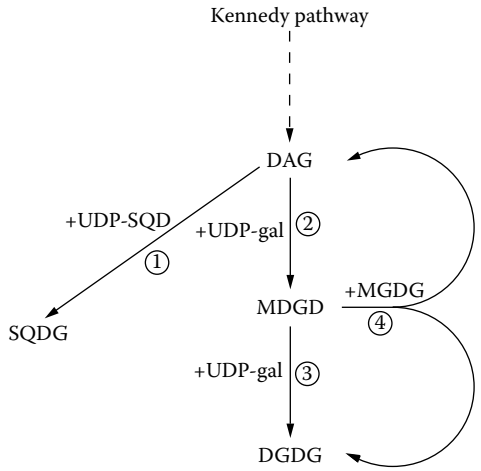


FIGURE 10.32 Biosynthesis of plant glycosylglycerides. Abbreviations: DAG, diacylglycerol; MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; SQD, sulfoquinovose; SQDG, sulfoquinovosyldiacylglycerol. Reactions: (1) UDP-SQD: diacylglycerol sulfoquinovosyltransferase; (2) UDP-galactose: diacylglycerol galactosyltransferase; (3) UDP-galactose:MGDG galactosyltransferase; (4) galactolipid: galactolipid galactosyltransferase.

fractions was due to its envelope content. Plastid envelope membranes from nonphotosynthetic tissues, such as the chromoplasts of *Narcissus pseudonarcissus* (Leidvogel and Kleinig, 1977) and potato tuber amyloplasts (Fishwick and Wright, 1980) are also active.

The first galactosyltransferase is specific for the formation of a β-glycosidic bond while the other forms an α-glycosidic bond (Douce and Joyard, 1980). There are two digalactosyldiacylglycerol synthetases in *Arabidopsis* and details of the formation of galactosyldiacylglycerol and the galactosyltransferases will be found in Dormann and Benning (2002) and in Dormann (2005).

As an alternative to the use of UDP-galactose, digalactosyldiacylglycerol may be formed by the action of galactolipid: galactolipid galactosyltransferase (see Figure 10.32), first demonstrated by Von Besouw and Wintermans (1978). Comparative aspects of these alternative pathways have been discussed (Joyard and Douce, 1987) and methods for the analysis of galactolipids and their metabolites reviewed (Douce et al., 1990). Recent evidence, discussed by Dormann (2005), suggests that the galactolipid transferase is not important for digalactosyldiacylglycerol synthesis *in vivo,* but can be activated under certain conditions, such as during membrane isolation.

The formation of polyunsaturated fatty acids (mainly α-linolenate) associated with the galactosylglycerides has been reviewed (Harwood, 1996). Depending on the plant type, there seem to be differences between the "16:3 species" and the "18:3 species," as discussed by Heinz and Roughan (1982). The distinctive features of the fatty acid distributions of galactolipids in 16:3 and 18:3 plants is illustrated in Section 2.10 and discussed thoroughly by

Browse and Somerville (1991). For 18:3 plants the desaturation of oleate in association with phosphatidylcholine is followed by release of the diacylglycerol for galactolipid formation (Joyard and Douce, 1987). Whether diacylglycerol itself or phosphatidylcholine moves from the endoplasmic reticulum to the chloroplast envelope is not known, although Tanaka and Yamada (1982) demonstrated the latter process mediated via a phospholipid transfer protein. After the formation of monogalactosyldiacylglycerol, further desaturation can then take place (Jones and Harwood, 1980; Harwood, 1996; see also Section 10.1.4). The fatty acid combinations (Heinz, 1977; Rullkötter et al., 1975) and turnover (Heinz et al., 1979) in galactosylglycerides have been studied thoroughly. In 16:3 plants and cyanobacteria the galactosylglycerides appear to be substrates for successive desaturations of oleate and linoleate to α-linolenate (see Harwood and Jones, 1989).

Initial breakdown of galactosylglycerides is catalysed by nonspecific acylhydrolase enzymes (see Galliard, 1980, for review). The enzymes from runner bean leaves (Burns et al., 1979) and potato tubers (Hirayama et al., 1975) have been purified. Two enzymes that differed slightly in substrate specificity were isolated from runner bean leaves (Burns et al., 1979) and the position of hydrolysis identified by mass spectrometry as the bond between the fatty acyl carboxy carbon and the oxygen of glycerol (Burns et al., 1980). Acyl hydrolase enzymes are particularly active; homogenization of potato tubers in water at 4°C for a few seconds being sufficient for most of the endogenous

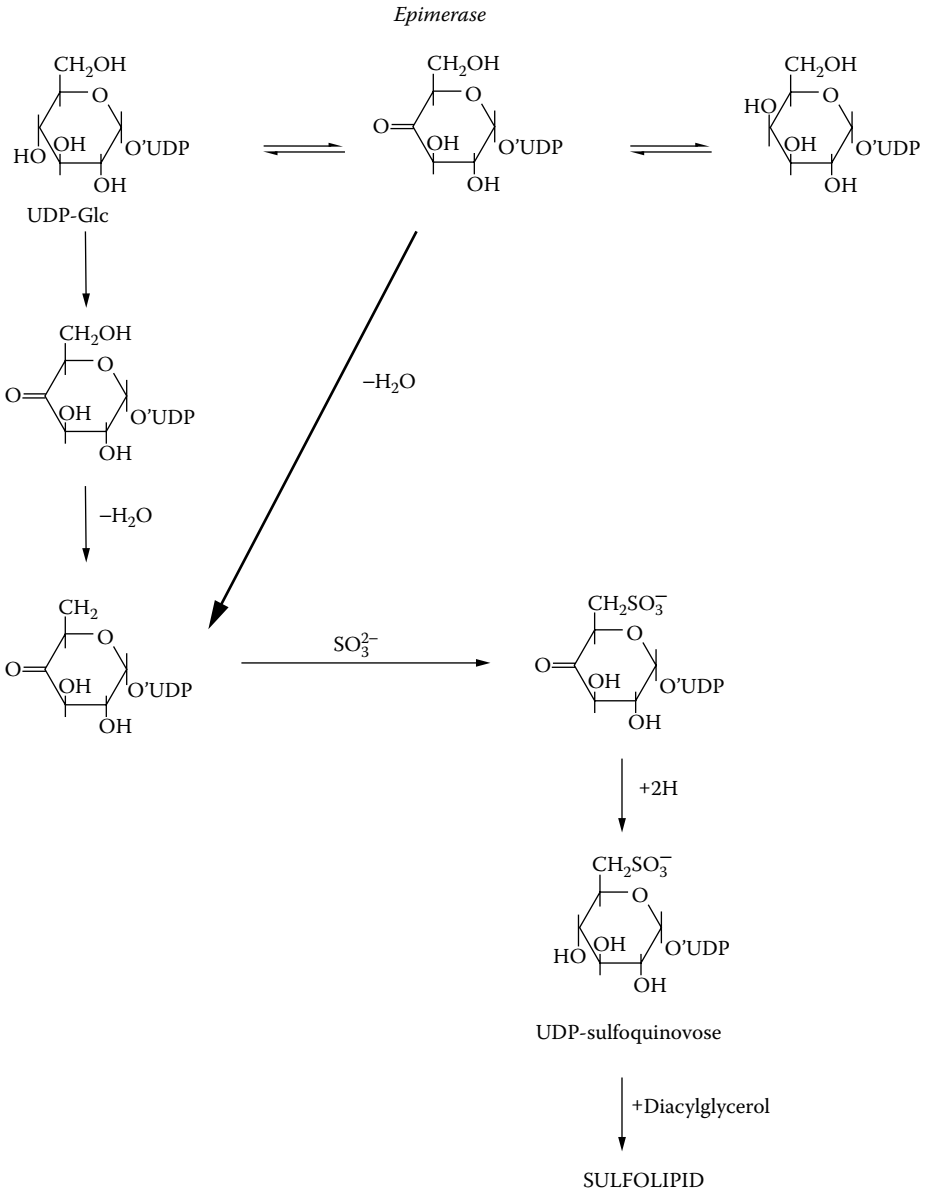


FIGURE 10.33 Synthesis of sulfoquinovosyldiacylglycerol. (See Pugh et al. (1995), Harwood and Okanenko, (2003) and Dormann (2005) for details.)

membrane lipids to be destroyed. Some recent information is given in Dormann (2005) and see Section 10.2.2.

10.4.2 Sulfolipid (diacylsulfoquinovosylglycerol)

Early work on possible pathways for the formation of the plant sulfolipid were reviewed by Harwood (1980) and by Mudd and Kleppinger-Sparace (1987). The immediate precursor, UDP-sulfoquinovose, was shown to transfer its sugar to diacylglycerol using SQDE synthase in the envelope membrane of chloroplasts (Heinz et al., 1989). The UDP-sulfoquinovose itself is synthesised from UDP-glucose and sulfite in a series of reactions whose theory is explained by Pugh et al. (1995). Apparently, a single gene codes for the protein needed to carry out all the conversions to UDP-sulfoquinovose (Sanda et al., 2001) (Figure 10.33).

The metabolism, genetics and possible physiological functions of sulfoquinovosyldiacylglycerol have been reviewed by Benning (1998) and by Harwood and Okanenko (2003).

Plant tissues are capable of, at least, partial catabolism of sulfolipid. Deacylation appears to be the first step and may take place with two enzymes, such as in green algae (e.g., Yagi and Benson, 1962) or by the action of a single acyl hydrolase (cf. Harwood, 1980). Cleavage of sulfoquinovosylglycerol to sulfoquinovose was reported in leaves of *Medicago sativa* (Lee and Benson, 1972) where sulfolactaldhyde and, later, sulfolactic acid accumulated. In contrast, catabolism by cell-free preparations from *Phaseolus multiforus* stopped at sulfoquinovose (Burns et al., 1980). Further aspects of sulfolipid catabolism are discussed by Harwood (1980) and in Harwood and Okanenko (2003).

Studies on the turnover of molecular species of sulfolipid have been reported in *Vicia faba* and *Hordeum vulgare.* In both these plants, the more saturated species were turned over at high rates, whereas the predominant 1-linolenoyl, 2-palmitoyl species had a low rate of turnover. These differences in metabolism may be related to the function of the sulfolipid — the fast turning over species being involved in a metabolic function, while the trienoic species have a structural role (cf. Harwood, 1980).

References

Benning, C. (1998). Biosynthesis and function of the sulfolipid sulfoquinovosyldiacylglycerol. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **49**, 53–75.

Benson, A.A. (1963). The plant sulfolipid. *Adv. Lipid Res.* **1**, 382–394.

Browse, J. and Somerville, C. (1991). Glycerolipid synthesis: biochemistry and regulation. *Annu. Rev. Plant Physiol.* **42**, 467–506.

Burns, D.D. et al. (1979). Purification of acyl hydrolase enzymes from the leaves of *Phaseolus multiflorus. Phytochemistry.* **18**, 1793–1797.

Burns, D.D. et al. (1980). Properties of acyl hydrolase enzymes from *Phaseolus multiflorus* leaves. *Phytochemistry*, **19**, 2281–2285.

Dormann, P. (2005). Membrane lipids. In *Plant Lipids:Biology, Utilisation and Manipulation*, Ed. D.J. Murphy, Blackwell Publishing, Oxford, U.K., pp. 123–161.

Dormann, P. and Benning, C. (2002). Galactolipids rule in seed plants. *Trends Plant Sci.* **7**, 112–118.

Douce, R. and Joyard, J. (1980). Plant galactolipids. In *Biochemistry of Plants,* vol. 4, Eds. P.K. Stumpf and E.E. Conn, Academic Press, New York, pp. 321–362.

Douce, R. et al. (1990). Glycolipid analyses and synthesis in plastids. In *Methods in Plant Biochemistry*, vol. 4, Eds. J.L. Harwood and J.R. Boyer, Academic Press, London, pp. 71–103.

Fishwick, M.J. and Wright, A.J. (1980). Isolation and characterization of amyloplast envelope membranes from *Solanum tuberosum*. *Phytochemistry*, **19**, 5559.

Galliard, T. (1980). Degradation of acyl lipids: hydrolytic and oxidative enzymes. In *Biochemistry of Plants,* vol. 4, Eds. P.K. Stumpf and E.E. Conn, Academic Press, New York, pp. 85–116.

Harwood, J.L. (1980). Sulpholipids. In *The Biochemistry of Plants,* vol. 4, Eds. P.K. Stumpf and E.E. Conn, Academic Press, New York, pp. 301–320.

Harwood, J.L. (1996). Recent advances in the biosynthesis of plant fatty acids. *Biochim. Biophys. Acta.* **1301**, 7–56.

Harwood, J.L. and Jones, A.L. (1989). Lipid metabolism in algae. *Adv. Bot. Res.* **16**, 1–53.

Harwood, J.L. and Okanenko, A.A. (2003). Sulphoquinovosyldiacylglycerol (SQDG) – the sulpholipid of higher plants. In *Sulphur in Plants,* Eds. Y.P. Abrol and A. Ahmad, Kluwer, Dordrecht, pp. 189–219.

Heinz, E. (1977). Enzymatic reactions in galactolipid biosynthesis. In *Lipids and Lipid Polymers of Higher Plants*, Eds. M. Tevini and H.K. Lictenthaler, Springer-Verlag, Berlin, pp. 102–120.

Heinz, E. and Roughan, P.G. (1982). De novo synthesis, desaturation and acquisition of monogalactosyldiacylglycerol by chloroplasts of 16:3 and 18:3 – plants. In *Biochemistry and Metabolism of Plant Lipids*, Eds. J.F.G.M. Wintermans and P.J.C. Kuiper, Elsevier, Amsterdam, pp. 169–182.

Heinz, E. et al. (1979). Investigations on the origin of diglyceride diversity in leaf lipids. In *Recent Advances in the Biochemistry and Physiology of Plant Lipids,* Eds. L.A. Appelqvist and C. Liljenberg, Elsevier, Amsterdam, pp. 99–120.

Heinz, E. et al. (1989). Synthesis of different nucleoside 5′-diphosphosulphoquinovoses and their use for studies of sulpholipid synthesis in chloroplasts. *Eur. J. Biochem.* **184**, 445–454.

Hirayama, O. et al. (1975). Purification and properties of a lipid acyl-hydrolase from potato tubers. *Biochim. Biophys. Acta.* **384**, 127–137.

Jones, A.V.M. and Harwood, J.L. (1980). Desaturation of linoleic acid from exogenous lipids by isolated chloroplasts. *Biochem. J.* **190**, 851–854.

Joyard, J. and Douce, R. (1976). Preparation and enzymatic activities of spinach chloroplast envelope. *Physiol. Veg.* **14**, 31–48.

Joyard, J. and Douce, R. (1987). Galactolipid synthesis. In *The Biochemistry of Plants,* vol. 9, Eds. P.K. Stumpf and E.E. Conn, Academic Press, New York, pp. 215–274.

Lee, R.F. and Benson, A.A. (1972). The metabolism of glyceryl ^{35}S-sulfoquinovoside by the coral tree, *Erythrina cristagalli*, and alfalfa, *Medicago sativa*. *Biochim. Biophys. Acta.* **261**, 35–37.

Leidvogel, B. and Kleining, H. (1977). Lipid metabolism in chromoplast membranes from daffodil — glycosylation and acylation. *Planta,* **133**, 249–253.

Mudd, J.B. and Kleppinger-Sparace, K. (1987). Sulfolipids. In *The Biochemistry of Plants,* vol. 9, Eds. P.K. Stumpf and E.E. Conn Academic Press, New York, pp. 275–289.

Pugh, C.E. et al. (1995). A new pathway for the synthesis of the plant sulpholipid, sulphoquinovosyldiacylglycerol. *Biochem. J.* **309**, 513–519.

Rullkötter, J. et al. (1975). Combination and positional distribution of fatty acids in plant digalactosyl diglycerides. *Z. Pflanzenphysiol.* **76**, 163–175.

Sanda, S. et al. (2001). Recombinant *Arabidopsis* SQD_1 converts UDP-glucose and sulphite to the sulfolipid head group precursor UDP-sulphoquinovose *in vivo*. *J. Biol. Chem.* **276**, 3941–3946.

Tanaka, T. and Yamada, M. (1982). Properties of phospholipid exchange proteins from germinated castor bean endosperm. In *Biochemistry and Metabolism of Plant Lipids,* Eds. J.F.G.M. Wintermans and P.J.C. Kuiper, Elsevier, Amsterdam, pp. 99–110.

Van Besouw, A. and Wintermans, J.F.G.M. (1978). Galactolipid formation in chloroplast envelopes. I. Evidence for two mechanisms in galactosylation. *Biochim. Biophys. Acta* **529**, 44–53.

Yagi, T. and Benson, A.A. (1962). Plant sulfolipid. V. Lysosulfolipid formation. *Biochim. Biophys. Acta* **57**, 601–603.

10.5 Sphingolipids

10.5.1 Biosynthesis

Sphingolipid chemistry and biosynthesis have been reviewed by Carter et al. (1965), Wiegandt (1971), Stoffel (1971), Kanfer and Hakomori (1983), Sweeley (1991), and Merrill and Sandhoff (2002).

Biosynthesis of long-chain bases has been studied in a series of experiments by Snell and coworkers (e.g., Brady et al., 1969) and by Stoffel and coworkers (see Stoffel, 1971). A condensing enzyme, requiring pyridoxal phosphate, is able to condense serine with palmitoyl-CoA to produce 3-oxosphinganine (3-keto sphinganine). The reaction, which is probably the rate-limiting step in sphingoid base biosynthesis, proceeds with overall retention of configuration of the C-2 carbon of serine. L-Cycloserine (4-amino-3-isoazolidinone) is an irreversible inhibitor of the palmitoyltransferase and depresses the level of central nervous system sphingolipids if administered to mice (Sweeley, 1991). Other inhibitors of this and succeeding reactions in sphingolipid metabolism are listed in Merrill and Sandhoff (2002). The next reaction is a reduction using NADPH to produce sphinganine (Figure 10.34). The reductase responsible is probably

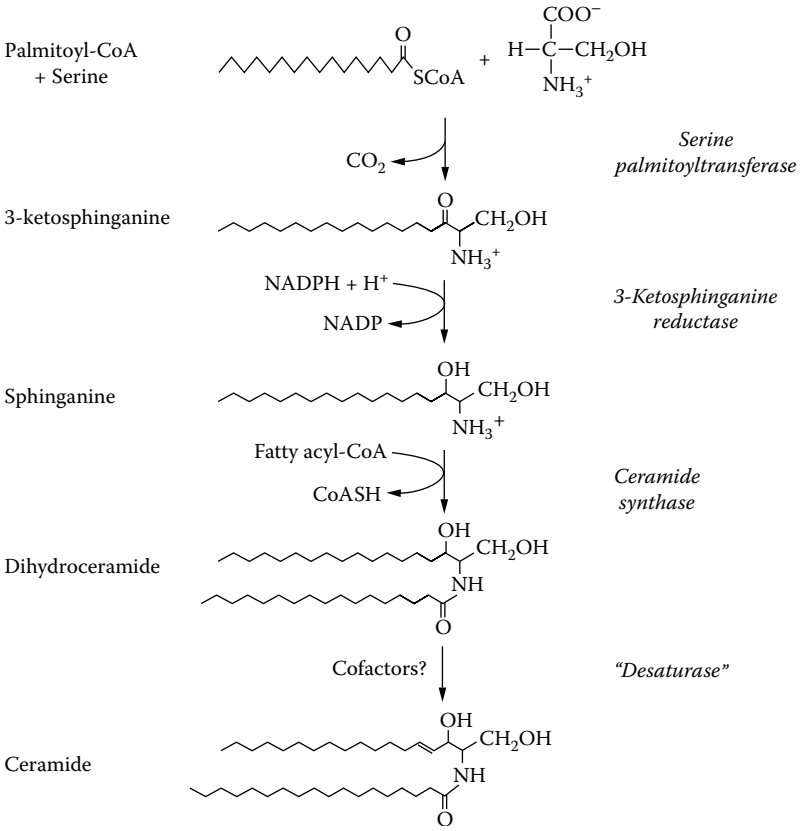


FIGURE 10.34 Biosynthesis of ceramide.

closely associated with the palmitoyltransferase in the endoplasmic reticulum. Both the palmitoyltransferase and the reductase exhibit chain-length specificity, with C_{14} to C_{18} CoA esters and C_{14} to C_{20} 3-dehydrosphinganines being utilized. The amino acid may form a Shiff-base complex with the pyridoxal phosphate coenzyme and Mg^{2+} during the condensation reaction. Some researchers have also suggested that palmitaldehyde is an intermediate in the reaction, although this has not been proven.

Stoffel et al. (1968) have studied the direct transformation of sphinganine into 4-hydroxysphinganine in the yeast, *Hansenula ciferri.* The origin of the hydroxyl group is obscure, since Thorpe and Sweeley (1967) concluded that it did not arise from either molecular oxygen or water. For further discussion of the formation of sphingoid bases, see Sweeley (1991) and for their structures and nomenclature, see Merrill and Sandhoff (2002).

Ceramides can be rapidly formed from *erythro* or *threo* long-chain bases. The specificity of this acylation by acyl-CoAs has been studied in brain and other tissues (see Stoffel, 1971). The significance of the nature of the fatty acid moiety in ceramides is well seen when the glycosylation of ceramides is considered: hydroxyl fatty acid-containing ceramides accept predominantly galactose, while nonhydroxy fatty acid ceramides accept glucose. Reversal of ceramidase to yield ceramide does not appear to be important (Merrill and Sandoff, 2002). Genes for ceramide synthase have been identified in yeast and animals. Microorganisms can produce inhibitors of the enzyme and fumonisins from *Fusaria* spp. are notable as causes of a number of human pathologies. The last step in ceramide synthesis is the insertion of a 4,5-*trans*-double bond into the sphingoid base (see Figure 10.34). Desaturase genes have been identified in plants (Sperling et al., 2000; Dunn et al., 2004) and animals. For the 4-hydroxysphinganines, insertion of the 4-hydroxyl group occurs at the level of sphinganine (see Dunn et al., 2004).

Cerebrosides are made from ceramide using glycosyltransferase enzymes, which are specific for UDP-galactose or UDP-glucose. The latter also recognises ceramides with nonhydroxy fatty acids, as mentioned above. Thus, galactocerebrosides (and sulfatides) are enriched in α-hydroxy fatty acids (Merrill and Sandhoff, 2002).

As expected, the formation of sulfatides involves transfer of sulfate from phosphoadenosine phosphosulfate (PAPS). Galactosylceramide and lactosylceramide were both acceptors for the sulfate from PAPS and the reaction is catalysed by the Golgi enzyme, galactosylceramide sulfotransferase. Sulfate transfer is preceded by receptor-mediated translocation of PAPS from the cytosol across the Golgi membrane, and this process can be inhibited by 3′-P-AMP, palmitoyl-CoA, or atractyloside (Sweeley, 1991). Sulfatide synthesis is most rapid in the period 20 to 25 days after birth in rat brain (Stoffel, 1971). The

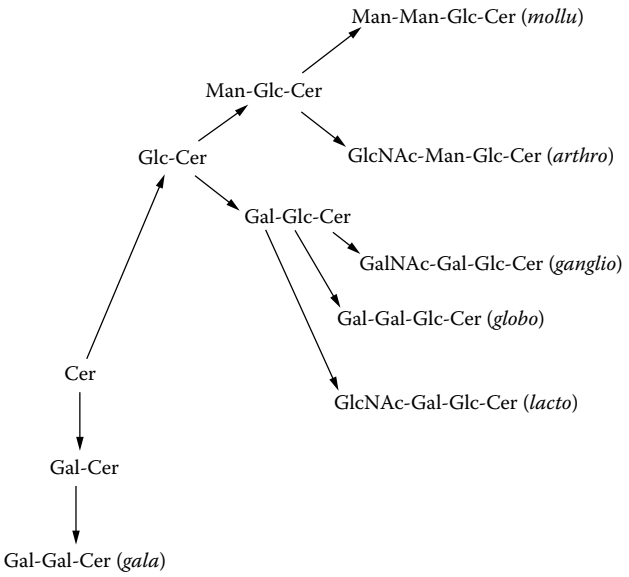


FIGURE 10.35 Biosynthesis of different root glycosphingolipids from ceramide. (From Sweeley, 1991.)

cDNA encoding the sulfotransferase has been cloned (Honke et al., 1997) and activity of this enzyme appears important in controlling overall sulfatide formation. See also Vos et al. (1994) for metabolic and functional aspects of sulfogalactolipids.

Synthesis of the neutral glycosphingolipids begins from glucosylceramide or galactosylceramide. Specific glycosyltransferases are involved, and the activated forms of the sugar substrates (UDP-Glc, UDP-Gal, UDP-GlcNAc, GDP-Man, and GDP-Fuc) are produced in the cytosol from nucleoside triphosphates and hexose 1-phosphates. The formation of different root glycosphingolipids from ceramide is illustrated in Figure 10.35 and occurs in the lumen of the Golgi apparatus. The active sites of the glycosyltransferases are localized on the lumenal surface of the Golgi membrane and may be organized in several multiglycosyltransferases, which could account for the various types of products that are formed (Sweeley, 1991). This contrasts to glycosylceramide, which is made on the cytosolic face of the endoplasmic reticulum and/or early Golgi membranes. Thus, glucosylceramide must undergo trans-bilayer movement before further metabolism (Merrill and Sandhoff, 2002).

Gangliosides are formed by stepwise elongation of the carbohydrate chain by the action of various glycosyltransferases. Several of the individual enzymes have been studied (see Stoffel, 1971) and assay methods are detailed by Basu et al. (1987). The individual sugars are transferred from their UDP derivatives, while sialic acid residues are donated by CMP-*N*-acetylneuraminic acid (CMP-NANA). CMP-NANA itself is produced by a reaction of NANA with CTP. Initial reactions in the formation of gangliosides are shown in Figure 10.36. Ganglioside synthesis involves the use of relatively few enzymes (Table 10.8), which can

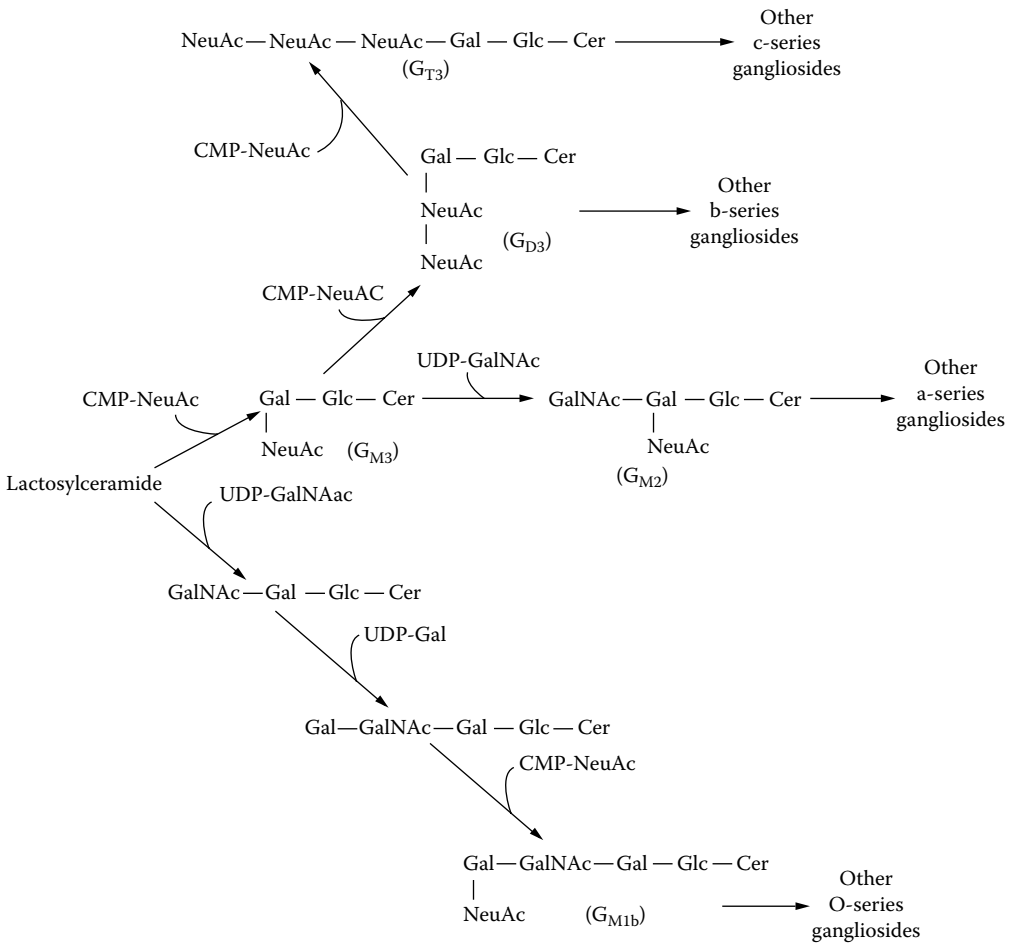


FIGURE 10.36 Key steps in the initial formation of gangliosides. (From Gurr, M.I., Harwood, J.L. and Frayn, K.N. (2002). *Lipid Biochemistry,* 5th ed., Blackwell Scientific, Oxford, U.K. With permission.)

TABLE 10.8 Glycosyltransferases catalysing the biosynthesis of gangliosides

Abbreviation	Name	Linkage created	Substrates
GalT-1	β-Galactosyltransferase	Gal(β1-4)Glc	Glc-Cer
SAT-1	Sialyltransferase	Neu5Ac(-2-3)Gal	Lac-Cer
SAT-2	Sialyltransferase	Neu5Ac(-2-8)Neu5Ac	G_{M3}
SAT-3	Sialyltransferase	Neu5Ac(-2-8)Neu5Ac	G_{D3}
SAT-4	Sialyltransferase	Neu5Ac(-2-3)Gal	G_{A1}, G_{M1a}, G_{D1b}
SAT-5	Sialyltransferase	Neu5Ac(-2-8)Neu5Ac	G_{M1}, G_{D1a}, G_{T1b}
GalNAcT	β-***N***-Acetylgalactosaminyltransferase	GalNAc(β1-4)Gal	Lac-Cer, G_{M3}, G_{D3}
GalT-2	β-Galactosyltransferase	Gal(β1-3)GalNAc	G_{A2}, G_{M2}, G_{D2}

From Sweeley (1991).

work in various combinations. Thus, the final ganglioside composition can be influenced by the relative activity of these enzymes as well as substrate availability (Muramatsu, 2000). Regulation of ganglioside biosynthesis occurs at transcriptional and post-transcriptional levels (Merrill and Sandhoff, 2002). As with neutral glycosphingolipid synthesis, the glycosyltransferases are membrane-bound and predominantly located in the Golgi apparatus.

Sphingomyelin, which is both a phospho- and sphingolipid, is synthesised by transfer of phosphorylcholine from phosphatidylcholine to ceramide, liberating diacylglycerol. *De novo* sphingomyelin synthesis occurs on both the plasma membrane and in the Golgi apparatus with the proportions depending on the cell type. An analogous sphingolipid, ceramide phosphorylehanolamine, is made in a similar way but using phosphatidylethanolamine as the donor. Inositolphosphoceramides are made similarly (Merrill and Sandhoff, 2002). A comprehensive review of sphingomyelin metabolism, intracellular transport and various aspects of its biological functions has been published (Koval and Pagano, 1991).

The biological function (Stoffel, 1971) and immunochemistry of the sphingolipids — especially the gangliosides — have been reviewed (Hakomori, 1981). Kanfer

and Hakomori (1983) have published a comprehensive review on sphingolipid biochemistry and see Bell et al. (1993) for aspects of metabolism. Merrill and Hannun (2000) have edited a useful volume of *Methods in Enzymology* and Lynch (1993) describes plant sphingolipids.

10.5.2 Breakdown

Breakdown of sphingolipids occurs by stepwise hydrolytic cleavage of the various substituents, starting with the terminal hydrophilic portions of the molecules. As an example, catabolism of ganglioside G_{M1}[Gal(β1→3) GalNAc(β1→4)Gal-(3→2αAcNeu)(β1→4)Glc(β1→1′) Cer] begins by the action of a β-galactosidase that cleaves the terminal galactose to give ganglioside G_{M2} as the other product. Ganglioside G_{M2} can be cleaved by two pathways. Either a hexosaminidase removes the molecule of GalNAc or a neuroaminidase hydrolyses AcNeu. These reactions have been demonstrated *in vivo*, but because more G_{M2}[GalNAc(β1→4) Gal-(3→2AcNeu) (β1→4) Glc(β1→1′)Cer] accumulates in Tay-Sachs disease than G_{A2}[GalNAc(β1→4)-Gal(β1→4)Glc(β1→1′)Cer], the hexosaminidase reaction appears the more important. The latter enzyme attacks G_{M2} to yield G_{M3}[(AcNeu2→3)-Gal(β1→4)Glc(β1→1′)Cer], which is then catalysed further by a neuraminidase to give ceramide lactoside (Figure 10.37) (see Brady, 1978).

The cleavage of ceramide lactoside needs a β-galactosidase. Two β-galactosidases have been demonstrated in mammalian tissues, both of which have acidic pH optima (pH 4.2 and 4.8). When assayed *in vitro*, either of these enzymes catabolizes ceramide lactoside, depending on the detergent used in the experiment. The pH4.2 galactosidase also hydrolyses galactose from galactocerebroside. The pH 4.8 enzyme preferentially catalyses the removal of the terminal galactose of G_{M1} as well as catabolizing ceramide lactoside. There are also two liver β-galactosidases with more neutral pH optima that catabolize ceramide lactoside, but are inactive with galactocerebroside or G_{M1} (Ben-Yoseph et al., 1977).

For degradation of sphingolipids with 4 or less carbohydrate residues, there is often a requirement for sphingolipid activator proteins (SAPs or saposins) *in vivo*. Some inherited diseases are caused by mutation of the domains of the exoglycosidases that interact with SAPs (Merrill and Sandhoff, 2002).

Glucocerebroside is hydrolysed by glucocerebrosidase to give glucose and ceramide. Catabolism of ceramide is catalysed by acid (Gatt, 1963), neutral (Sugita et al., 1975) or alkaline ceramidases in mammalian tissues. A sphingosine

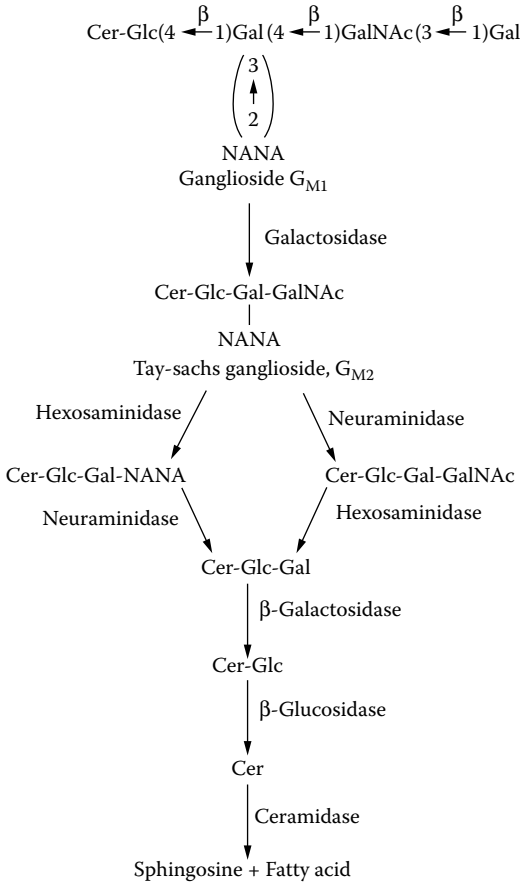


FIGURE 10.37 Breakdown of ganglioside G_{MI}-galactosidase.

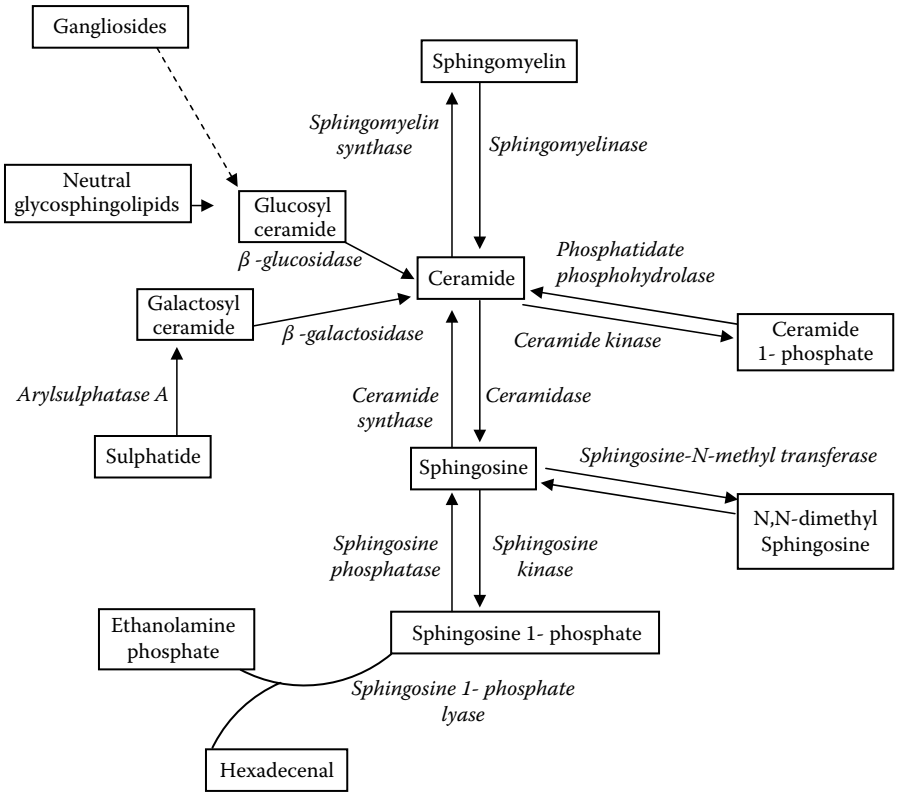


FIGURE 10.38 Interconversions between and catabolism of simple sphingolipids.

base and a nonesterified fatty acid are liberated. The former is phosphorylated to give a 1-phosphate derivative and then hydrolysed to yield a long-chain aldehyde and phosphoethanolamine (Stoffel, 1971). Sulfatides are catabolised by a sulfatase and the resultant galactocerebroside hydrolysed by a β-galactosidase. In addition, sphingomyelin is hydrolysed by sphingomyelinase to yield ceramide and phosphocholine (see Barenholz and Gatt, 1982) (Figure 10.38).

Sphingolipid breakdown is reviewed by Kanfer and Hakomori (1983) and deficiency diseases of sphingolipid catabolism are covered in Section 11.5.

Sweeley (1991) and Merrill and Sandhoff (2002) discuss some aspects of sphingolipid breakdown and regulation of their turnover. In the latter connection, glycosphingolipids have important functions during cellular differentiation and oncogenic transformation. Recent interest has also included their function as modulators of transmembrane signalling and as mediators for cellular interactions (Hakomori, 1990). Sphingolipids as cell signalling molecules are discussed in Section 10.6 (see also Aue et al., 2000). Apart from the main compounds (ceramide, sphingoid bases, and sphingosine-1-phosphate), a number of other lysosphingolipids have acute activity. Thus, lysosphingomyelin is a potent mitogen while psychosine (lyso-GlcCer or lyso-GalCer) is highly cytotoxic. Ceramide-1-phosphate is an active Ca^{++}-mobilising agent (see Merrill and Sandhoff, 2002).

For a simple review of sphingolipid metabolism and function see Gurr et al. (2002).

References

Aue, N. et al. (2000). Sphingomyelin metabolites in vascular cell signalling and atherogenesis. *Prog. Lipid Res.* **39**, 207–229.

Barenholz, Y. and Gatt, S. (1982). Sphingomyelin: metabolism, chemical synthesis, chemical and physical properties. In *Phospholipids* (Eds. J.N. Hawthorne and G.B. Ansell), Elsevier, Amsterdam, pp. 129–177.

Basu, M. et al. (1987). Glycolipids. In *Methods in Enzymology,* vol. 138 (Ed. V. Ginsburg), Academic Press, Orlando, FL, pp. 575–607.

Bell, R.M. et al. Eds. (1993). Sphingolipids, Part B: regulation and function of metabolism. *Adv. Lipid Res.* **26** (special issue).

Ben-Yoseph, Y. et al. (1977). Purification and properties of neutral beta-galactosidases from human liver. *Arch. Biochem. Biophys,* **184**, 373–380.

Brady, R.N. et al. (1969). Biosynthesis of sphingolipid bases. 3. Isolation and characterization of ketonic intermediates in the synthesis of sphingosine and dihydrosphingosine by cell-free extracts of *Hansenula ciferri*. *J. Biol. Chem.* **244**, 491–496.

Brady, R.O. (1978). Sphingolipidoses. *Annu. Rev. Biochem.* **47**, 687–713.

Carter, H.E. et al. (1965). Glycolipids. *Annu. Rev. Biochem.* **34**, 109–142.

Dunn, T.M. et al. (2004). A post-genomic approach to understanding sphingolipid metabolism in *Arabidopsis thaliana. Ann. Bot.*. **93**, 483–497.
Gatt, S. (1963). Enzymic hydrolysis and synthesis of ceramides. *J. Biol. Chem.* **238**, 3131–3133.
Gurr, M.I. and James, A.T. (1980). *Lipid Biochemistry,* Chapman & Hall, London.
Gurr, M.I. et al. (2002). *Lipid Biochemistry,* 5th ed., Blackwell Scientific, Oxford, U.K.
Hakomori, S.-I. (1981). Glycosphingolipids in cellular interaction, differentiation, and oncogenesis. *Annu. Rev. Biochem.* **50**, 733–764.
Hakomori, S.-I. (1990). Bifunctional role of glycosphingolipids. Modulators for transmembrane signaling and mediators for cellular interactions. *J. Biol. Chem.* **265**, 18713–18716.
Honke, K. et al. (1997). Molecular cloning and expression of cDNA encoding human 3-phosphoadenylsulfate: galactosylceramide 3-sulfotransferase. *J. Biol. Chem.* **272**, 4864–4868.
Kanfer, J.N. and Hakomori, S-I. (1983). *Sphingolipid Biochemistry,* Plenum, New York.
Koval, M. and Pagano, R.E. (1991). Intracellular transport and metabolism of sphingomyelin. *Biochim. Biophys. Acta.* **1082**, 113–125.
Lynch, D.V. (1993). Sphingolipids. In *Lipid Metabolism in Plants,* Ed. T.S. Moore, CRC Press, Boca Raton, FL, pp. 285–308.
Merrill, A.H. and Hannun, Y.A. Eds. (2000). Sphingolipid metabolism and function Part A, Part B. In *Methods of Enzymology,* vols. 311 and 312.
Merrill, A.H. and Sandhoff, K. (2002). Sphingolipids: metabolism and cell signalling. In *Biochemistry of Lipids, Lipoproteins and Membranes*, Eds. D.E. Vance and J.E. Vance, 4th ed., Elsevier, Amsterdam, pp. 373–407.
Muramatsu, T. (2000). Essential roles of carbohydrate signals in development, immune response and tissue functions, as revealed by gene targeting. *J. Biochem* (Tokyo). **127**, 171–176.
Spence, M.W. (1989). Sphingomyelin biosynthesis and catabolism. In *Phosphatidylcholine Metabolism,* Ed. D.E. Vance, CRC Press, Boca Raton, FL, pp. 185–203.
Sperling, P., Blume, A., Zahringer, U. and Heinz, E. (2000). Further characterisation of Δ8-desaturases from higher plants. *Biochem. Soc. Trans.* **28**, 638–641.
Stoffel, W. (1971). Sphingolipids. *Annu. Rev. Biochem*. **40**, 57–82.
Stoffel, W. et al. (1968). Metabolism of sphingosine bases. IX. Degradation *in vitro* of dihydrospingosine and dihydrospingosine phosphate to palmitaldehyde and ethanolamine phosphate. *Hoppe-Seyler's Z. Physiol. Chem.* **349**, 1745–1748.
Sugita, M. et al. (1975). Ceramidase and ceramide synthesis in human kidney and cerebellum. Description of a new alkaline ceramidase. *Biochim. Biophys. Acta.* **398**, 125–131.
Sweeley, C.C. (1991). Sphingolipids. In *Biochemistry of Lipids, Lipoproteins and Membranes,* Eds. D.E. Vance and J. Vance, Elsevier, Amsterdam, pp. 327–361.
Thompson, G.A. (1973). Phospholipid metabolism in animal tissues. In *Form and Function of Phospholipids*, Eds. G.B. Ansell, R.M.C. Dawson, and J.N. Hawthorne, Elsevier, Amsterdam, pp. 67–96.
Thorpe, S.R. and Sweeley, C.C. (1967). Chemistry and metabolism of sphingolipids. On the biosynthesis of phytosphingosine by yeast. *Biochemistry,* **6**, 887–897.
Wiegandt, H. (1971). Glycosphingolipids. *Adv. Lipid Res.* **9**, 249–289.

10.6 Lipids as signalling molecules

The first lipids to be recognised as giving rise to lipid second messengers were the inositol-containing phosphoglycerides. Since that time a multitude of lipids with acute biological activity have been recognised and this general area is one of the most active in biochemistry. Signalling molecules include intact phosphoglycerides (e.g., phosphatidic acid (PA), platelet-activating factor (PAF), membrane-soluble hydrolysis products (e.g., diacylglycerol (DAG)), water-soluble products (e.g., inositol-1,4,5-*tris*-phosphate) and other degradative metabolities (e.g., lysoPA, fatty acids). The role of polyunsaturated fatty acids themselves is described in Section 10.1 and Section 11.1.

Two general reviews about lipids as signalling molecules or bioactive lipids are Bell et al. (1996) and Nicolaou and Kokotos (2004). Methods for analysis are described in Christie (2003) and in Laychock and Rubin (1999). Simpler summaries of some aspects of lipid signalling are in Gurr et al. (2002) and Vance and Vance (2002).

10.6.1 Platelet activating factor (PAF)

Intact phospholipids have various important roles in signalling. One of the first such lipids to be recognised was platelet activating factor (PAF; 1-*O*-alkyl-2-acetyl-*sn*-glycerol-3-phosphocholine). PAF is produced by many types of cells in response to stimuli. In inflammatory cells, such as monocytes or macrophages, it can be rapidly synthesised by a deacylation-acetylation pathway (Tokumura, 1995). It can also be produced by *de novo* synthesis, which seems particularly important for maintaining PAF levels in the central nervous and reproductive systems.

Because of its very potent biological activity, PAF levels in cells and in the circulation are strictly regulated (Tokomura, 1995). PAF-acetylhydrolase plays a primary role in inactivation. There are a family of PAF-acetylhydrolases that consist of two intracellular isoforms and one secreted (plasma) isoform. Details of these enzymes, physiological function and role in human disease are given by Karasawa et al. (2003).

PAF elicits responses in many cells and organs. Originally it was shown to aggregate platelets (hence, the name), but it is now known to affect vasodilation/constriction, bronchial responsiveness and many acute responses connected to inflammation. PAF acts by binding to a unique G-protein-coupled seven transmembrane receptor that links it to various signalling pathways (Ishii and Shimizu, 2000).

A series of PAF-like lipids have been identified, many of which have biological activity and some can also be formed under oxidising conditions such as those produced by smoking (see Tokumura, 1995).

A general review of the biochemistry of PAF is that by Synder (1995) and Honda et al. (2002) describe PAF

receptors. Farooqui and Horrocks (2004) discuss general aspects of PAF and provide up-to-date references.

10.6.2 Lysophosphatidic acid (LPA)

Lysophosphatidic acid (LPA) was first identified as the active ingredient of Darmstoff (smooth muscle-stimulating substance) in 1957. Tokumura (1995) reviewed the activity of LPAs as a vasopressor, a platelet agonist, growth factor, and putative second messenger. LPA can be generated from phosphatidic acid (phospholipase A_1 or A_2), from lysophospholipids (phospholipase D), or by oxidative modification of low-density lipoprotein. LPA can bind to receptors, a number of which have been reported (Fukushima et al., 2001). It is thought to have important pathophysiological roles in cancer, cell survival apoptosis, and vascular activity (Tigyi and Parrill, 2003). See Pyne (2004) for an update on the metabolism and function of LPA.

The biochemistry of the parent compound for LPA, phosphatidic acid, is discussed thoroughly by Bocckino and Exton (1996), who pointed out that many of the purported physiological effects of phosphatidic acid can be explained by traces of LPA.

Phosphatidic acid, itself, has many important functions in plants that are well reviewed by Wang (2006) and by Testerink and Munnik (2005).

10.6.3 Inositol lipids

Although phosphatidylinositol-4,5-*bis*-phosphate (PIP_2) is an important precursor of the twin second messengers inositol-1,4,5-*tris*phosphate and diacyglycerol, it has functions as an intact lipid also. These include functions in ion channel function (e.g., Kobrinsky et al., 2000) and in membrane trafficking (see Hilgemann, 2003). There are also roles for phosphoinositides in cytoskeletal function (Yin and Janmey, 2003) and in the activity and binding of phospholipase D. Phosphatidylinositol 4-phosphate may have a function in binding the cytoskeletal protein, talin (Payrastre, 2004).

Recent work has also revealed a number of important signalling roles for phosphoinositides that does not involve their hydrolysis. Thus, in addition to PIP_2, there are well-evidenced roles for phosphatidylinositol-3-phosphate and phosphatidylinositol-3,4,5-*tris*phosphate as membrane lipids for the recruitment and/or activation of various proteins. In turn, this can influence the action of a large number of proteins and, hence, signalling pathways that underpin mechanisms for signal transduction, cytoskeletal, and membrane trafficking events (Martin, 1998). Additional information on the synthesis and function of 3-phosphorylated inositol phospholipids is given by Vanhaesebroeck et al. (2001) and Katso et al. (2001). Vivanco and Sawyers (2002) discuss phosphatidylinositol 3-kinases and cancer.

Reviews of the roles of different intact inositol phospholipids (as well as their biologically active metabolites) in plants have been made recently (Meijer and Munnik, 2003; Drøbak, 2005). Although plant proteins, such as those with PH- or PX-domains, can bind to certain phosphoinositides, there are unique features of plants compared to mammals. Moreover, the pathways for phosphorylation and, hence, the spectrum of plant phosphoinositides (Drøbak, 2005) differ from animal systems (Vanhaesebroeck et al., 2001).

Phosphoinositides can also exert their role in the control of cellular processes by being hydrolysed and giving rise to second messengers. The classic reaction is the hydrolysis of PIP_2 to give rise to the dual second messengers diacylglycerol and inositol 1,4,5-*tris*phosphate (Berridge, 1987). For more recent description of these effectors and general descriptions of the importance of phosphoinositides, refer to Payrstre et al. (2001), Toker (2002), and Payrastre (2004).

The product of phosphoinositide hydrolysis with phospholipase C (above) is diacylglycerol (DAG), which has a well-known function with protein kinase C (Takai et al., 1979). While hydrolysis of phosphoinositides produces a quick elevation of DAG, it can be produced from phosphatidylcholine in a more sustained manner via phosphatidic acid (Nishizuka, 1995). Other aspects of DAGs are covered in Becker and Hannun (2004).

10.6.4 Plasmalogens

Plasmalogens are thought to have a number of important roles in controlling tissue functions. General reviews are those of Farooqui and Horrocks (2001) and Nagan and Zoeller (2001). Functions include those in ion transport, membrane fusion, protection of membranes against oxidative stress, cholesterol efflux, and cellular differentiation. Other ether lipids have been reported to have various physiological functions and to be involved in different human diseases (Farooqui and Horrocks, 2004).

10.6.5 Sphingolipids

Sphingolipids can have various signalling roles or can act as bioactive lipids. Their relationship with each other is illustrated in Figure 10.39 (see also Gurr et al., 2002).

Ceramide was the first such lipid to be described as a second messenger (Hannun et al., 1996). Its biochemistry and signalling actions have been reviewed by Hannun and Obeid (2002) and by de Avalos et al. (2004). Ceramide plays a very important role in apoptosis (Pettus et al., 2002), in oxidative and heat stress, and in various diseases (de Avalos et al., 2004).

Ceramides can be hydrolysed to produce sphingosine, which itself has important properties in regulating cellular systems (Merrill et al., 1996). Importantly, sphingosine can be phosphorylated to produce another signalling

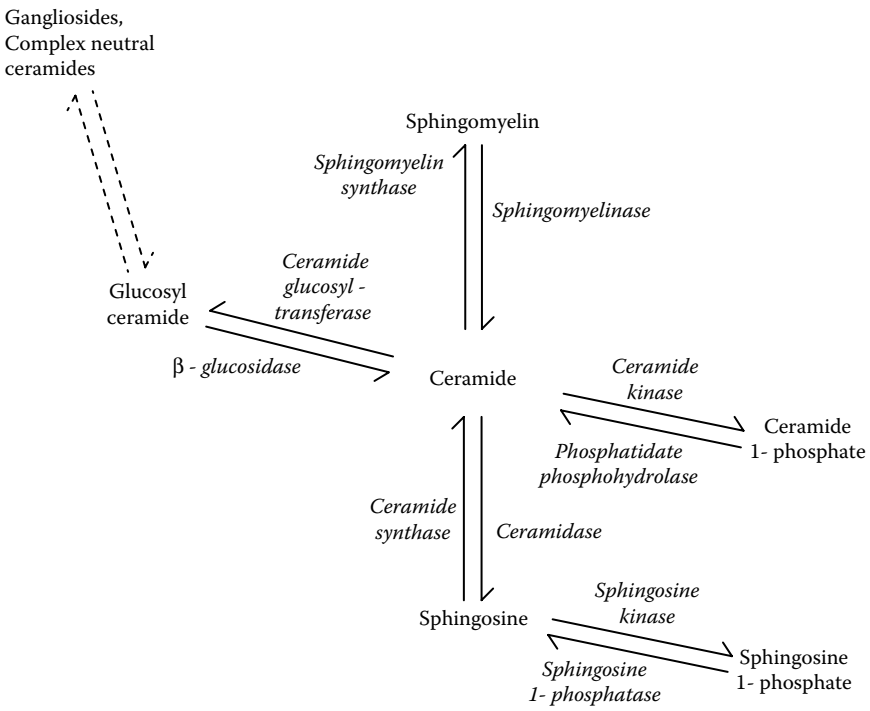


FIGURE 10.39 Biosynthetic and catabolic pathways of the core sphingolipid signaling molecules.

molecule, sphingosine 1-phosphate. This compound was reviewed by Pyne and Pyne (2000) and has two major intracellular functions. First, it acts in a "sphingolipid rheostat" where the balance between such lipids determines cellular fate (Pyne, 2004). Second, it is involved in calcium homeostatis. Sphingosine 1-phosphate binds to specific G-protein coupled receptors (Kluk and Hla, 2002).

The general aspect of sphingolipids and signalling was reviewed by Smith and Merrill (2002).

A large proportion of the sphingolipids that are present in cells and tissues are the complex glycosphingolipids. These lipids accumulate in particular patterns in different cell types and species (Hakomori, 1981). The pattern of glycosphingolipids changes with cell growth, differentiation, viral transformation, ontogenesis, and oncogenesis (Kolter, 2004). Because of their widespread functions, there is increasing interest in them as therapeutic agents or targets (Gagnon and Saragovi, 2002).

The role of sphingolipids in storage diseases is covered in Section 11.5.

10.6.6 Endocannabinoids

The identification, in the 1960s, of brain-specific receptors for one of the major components of *Cannabis sativa* preparations led to the discovery of endogenous ligands for such receptors. These are known as the endocannabinoids.

The first determination and characterisation of a cannabinoid receptor in brain was by Devane et al. (1988). The first endocannabinoid identified and the best studied is anandamide (Devane et al., 1992). The second endocannabinoid found was 2-arachidonylglycerol and most other compounds contain the arachidonyl component (Kokotos, 2004). The endocannabinoids can be chemically synthesised (Razdan and Mahadevan, 2002) and have therapeutic possibilities (Goutopoulos and Makriyannis, 2002). A general review is that of Kokotos (2004). *N*-acylethanolamines that occur in significant amounts in certain plant tissues and which also bind to endocannabinoid receptors appear to have a role in plant defence (for review, see Chapman, 2004).

References

Becker, K.P. and Hannun, Y.A. (2004). Diacylglycerols. In *Bioactive Lipids* (Eds. A. Nicolaou and G. Kokotos), The Oily Press, Bridgwater, U.K., pp. 37–61.

Bell, R.M. et al. Eds. (1996). *Lipid Second Messengers, Handbook of Lipid Research,* Plenum Press, New York.

Berridge, M.J. (1987) Inositol triphosphate and diacylglycerol: two interacting second messengers. *Annu. Rev. Biochem.* **56**, 159–193.

Bocckino, S.B. and Exton, J.H. (1996). Phosphatidic acid. In *Lipid Second Messengers*, Eds. R.M. Bell, J.H. Exton and S.M. Prescott, Plenum Press, New York, pp. 1–58.

Chapman, K.D. (2004). Occurrence, metabolism and prospective function of *N*-acylethanolamines in plants. *Prog. Lipid Res.* **43**, 302–327.

Christie, W.W. (2003). *Lipid Analysis,* 3rd ed., The Oily Press, Bridgwater, U.K.

De Avalos, S.V. et al. (2004). Ceramides. In *Bioactive Lipids*, Eds. A. Nicolaou and. G. Kokotos, The Oily Press, Bridgwater, U.K., pp. 135–168.

Devane, W.A. et al. (1988) Determination and characterization of a cannabinoid receptor in rat brain. *Mol. Pharmacol*., **34**, 605–613.

Devane, W.A. et al. (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science*, **258**, 1946–1949.

Drøbak, B.K. (2005). Inositol-containing lipids: roles in cellular signalling. In *Plant Lipids: Biology, Utilisation and Manipulation*, Ed. D.J. Murphy, Blackwell Publishing, Oxford, U.K., pp. 303–328.

Farooqui, A.A. and Horrocks, L.A. (2004). Plasmalogens, platelet-activating factor and other ether glycerophospholipids. In *Bioactive Lipids*, Eds. A. Nicolaou and G. Kokotos, The Oily Press, Bridgwater, U.K., pp. 107–134.

Fukushima, N. et al. (2001) Lysophospholipid receptors. *Ann. Rev. Pharmacol. Toxicol.* **41**, 507–534.

Gagnon, M. and Saragovi, H.U. (2002). Gangliosides: therapeutic agents or therapeutic targets? *Expert Opin. Ther. Patents,* **12**, 1215–1223.

Goutopoulos, A. and Makriyannis, A. (2002) From cannabis to cannabinergics: new therapeutic opportunities. *Pharmacol. Ther.* **95**, 103–117.

Gurr, M.I. et al. (2002). *Lipid Biochemistry,* 5th ed., Blackwell Scientific, Oxford, U.K.

Hakomori, S. (1981) Glycosphingolipids in cellular interaction, differentiation and oncogenesis. *Annu. Rev. Biochem.* **50**, 733–764.

Hannun, Y.A. et al. (1996). Ceramide: a novel second messenger and lipid mediator. In *Lipid Second Messengers*, Eds. R.M. Bell, J.H. Exton and S.M. Prescott, Plenum Press, New York, pp. 75–123.

Hannun, Y.A. and Obeid, L.M. (2002) The ceramide-centric universe of lipid-mediated cell regulation: stress encounters of the lipid kind. *J. Biol. Chem.* **277**, 25847–25850.

Hilgemann, D.W. (2003). Getting ready for the decade of the lipids. *Annu. Rev. Physiol.* **65**, 697–700.

Honda, Z. et al. (2002) Platelet-activating factor receptor. *J. Biochem*. **131**, 773–779.

Ishii, S. and Shimizu, T. (2000). Platelet-activating factor (PAF) receptor and genetically engineered PAF receptor mutant mice. *Prog. Lipid Res.* **39**, 41–82.

Katso, R. et al. (2001) Cellular function of phosphoinositide 3-kinase: implication for development, immunity, homeostasis and cancer. *Ann. Rev. Cell Dev. Biol.* **17**, 615–675.

Karasawa, K. et al. (2003). Plasma platelet activating factor-acetylhydrolase (PAF-AH). *Prog. Lipid Res.* **42**, 93–114.

Kluk, M.J. and Hla, T. (2002) Signalling of sphinosine 1-phosphate via the SIP/EDG-family of G-protein coupled receptors. *Biochim. Biophys. Acta.* **1582**, 72–80.

Kobrinsky, E. et al. (2000). Reception-mediated hydrolysis of plasma membrane messenger PIP_2 leads to K^+ current desensitisation. *Nat. Cell Biol.* **2**, 507–514.

Kokotos, G. (2004). Endocannabinoids. In *Bioactive Lipids*, Eds. A. Nicolaou and G. Kokotos, The Oily Press, Bridgwater, U.K., pp. 245–264.

Kolter, T. (2004). Glycosphingolipids. In *Bioactive Lipids* (Eds. A. Nicolaou and G. Kokotos), The Oily Press, Bridgwater, U.K., pp. 169–196.

Laychock, S. and Rubin, R.P. Eds. (1999). *Lipid Second Messengers.* CRC Press, Boca Raton, FL.

Maijer, H.J.G. and Munnik, T. (2003). Phospholipid-based signalling in plants. *Annu. Rev. Plant Biol.* 54, 265–306.

Martin, T.F.J. (1998). Phosphoinositide lipids as signalling molecules: common themes for signal transduction, cytoskeletal regulation and membrane trafficking. *Annu. Rev. Cell Dev. Biol.* **14**, 231–264.

Merrill, A.H. et al. (1996). Bioactive properties of sphingosine and structurally related compounds. In *Lipid Second Messengers*, Eds. R.M. Bell, J.H. Exton and S.M. Presscott, Plenum Press, New York, pp. 205–237.

Nagan, N. and Zoeller, R.A. (2001) Plasmalogens: biosynthesis and functions. *Prog. Lipid Res.* **40**, 199–229.

Nicolaou, A. and Kokotos, G. Eds. (2004). Bioactive Lipids, The Oily Press, Bridgwater, U.K.

Nishizuka, Y. (1995) Protein kinase C and lipid signalling for sustained cellular responses. *FASEB J.* **9**, 484–496.

Payrastre, B. et al. (2001) Phosphoinositides: key players in cell signalling, in time and space. *Cell Signal*. **13**, 377–387.

Payrastre, B. (2004). Phosphoinositides. In *Bioactive Lipids*, Eds. A. Nicolaou and G. Kokotos, The Oily Press, Bridgwater, U.K., pp. 63–84.

Pettus, B.J. et al. (2002) Ceramide in apoptosis: an overview and current perspectives. *Biochim. Biophys. Acta.* **1585**, 114–125.

Pyne, S. and Pyne, N.J. (2002) Sphingosine 1-phosphate signalling and termination at lipid phosphate receptors. *Biochim. Biophys. Acta.* **1582**, 121–131.

Pyne, S. (2004). Lysolipids: sphingosine 1-phosphate and lysophosphatidic acid. In *Bioactive Lipids*, Eds. A. Nicolaou and G. Kokotos, The Oily Press, Bridgwater, U.K., pp. 85–106.

Razdan, R.K. and Mahadevan, A. (2002). Recent advances in the synthesis of the endocannabinoid-related ligands. *Chem. Phys. Lipids.* **121**, 21–33.

Rubin, R.P. and Laychock, S. Eds. (1999). *Lipid Second Messengers.* CRC Press, Boca Raton, FL.

Smith, W.L. and Merrill, A.H. (2002) Sphingolipid metabolism and signalling, mini review series. *J. Biol. Chem.* **277**, 25841–25842.

Snyder, F. (1995) Platelet-activating factor: the biosynthetic and catabolic enzymes. *Biochem. J.* **305**, 689–705.

Takai, Y. et al. (1979) Unsaturated diacylglycerol as a possible messenger for the activation of calcium-activated, phospholipid-dependent protein kinase system. *Biochem. Biophys. Res. Commun.* **91**, 1218–1224.

Testerink, C. and Munnik, T. (2005). Phosphatidic acid: a multifunctional stress signalling lipid in plants. *Trends Plant Sci.* **10**, 368–375.

Tigyi, G. and Parrill, A.B. (2003). Molecular mechanisms of lysophosphatidic acid action. *Prog. Lipid Res.* **42**, 93–114.

Toker, A. (2002) Phosphoinositides and signal transduction. *Cell Mol. Life Sci.* **59**. 761–779.

Tokumura, A. (1995). A family of phospholipid autacoids: occurrence, metabolism and bioactions. *Prog. Lipid Res.* **34**, 151–184.

Vance, D.E. and Vance, J.E. Eds. (2002). *Biochemistry of Lipids, Lipoproteins and Membranes,* 4th ed., Elsevier, Amsterdam.

Vanhaesebroceck, B. et al. (2001). Synthesis and function of 3-phosphorylated inositol lipids. *Annu. Rev. Biochem.* **70**, 535–602.

Vivanco, I. and Sawyers, C.L. (2002) The phosphatidylinositol 3-kinase-Akt pathway in human cancer. *Nature Rev.* **2**, 489–501.

Wang, X. et al. (2006). Signalling functions of phosphatidic acid. *Prog. Lipid Res.* **45**, 250–278.
Yin, H.L. and Janmey, P.A. (2003). Phosphoinositide regulation of the actin cytoskeleton. *Annu. Rev. Physiol.* **65**, 761–789.

10.7 Sterol esters

The esterification of cholesterol in animals has attracted considerable research because of the possible involvement of cholesterol and its ester in various disease states (see Glomset and Norum, 1973; Section 11.1, Section 11.3 and Section 11.6). Cholesteryl esters are formed by the action of lecithin cholesterol acyltransferase (LCAT, EC 2.3.1.43), which is particularly active in plasma; see Sabine (1977) for a review of cholesterol metabolism. The reaction involves transfer of a fatty acid from position-2 of the lecithin (phosphatidylcholine) to the 3-hydroxyl group of cholesterol with the formation of monoacylphosphatidylcholine. LCAT is a glycoprotein of mass 60 kDa (Fielding, 1990). LCAT consumes unesterified cholesterol and the cholesteryl ester is retained in the high-density lipoprotein (HDL) core while lysophosphatidylcholine is transferred to albumin. LCAT plays a critical role in the genesis of HDL. In addition, it may be able to directly reactivate lipid-poor HDL (Kendrick et al., 2001). The enzyme is a 416-amino acid serine hydrolase with rather limited sequence homology to other plasma lipases. Aspects of its structure and regulation are discussed by Fielding and Fielding (2002). ApoA1 is needed for both its acyltransferase and phospholipase activities. When LCAT interacts with low-density lipoprotein (LDL), it can catalyse phosphatidylcholine acyl exchange. LCAT is reviewed by Jonas (2000).

Cholesterol ester transfer protein (CETP) catalyses the movement of cholesteryl esters, triacylglycerols, and nonpolar lipids (such as retinyl esters) between plasma lipoproteins. CETP expression in hepatocytes is PPAR-dependent (Luo et al., 2001). Physiologically, the main effect of CETP may be to promote the transfer of LCAT-derived cholesteryl esters out of HDL (where they were formed) into VLDL and LDL, in exchange for triacylglycerol (see Fielding and Fielding, 2002); CETP is a glycoprotein of mass 53 kDa (Drayna et al., 1987). Its physiological function and mechanism of activity are discussed by Fielding and Fielding (1991). Activity of CETP in plasma is regulated by an inhibitor protein, which acts by displacing it from its lipoprotein binding sites (Morton and Zilverschmidt, 1981). It is structurally related to phospholipid transfer protein (PLTP), which transfers phospholipids between serum lipoprotein classes.

Cholesteryl esters, in contrast to free cholesterol, are taken up by cells mostly via specific receptor pathways (Brown et al., 1981), are hydrolysed by lysosomal enzymes and eventually re-esterified and stored within cells. Scavenger receptor B1 (SR-B1) is the important trans-membrane protein (Gu et al., 2000). Selective uptake from HDL needs SR-B1 binding. The receptor also promotes cholesterol efflux from the plasma membrane by an unknown mechanism. SR-B1 has been localised to cholesterol-rich microdomains called calveoli (Fielding and Fielding, 2002). LCAT may also participate in the movement of cholesterol out of cells by esterifying excess cholesterol in the intravascular circulation (cf. Marcel, 1982).

Schneider (2002) has written a useful review on lipoprotein receptors and their importance in plasma lipid metabolism. The purification and properties of LCAT, together with a discussion of its mechanism of reaction, are given by Marcel (1982). A number of disease states involve LCAT activity. Two LCAT deficiencies have been found. In one, no cholesteryl esters are formed in plasma and cholesterol accumulates as droplets in peripheral tissues. In a second disease, LCAT can transesterify cholesterol from VLDL and LDL, but not from exogenous HDL (Fielding and Fielding, 2002). A discussion of cholesteryl ester metabolism in relation to other liver diseases and dyslipoproteinaemia has been reported (Marcel, 1982). Similarly, the metabolism of cholesteryl esters in relation to arteries and arterial disease has been fully discussed (Kritchevsky and Kothari, 1978). Mammalian steroid sulfates have been reviewed by Farooqui (1981).

Sterol esters and acylated sterol glycosides have been detected in a number of plant tissues and, in some cases, can be quite significant components (Mudd, 1980). For sterol esters any one of palmitate, oleate, linoleate, or α-linoleate could be the principal fatty acyl component depending on the tissue. In acylated sterol glycosides, palmitate or linoleate are the most abundant fatty acids.

Synthesis of sterol esters by preparations from spinach leaves have been studied by Mudd's group (Mudd, 1980). Enzyme activity may have been associated with mitochondria and diacylglycerol was found to be the best acyl donor, although other lipids including phosphatidylcholine could also serve. The acyl donor for sterol ester synthesis in *Phycomyces blakesleanus* was also found to be phosphatidylcholine, as in animal tissues (Bartlett et al., 1974). For a summary of plant sterol ester biochemistry, refer to Goad et al. (1987).

The acylation of sterol glucosides in plant tissues has been studied by several workers. The research has been reviewed by Eichenberger (1977) and by Mudd (1980). The acylating enzymes are usually particulate and have been partly purified following solubilization. The best purification has been with an enzyme from *Gossipium* spp. (Forsee et al., 1976). Soluble preparations have been studied from carrot roots (cf. Eichenberger, 1977) and bean leaves (Heinz et al., 1975). The acyl donor for acylated sterol glucoside synthesis seems to vary with the preparation being studied. The particulate enzymes tend to use various phosphoglycerides, whereas the soluble enzymes utilise diacyldigalactosylglycerol much better. The bean leaf

enzyme would also use diacylgalactosylglycerol efficiently. The overall pathway seems to involve glucosylation of the sterol before acylation (see Mudd, 1980).

Work on sterol ester metabolism in insects has been reviewed by Thompson et al. (1973).

References

Bartlett, K. et al. (1974). Biosynthesis of sterol esters in *Phycomyces blakesleanus*. *Phytochemistry*, **13**, 1107–1113.

Brown, M.S. et al. (1981). Regulation of plasma cholesterol by lipoprotein receptors. *Science.* **212**, 628–635.

Drayna, D. et al. (1987). Cloning and sequencing of human cholesteryl ester transfer protein cDNA. *Nature.* **327**, 632–634.

Eichenberger, W. (1977). Steryl glycosides and acylated steryl glycosides. In *Lipids and Lipid Polymers from Higher Plants*, Eds. M. Tevini and H.K. Lichtenthaler, Springer-Verlag, Berlin, pp. 167–179.

Farooqui, A.A. (1981). Metabolism of sulfolipids in mammalian tissues. *Adv. Lipid Res.* **18**, 159–202.

Fielding, C.J. (1990). Lecithin: cholesterol acyltransferase. In *Advances in Cholesterol Research,* Eds. M. Esfahani and J.B. Swaney, Telford, Caldwell, NJ, pp. 271–314.

Fielding, P.E. and Fielding, C.J. (1991). Dynamics of lipoprotein transport in the circulatory system. In *Biochemistry of Lipids*, Eds. D.E. Vance and J. Vance, Elsevier, Amsterdam, pp. 427–459.

Fielding, P.E. and Fielding, C.E. (2002). Dynamics of lipoprotein transport in the human circulatory system. In *Biochemistry of Lipids, Lipoproteins and Membranes*, Eds. D.E. Vance and J.E. Vance, 4th ed., Elsevier, Amsterdam, pp. 527–552.

Forsee, W.T. et al. (1976). Acylation of steryl glucosides by phospholipids. Solubilization and properties of the acyl transferase. *Arch. Biochem. Biophys.*, **172**, 410–418.

Goad, L.J. et al. (1987). The steryl esters of higher plants. In *The Metabolism, Structure and Function of Plant Lipids*, Eds. P.K. Stumpf, J.B. Mudd and W.D. Nes, Plenum, New York, pp. 95–102.

Glomset, J.A. and Norum, K.R. (1973). The metabolic role of lecithin: cholesterol acyltransferase: perspectives from pathology. *Adv. Lipid Res.* **11**, 1–65.

Gu, X. et al. (2000) Scavenger receptor, class B type 1-mediated ^{3}H-cholesterol efflux to high and low density lipoproteins is dependent on lipoprotein binding to the receptor. *J. Biol. Chem.* **275**, 29993–30001.

Heinz, E. et al. (1975). Enzymatic acylation of steryl glycoside. *Z. Pflanzenphysiol.*, **75**, 78–87.

Jonas, A. (2000) Lecithin cholesterol acyltransferase. *Biochim. Biophys. Acta.* **1529**, 245–256.

Kendrick, J.S. et al. (2001) Superior role of apolipoprotein B-48 over apolipoprotein B100 in chylomicron assembly and fat absorption: an investigation of apobec-1 knockout and wild type mice. *Biochem. J.* **356**, 821–827.

Kritchevsky, D. and Kothari, H.V. (1978). Arterial enzymes of cholesteryl ester metabolism. *Adv. Lipid Res.* **16**, 221–266.

Luo, Y. et al. (2001) The orphan nuclear receptor LRH-1 potentiates the sterol-mediated induction of the human CETP gene by liver X receptor. *J. Biol. Chem.* **276**, 24767–24773.

Marcel, Y.L. (1982). Lecithin: cholesterol acyltransferase and intravascular cholesterol transport. *Adv. Lipid Res.* **19**, 85–136.

Morton, R.E. and Zilverschmidt, D.B. (1981). A plasma inhibitor of triglyceride and cholesteryl ester transfer activities. *J. Biol. Chem.* **256**, 11992–11995.

Mudd, J.B. (1980). Sterol interconversions. In *Biochemistry of Plants,* vol. 4, Eds. P.K. Stumpf and E.E. Conn, Academic Press, New York, pp. 509–534.

Sabine, J. R. (1977). *Cholesterol,* Marcel Dekker, New York.

Schneider, W.J. (2002) Lipoprotein receptors. In *Biochemistry of Lipids, Lipoproteins and Membranes*, Eds. D.E. Vance and J.E. Vance, 4th ed., Elsevier, Amsterdam, pp. 553–572.

Thompson, M.J. et al. (1973). Metabolism of steroids in insects. *Adv. Lipid Res.* **11**, 219–265.

10.8 Control mechanisms

It is clearly important for organisms to be able to control the activity of individual enzymes and, hence, the rate of flux down a pathway. This may be as an adaptation or response to an environmental stress, during development or because of disturbances in the normal *status quo.* One important point to note is that there is (contrary to what is said in many textbooks) no such thing as a rate-controlling enzyme in a pathway. All enzymes can contribute to control, but their contribution can vary with circumstances. An excellent review of the control of metabolism is that by Fell (1997). General comments on the regulation of lipid metabolism will be found in Gurr et al. (2002).

Individual aspects of control mechanisms are detailed in the earlier sections of this chapter, but a few additional remarks will be made here. For a recent summary of the regulation of different aspects of lipid metabolism, see Vance and Vance (2002); for plant metabolism, refer to Browse and Somerville (1991), Quinn and Harwood (1990), and Murphy (2005); and for microbial aspects, see Ratledge and Wilkinson (1988, 1989).

The overall control of lipogenesis has been reviewed by Saggerson (1980) and the mechanisms by which carbohydrates regulate expression of lipogenic genes, by Girard et al. (2002). So far as saturated fatty acid synthesis is concerned, the activity of both acetyl-CoA carboxylase and fatty acid synthase can be altered in various ways (Gurr et al., 2002). Short-term or acute control involves metabolic or allosteric regulation and the covalent modification of enzymes. Long-term control involves alterations in the amounts of enzyme protein (Wakil et al., 1983).

Because acetyl-CoA carboxylase catalyses the first committed step in lipid synthesis and because its substrate lies at a crossroads between carbohydrate and lipid metabolism, its regulation is clearly important. Mammalian acetyl-CoA carboxylase is regulated both acutely (by phosphorylation/dephosphorylation, by acyl-CoAs, and by tricarboxylic acids) and chronically due to changes in enzyme amounts (see Gurr et al., 2002). Citrate not only

causes polymerisation of acetyl-CoA carboxylase, but also can overcome inhibition caused by the enzyme product malonyl-CoA or the overall products of fatty acid synthesis, acyl CoAs (Allred and Reilly, 1997). See also Kim (1997) for a general discussion.

The overall regulation of fatty acid synthesis in plants has been reviewed (Ohlrogge and Jaworski, 1997). The role of acetyl-CoA carboxylase in leaves was specifically addressed by flux control experiments and it was shown that this enzyme could exert up to 60% of the total control of flux towards lipid synthesis in the light (Page et al., 1994). The mechanism of regulation may involve changes in the enzyme's redox state (Harwood, 1996; Rawsthorne, 2002).

Cronan and Waldrop (2002) have given a very good recent survey of multisubunit acetyl-CoA carboxylases with particular emphasis on that from *E. coli.* They discuss the physiology, catalytic mechanism and function of the enzyme. Heath et al. (2003) also describe the regulation of *E. coli* acetyl-CoA carboxylase.

Mammalian fatty acid synthase is also subject to adaptive changes in enzyme content. Any short-term metabolic control is ill defined (Wakil et al., 1983) and generally thought to be unimportant (Semenkovich, 1997). However, diet, triiodothyronine, hydrocortisone and insulin effects have been noted on the amount of synthase protein. Hydrocortisone and triiodothyronine have no effect alone, but potentiate the insulin induction of synthase (Wakil et al., 1983). The increases in synthase activity on refeeding or insulin administration are due to an increase in transcription of mRNA, which is elevated 70-fold (Morris et al., 1982). The mechanisms underlying fatty acid synthase regulation are reviewed by Semenkovich (1997). Rangan and Smith (2002) also discuss the regulation of fatty acid synthesis.

A thorough discussion of the regulation of fatty acid synthase in plants has been made by Ohlrogge and Jaworski (1997) and recently updated (Harwood, 2005). Rawsthorne (2002) also covered some general aspects of fatty acid synthesis in relation to seed oil production.

Heath et al. (2002) have described the overall regulation of fatty acid formation in *E. coli* from a quantitative and qualitative viewpoint. This article mentions the acetyl-CoA carboxylase accBC operon as well as the fab (fatty acid synthase) cluster. The role of the FadR protein in transcriptional control through the regulation of acyl-CoA concentrations is important in altering the balance between fatty acid synthesis and oxidation.

It is well known that changes in growth temperature lead frequently to a modification in the pattern of fatty acids made and in those accumulated in the membranes of poikilotherms. Typical changes include an increase in unsaturated or of shorter chain-length fatty acids at lower growth temperatures. The adaptation has been studied in a large number of organisms. In anaerobic bacteria, such as *E. coli,* it is not possible for desaturases to be induced, so that alterations in saturated/unsaturated fatty acid synthesis have to be controlled via fatty acid synthase. At lower temperatures the amount of *cis*-vaccenic acid is rapidly increased due to increased activity of β-ketoacyl-ACP synthase II. Overexpression of β-ketoacyl-ACP I can also increase the amount of vaccinate, but in a temperature-independent manner. At low temperatures, vaccenate is also transferred to the *sn*-1 position of glycerolipids (where it competes with palmitate), whereas it is usually concentrated at the *sn*-2 position. However, the mechanism for the apparent change in acyltransferase selectivity is unknown (Heath et al., 2002). Growth temperature has also been reported to alter the fatty acid products of other fatty acid synthases. For example, the *Saccharomyces cerevisiae* synthase produces more palmitate rather than stearate at lower growth temperatures (Okuyama et al., 1979).

There has been a considerable advance in our understanding of the control of aerobic fatty acid desaturases in recent years. In the simple protozoa, *Tetrahymena* and *Acanthamoeba*, low temperatures induce an increase in desaturase activity in order that the organisms can maintain membrane fluidity (Gurr et al., 2002). In the case of *Acanthamoeba*, the desaturase concerned with temperature adaptation is a Δ12 (ω6) oleate desaturase which produces linoleate (Avery et al., 1995). It is also induced independently by oxygen (Rutter et al., 2002).

Another class of organism where there has been considerable study of temperature adaptation is the cyanobacteria. Induction of a number of individual desaturases and their mechanism of control have been well reviewed (Murata and Wada, 1995; Mikami and Murata, 2003). General aspects of temperature adaptation in different organisms will be found in Cossins (1994).

For animals, considerable attention has been paid to the effects of diet on desaturase induction. In particular, there has been much study of stearoyl-Δ9-desaturase, which can show extreme responses (100-fold changes in activity) to dietary manipulation. A thorough review of the earlier work in this area was made by Ntambi (1995), who discussed tissue-specific expression and the ability of carbohydrate or unsaturated fatty acids to regulate the stearoyl-CoA desaturase genes. The molecular mechanisms by which dietary polyunsaturated fatty acids could regulate genes (including those of glucose or fatty acid metabolism) is discussed by Jump et al. (1996) and developed further by Clarke (2000).

Recently Ntambi and Miyazaki (2004) have revisited the topic of the mammalian stearoyl-CoA desaturases including aspects of the specific functions of isoforms and contribution of the enzyme activity to the regulation of metabolism.

In addition to fatty acid synthesis, the assembly of complex acyl lipids is also under careful metabolic control. For general remarks, see Gurr et al. (2002) and Vance and Vance (2002).

The regulation of the enzymes of triacylglycerol synthesis has been well reviewed by Coleman and Lee (2004) with reference to earlier work and to the use of sterol regulatory element-binding protein (SREBP), the liver X receptors and PPARs (peroxisome proliferator-activated receptors) in the regulation. SREBP is, of course, also involved in the relative rates of fatty acid and cholesterol biosynthesis (Gibbons, 2003). Interactions between phospholipids and sterol metabolism in mammalian cells are reviewed by Ridgway et al. (1999), while a comprehensive account of the regulation of phosphoglyceride synthesis and degradation in different organisms will be found in Hawthorne and Ansell (1982); see also Kent (1995) and Vance (1998); and see also various chapters in Vance and Vance (2002).

Flux control analysis has been applied to the study of lipid accumulation in oil crops. Experiments with olive and palm have shown that fatty acid synthesis exerts more control than lipid assembly (Ramli et al., 2002), but that, within the Kennedy pathway, diacylglycerol acyltransferase may be important in some crops (Ramli et al., 2005). The regulation of storage oil accumulation has been discussed by Voelker and Kinney (2001), Rawsthorne (2002) and, recently, by Weselake (2005).

The regulation of plant lipid metabolism was reviewed by Harwood (1989) and, with respect to environmental stress, by Harwood (1994, 1998).

For a review of phospholipid metabolism in yeast and its interrelationship with other metabolic processes, see Carman and Henry (1999), and for metabolic regulation of phospholipids in *E. coli* consult Shibuya (1992) and Cronan (2003).

References

Allred, J.B. and Reilly, K.E. (1997). Short-term regulation of acetyl-CoA carboxylase in tissues of higher animals. *Prog. Lipid Res.* **35**, 371–385.

Avery, S.V. et al. (1995). Temperature dependent changes in plasma membrane lipid order and phagocytotic activity of the amoeba *Acanthamoeba castellanii* are closely related. *Biochem. J.* **312**, 811–816.

Browse, J. and Somerville, C.R. (1991). Glycerolipid synthesis-biochemistry and regulation. *Annu. Rev. Plant Physiol*. **42**, 467–506.

Carman, G.M. and Henry, S.A. (1999). Phospholipid synthesis in the yeast *Saccharomyces cerevisiae* and interrelationships with other metabolic processes. *Prog. Lipid Res.* **38**, 361–399.

Clarke, S.D. (2000). Polyunsaturated fatty acid regulation of gene transcription: a mechanism to improve energy balance and insulin resistance. *Brit. J. Nutr.* **83** (Supplement 1) S59–S66.

Coleman, R.A. and Lee, D.P. (2004) Enzymes of triacylglycerol synthesis and their regulation. *Prog. Lipid Res.* **43**, 134–176.

Cossins, A.J. Ed. (1994). *The Temperature Adaptation of Biological Membranes*, Portland Press, London.

Cronan, J.E. (2003) Bacterial membrane lipids: where do we stand? *Annu. Rev. Microbiol.* **57**, 203–224.

Cronan, J.E. and Waldrop, G.L. (2002) Multi-subunit acetyl-CoA carboxylases. *Prog. Lipid Res.* **41**, 407–435.

Fell, D.A. (1997) *Understanding the Control of Metabolism.* Portland Press, London.

Gibbons, G.F. (2003) Regulation of fatty acid and cholesterol synthesis: co-operation or competition? *Prog. Lipid Res.* **42**, 479–497.

Girard, J. et al. (1997) Mechanisms by which carbohydrates regulate expression of genes for glycolytic and lipogenic enzymes. *Annu. Rev. Nutr.* **17**, 325–352.

Gurr, M.I. et al. (2002) *Lipid Biochemistry,* 5th ed., Blackwell Scientific, Oxford, U.K.

Hardie, D.G. (1992) Regulation of fatty acid and cholesterol metabolism by the AMP-activated protein kinase. *Biochim. Biophys. Acta.* **1123**, 231–238.

Harwood, J.L. (1989) Lipid metabolism in plants. *Crit. Rev. Plant Science.* **8**, 1–44.

Harwood, J.L. (1994) Environmental factors affecting lipid metabolism. *Prog. Lipid Res.*, **33**, 193–202.

Harwood, J.L. (1996) Recent advances in the biosynthesis of plant fatty acids. *Biochim. Biophys. Acta.* 1210, 369–372.

Harwood, J.L. (1998) Involvement of chloroplast lipids in the reaction of plants submitted to stress. In *Lipids in Photosynthesis: Structure, Function and Genetics*, Eds. P-A Siegenthaler and N. Murata, Kluwer, Dordrecht, pp. 287–302.

Harwood, J.L. (2005) Fatty acid biosynthesis. In *Plant Lipids: Biology, Utilisation and Manipulation*, Ed. D.J. Murphy, Blackwell Publishing, Oxford, U.K., pp. 27–66.

Hawthorne, J.N. (1982). Inositol phospholipids. In *Phospholipids*, Eds. J.N. Hawthorne and G.B. Ansell, Elsevier Biomedical Press, Amsterdam, pp. 263–278.

Hawthorne, J.N. and Ansell, G.B. Eds. (1982). *Phospholipids,* Elsevier, Amsterdam.

Heath, R.J. et al. (2002) Fatty acid and phospholipid metabolism in prokaryotes. In *Biochemistry of Lipids, Lipoproteins and Membranes*, Eds. D.E. Vance and J.E. Vance, 4th ed., Elsevier, Amsterdam, pp. 55–92.

Jump, D.B. et al. (1996) Dietary polyunsaturated fatty acid regulation of gene transcription. *Prog. Lipid Res.* **35**, 227–241.

Kent, C. (1995) Eukaryotic phospholipid biosynthesis. *Annu. Review Biochem.* **64**, 315343.

Kim, K-H. (1997) Regulation of mammalian acetyl-CoA carboxylase. *Annu. Rev. Nutr.* **17**, 77–99.

Lane, M.D. et al. (1979). Hormonal regulation of acetyl-CoA carboxylase activity in the liver cell. CRC *Crit. Rev. Biochem.* **2**, 121–141.

Mikami, K. and Murata, N. (2003) Membrane fluidity and the perception of environmental signals in cyanobacteria and plants. *Prog. Lipid Res.* **42,** 527–543.

Morris, S.M. et al. (1982). Molecular cloning of gene sequences for avian fatty acid synthase and evidence for nutritional regulation of fatty acid synthase mRNA concentration. *J. Biol. Chem.* **257**, 3225–3229.

Murata, N. and Wada, H. (1995) Acyl-lipid desaturases and their importance in the tolerance and acclimatization to cold of cyanobacteria. *Biochem. J.* **308**, 1–8.

Murphy, D.J. Ed. (2005) *Plant Lipids: Biology, Utilisation and Manipulation.* Blackwell Publishing, Oxford, U.K.

Ntambi, J.M. (1995) Regulation of stearoyl-CoA desaturase. *Prog. Lipid Res.* **34**, 139–150.

Ntambi, J.M. and Miyazaki, M. (2004) Regulation of stearoyl-CoA desaturases and their role in metabolism. *Prog. Lipid Res.* **43**, 91–104.

Numa, S. (1991). Two long-chain acyl-coenzyme A synthetases: their different roles in fatty acid metabolism and its regulation. *Trends Biochem. Sci.* **6,** 113–115.

Ohlrogge, J.B. and Jaworski, J.G. (1997) Regulation of fatty acid synthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **48**, 109–136.

Okuyama, H. et al. (1979). Regulation by temperature of the chain length of fatty acids in yeast. *J. Biol. Chem.* **254**, 12281–12284.

Quinn, P.J. and Harwood, J.L. Eds. (1990) *Plant Lipid Biochemistry, Structure and Utilization,* Portland, London.

Ramli, U.S. et al. (2002) Control analysis of lipid biosynthesis in tissue cultures from oil crops shows that flux control is shared between fatty acid synthesis and lipid assembly. *Biochem. J.* **364**, 393–401.

Ramli, U.S. et al. (2005) Metabolic control analysis reveals an important role for diacylglycerol acyltransferase in olive but not in oil palm lipid accumulation. *FEBS J.* **272**, 5764–5770.

Rangan, V.S. and Smith, S. (2002) Fatty acid synthesis in eukaryotes. In *Biochemistry of Lipids, Lipoproteins and Membranes*, Eds. D.E. Vance and J.E. Vance, 4th ed., Elsevier, Amsterdam, pp. 151–179.

Ratledge, C. and Wilkinson, S.G. Eds. (1988) *Microbial Lipids*, vol. 1, Academic Press, London.

Ratledge, C. and Wilkinson, S.G. Eds. (1989) *Microbial Lipids*, vol. 2, Academic Press, London.

Rawsthorne, S. (2002) Carbon flux and fatty acid synthesis in plants. *Prog. Lipid Res.* **41**, 182–196.

Ridgway, N.D. et al. (1999) Integration of phospholipid and sterol metabolism in mammalian cells. *Prog. Lipid Res.* **38**, 337–360.

Rutter, A.J. et al. (2002) Oxygen induction of a novel fatty acid *n*-6 desaturase in the soil protozoan, *Acanthamoeba castellanii. Biochem. J.* **368**, 57–67.

Saggerson, E.D. (1980). Regulation of lipid metabolism in adipose and liver cells. *Biochemistry of Cellular Regulation*, vol. 2, CRC Press, Boca Raton, FL, pp. 207–256.

Semenkovich, C.F. (1997) Regulation of fatty acid synthase. *Prog. Lipid Res.* **36**, 43–53.

Shibuya, I. (1992) Metabolic regulation and biological functions of phospholipids in *E. coli*. *Prog. Lipid Res.* **31**, 245–300.

Stubbs, C.D. and Smith, A.D. (1984). The modification of mammalian membrane polyunsaturated fatty acid composition in relation to membrane fluidity and function. *Biochim. Biophys. Acta.* **779**, 89–137.

Vance, D.E. and Vance, J.E. Eds. (2002) *Biochemistry of Lipids, Lipoproteins and Membranes*. 4th ed., Elsevier, Amsterdam.

Vance, J.E. (1998) Eukaryotic lipid biosynthetic enzymes: the same but not the same. *Trends Biochem. Sci.* **23**, 423–428.

Voelker, T. and Kinney, A.J. (2001) Variations in the biosynthesis of seed storage lipids. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **52**, 335–361.

Volpe, J.J. and Vagelos, P.R. (1976). Mechanisms and regulation of biosynthesis of saturated fatty acids. *Physiol. Rev.* **56**, 339–417.

Wakil, S.J. et al. (1983). Fatty acid synthesis and its regulation. *Annu. Rev. Biochem*. **52**, 537–539.

Yeh, L.A. et al. (1981). Coenzyme A activation of acetyl-CoA carboxylase. *J. Biol. Chem.* **256**, 2289–2296.

11

MEDICAL AND AGRICULTURAL ASPECTS OF LIPIDS

J.L. Harwood, M. Evans, D.P. Ramji, D.J. Murphy and P.F. Dodds

11.1 Human dietary requirements

11.1.1 Introduction

There are two aspects to lipid requirements in the human diet — qualitative and quantitative. First, certain lipids are needed for good health — essential fatty acids and fat-soluble vitamins are good examples. Secondly, it is usually considered that, in the normal diet, some 25 to 30% of the total calories are conveniently supplied as fat (Jones, 1974). Such lipids (in reasonable amounts) also usually make food more palatable. Some comprehensive sources of information on the role of fats in nutrition are Gurr (1992b, 1999), Vergroesen and Crawford (1989), and Akoh and Lai (2005).

The human diet has always contained fat, but the amounts and types vary. Typical intakes in Europe and North America are between 80 and 150 g/day, which represents 30 to 40% of dietary calories. The nutrient fat per capita has been maintained, with a slight overall rise since 1900. However, the same foods are not always responsible for the fat consumed (Rizek et al., 1974). Salad and cooking oils have always been major contributors, followed by dairy products and shortening in the period 1910 to 1930, but by margarine, shortening, and meat in the period 1930 to 1960. Since that time, the increase in dietary fat has been due almost entirely to a rise in meat consumption. Moreover, current consumption of fat in different parts of the world varies markedly. In Asian countries, there is minimal dietary fat, whereas Inuits (North American Eskimos) consume 300 g/day, an amount that would nauseate a European.

Because a diet lacking in fat tends to be bulky, some rough rules can apply. If total intake is <3000 kcal/day, the fat should make up at least 25% of the calories and for intakes of >3000 kcal/day, it should represent at least 30% of the calories. This gives minimum intakes of 56 to 140 g/day. Of course, there are circumstances when dietary fat should be limited (see below and Section 11.2 and Section 11.3). In a survey in the U.S., it was found that intake of fat increased with higher incomes. This correlation may reflect a greater consumption of food or, alternatively, the selection of more expensive foods, such as meat or dairy products instead of cereals (Rizek et al., 1974). Pearson and Dutson (1990) give information on meat lipids, including several chapters dealing with dietary lipids and health, while Gregory et al. (1990) provide data on fat intakes and blood lipid levels for U.K. subjects.

Major food types that contribute to fat consumption in the U.K. are indicated in Table 11.1. For the U.S., the intake from fats given by Rizek et al. (1974) was 35% for salad and cooking oil, 23% for shortening, 15% for margarine, 8% for butter, and the remaining 19% from animal sources (meat, lard, etc.). It will be clearly seen from Table 11.1 that dairy products, meats, spreads, and cooking oils are the major contributors. Although not reflected in the table, there has been a significant shift in the sources of fats consumed in recent years. Thus, vegetable fats (margarine, cooking oils) are increasingly important when compared to animal fats (dairy products, lard). For example, the contribution of vegetable fats to the total in the U.S. increased from 17 to 38% in the period 1900 to 1950. This shift has raised the contribution of *n*-6 polyunsaturated fatty acids from 3 to 6% of the total dietary calories. In addition, the reduction in animal fats has lowered cholesterol consumption (cf. Section 11.3). Gold et al. (1992) have discussed the possible connections between cholesterol and coronary heart disease and Gurr (1992a) has

TABLE 11.1 Daily per capita fat consumption in the U.K.

Food component	Total fat		Fatty acids			Vitamin A or equivalent	Vitamin D (μg)
	(g)	(% total)	Saturated	Monounsaturated	Polyunsaturated	(μg)	
Milk/cream	16.5	15.6	10.4	5.0	0.4	178	0.33
Cheese	4.3	4.1	2.7	1.3	0.1	62	0.05
Meat	29.0	27.4	12.5	12.7	2.5	283	0.02
Fish	1.1	1.0	0.3	0.4	0.4	2	0.48
Eggs	3.2	3.0	1.2	1.4	0.4	41	0.44
Butter	18.7	17.7	11.6	5.6	0.5	228	0.28
Margarine	8.6	8.1	3.1	3.4	1.7	101	0.83
Cooking fats	10.9	10.3	3.9	4.8	1.7	–	0.02
Vegetables	1.5	1.5	0.2	0.6	0.7	280	Trace
Fruits	0.5	0.5	0.2	0.2	0.1	13	–
Bread	1.5	1.4	0.5	0.4	0.5	1	–
Cakes, pastries	2.7	2.5	1.0	1.1	0.5	9	0.09
Biscuits	4.5	4.3	2.1	1.9	0.3	–	–
Other cereals	1.3	1.2	0.5	0.5	0.3	6	0.08

Source: Household Food Consumption and Expenditure, HMSO, London.

critically reviewed the epidemiological evidence for such interactions.

Fats are broken down by digestion and absorbed through the alimentary tract. They are moved around the body in association with various plasma proteins (see Gurr et al. (2002) for a general description). Disorders in digestion and absorption will lead to impaired fat intake (Sickinger, 1975). Digestive problems can be caused by pancreatic insufficiency, hepatic insufficiency, gastric disturbances, or bacterial proliferation. In addition, malabsorption can be due to gut sensitisation or failure to adequately produce chylomicrons (Gurr et al., 2002). Principal fat components of a typical European or North American diet are triacylglycerol >> phospholipid = glycolipid > sterols. In processed food, there is often a small amount of monoacylglycerol and antioxidants, such as α-tocopherol, may be added. Fat-soluble vitamins may be present naturally or added. The process of digestion is beyond the scope of this review, but a clear, simple description of it will be found in Gurr and Harwood (1991). The use of medium-chain triacylglycerols in patients with digestive problems is discussed in Babayan (1974), Vergroesen (1975) and Thomas and Holub (1994).

Triacylglycerols are usually 97 to 100% digested and the products of this process are absorbed into intestinal cells where resynthesis takes place. Unsaturated triacylglycerols are hydrolysed faster than saturated ones. Cholesterol is absorbed as the free sterol and then re-esterified, as necessary, for transport in the lymph. Bile may act as a cofactor for cholesterol esterase and improves cholesterol absorption, but plant sterols inhibit this absorption. Indeed, plant sterols (sitosterol, stigmasterol) and ergosterol are themselves rather poorly absorbed (Boyd, 1975). Thus, commercial margarines containing plant sterols and/or their derivatives are increasingly important as a means of lowering blood cholesterol (see Section 11.2 and Section 11.3). Part of their action is to lower cholesterol absorption (see Moreau et al. (2002) for review).

Although dietary fats increase cholesterol absorption by raising bile flow, saturated lipids are more effective in *net* terms because unsaturated fats also increase cholesterol excretion. The rate of cholesterol absorption is influenced by the age of the subject, their previous dietary history, genetic factors, the amount of cholesterol, the type of fat consumed simultaneously and the frequency of cholesterol intake. Similarly, overall fat absorption is affected by the type and amount of fat consumed, the age of the individual, the presence of emulsifying agents, whether the food has been heated above 250°C (when fat becomes less digestible), and the presence of calcium (Jones, 1974; Vergroesen, 1975).

Malabsorption of fats can be measured with ^{131}I-labelled fats. Major problems of maldigestion or malabsorption, which can give rise to poor assimilation of dietary lipids, are listed in Table 11.2. Of the complaints listed, steatorrhea is the most common, but does not have pathological significance until the stool fat is greater than 10%. However, it causes distress and inconvenience and, when severe, wasting (loss of salts and liquid, and insufficient uptake of nutrients) and deficiencies (e.g., of fat-soluble vitamins, with vitamin D the first to show). For a fuller discussion of these medical aspects, see Petersdorf et al. (1983).

11.1.2 Food processing

The fatty acid of composition of foods can be affected by agricultural practice (see Section 11.8), but, usually, the biggest effects are those produced by industrial processing. Catalytic hydrogenation (see Section 4.2) is carried out to improve oxidative stability and physical properties. If oxidative stability is increased, then there is less chance of oxidation creating poor flavour and colour and giving rise to toxic compounds. Physical properties are mainly changed by "hardening" so that there are better textural

TABLE 11.2 Disorders leading to poor assimilation of dietary fats

Maldigestion	Pancreatic insufficiency	Pancreatitis
		Pancreatic tumour
		Malnutrition (e.g., Kwashiorkor)
		Cystic fibrosis side-effect
		Pancreatic lipase mutation
	Hepatic insufficiency	Liver disease
		Bilary obstruction
	Gastric disturbances	Abnormal acid secretion
	Ileum abnormality	Poor reabsorption of bile
Malabsorption	Intestinal defects	Bacterial invasion (Tropical spruce, Whipple's disease)
		Sensitisation (e.g., gluten sensitivity in coeliac disease)
		Impared chylomicron formation (e.g., Anderson's disease)
	Steatorrhea	Bacterial invasion

properties (Gurr et al., 2002). The analysis of lipid oxidation is covered in Kamal-Eldin and Pokorny (2005).

Chemically, there are three main results of hydrogenation: (1) the total number of double bonds are reduced, (2) some of the *cis* double bonds are isomerised to *trans,* and (3) the double bonds may be shifted from their original positions. Of these effects, it is the increase in *trans*-unsaturated fatty acids that has attracted the most attention. Although *trans* fatty acids are found naturally and are consumed at the rate of 5 to 7 g/day in the U.K. (British Nutrition Foundation, 1987), there is considerable evidence for adverse effects on health (Gurr, 1996). This has led to the increasing use of interesterification for triacylglycerol modification (Gunstone, 1998) or to the use of different dietary lipid sources (see Section 11.8). It has also led to a requirement for labelling food packaging with the *trans* fatty acid content and to attempts to minimise their intake (Hunter, 2004).

Other processes that can cause lipid changes in food include heating and irradiation. Heating, where there is little contact with air (e.g., deep fat fryer), gives rise to a gradual accumulation of polymeric products (see, e.g., Varela and Ruiz-Rozo, 2000). Provided this oil is not reused excessively, these polymers do not cause problems (Gurr et al., 2002). Heating in the presence of oxygen and, particularly, if there are trace metal catalysts (e.g., iron, copper) can cause lipid peroxidation and production of reactive oxygen species. A major harmful side effect is the loss of antioxidant nutrients, such as vitamin E or carotenes (Gurr, 1988). Little peroxidised lipid is thought to be absorbed intact, but there is some evidence for liver toxicity and gut damage (Gurr, 1999). Any absorbed oxidised cholesterol may have health implications (Gurr et al., 2002). Lipid oxidation may be reduced by the presence of lipid-soluble antioxidants, either natural (e.g., carotenoids, vitamin E) or synthetic (butylated hydroxytoluene, BHT or butylated hydroxyanisole, BHA).

Irradiation is used to kill pathogens in some types of food and may generate lipid radicals. Vitamins E and K are particularly susceptible to radiation damage, but not, apparently, carotenoids (Gurr et al., 2002).

11.1.3 Specific dietary lipids that may be harmful

The possible deleterious effects of high quantities of medium- or long-chain saturated fatty acids or cholesterol in the diet is touched on in several sections of Chapter 11; however, there are also specific lipids that are not known to have any nutritional benefit and that may be present in the diet.

Trans unsaturated fatty acids were mentioned above (Section 11.1.2) and epidemiological studies of human population and controlled dietary experiments with human subjects have been reported. Some, but not all, of these studies show a correlation of the intake of certain types of *trans* fatty acids and increased risk of coronary heart disease (see Recommendations of the European Atherosclerosis Society, 1992 and Willett et al., 1993). In general, though, the evidence for harmful effects of *trans* unsaturated fatty acids (at least, at normal dietary concentrations) is not very persuasive (Gurr, 1999; Gurr et al., 2002). Despite this, in 2006 the U.S. Food and Drug Administration (FDA) made it mandatory to declare the amount of *trans* fat present in foods. These concerns have led to research on solutions or alternatives to *trans* fatty acids in foods (see Kodali and List, 2005).

Cottonseed oil is the only important oil in the human diet that contains cyclopropene fatty acids. Because such acids (as sterculic acid) fed at 5% of dietary energy to rats caused death and at the 2% level caused disturbances of reproduction, there has been concern about the effect in humans. However, their concentration is low (0.6 to 1.2%) in cottonseed oil and reduced to 0.1 to 0.5% by processing. There has been no evidence that consumption of cottonseed oil in manufactured products has any harmful nutritional effects (Gurr et al., 2002).

Very long-chain monounsaturated fatty acids (such as erucic acid, 22:1*n*-9), when fed to rats at 5% or more of their total energy requirements, caused a buildup of triacylglycerols in heart muscle. Other pathological changes were also noticeable (Gurr et al., 2002). Despite lack of evidence for harmful effects in man, breeding programmes were intitiated to replace older varieties of oilseed rape (up to 45% erucate in its oil) with zero erucate or canola

varieties (see Section 11.8.2.3). The use of such varieties in most industrial countries is now mandatory; however, high erucate varieties are still used extensively in China. Furthermore, although fish oils are consumed for their desirable enrichment with the *n*-3 PUFAs (polyunsaturated fatty acids), eicosapentaenoic acid, and decosahexaenoic acid, they often contain high concentrations of 20:1*n*-9 and 22:1*n-9.* Moreover, certain fat spreads that incorporate hardened fish oils may also have significant concentrations of such acids. The long-term consequences (beneficial or otherwise) of consuming marine long-chain monoenes have been poorly researched (Gurr et al., 2002).

Conjugated linolenic acids (CLAs) are a group of geometric and positional isomers of linolenic acid and can be formed by biohydrogenation and oxidation processes in nature. They are significant components of dairy products where they are produced in the rumen by microbiological action. The main form of CLA is usually *cis*-9, *trans*-11-18:2, with *trans*-10, *cis*-12-18:2 the next most abundant. Adverse effects of CLAs have been demonstrated in animal studies, but it is not clear whether similar actions are applicable to humans. On the other hand, a surprising number of health benefits have been attributed to CLAs. This topic is well reviewed by Wahle et al. (2004).

We should not close this section without referring to the huge amount of evidence in the literature that claims (or not) to correlate dietary saturated fatty acids or cholesterol with an increase in plasma cholesterol (particularly LDL-cholesterol; see Section 11.2) and, then, a rise in cardiovascular disease. This work began with the classic study of Keys et al. (1957). Part of the difficulty in interpreting the various studies is that, often, an individual paper concludes that there are rather poor (and/or statistically not significant) correlations between the various components. This has led to the use of meta-analyses with all the inherent problems of selectivity and trying to compare studies where the measurements are not strictly comparable. Gurr (1992) discussed this subject very comprehensively and for an update of his views, see Gurr (1999). He "expresses scepticism for a major role for dietary lipids in the development of ischaemic heart diesease." Such a view is, of course, contrary to dietary advice as practiced currently by physicians in North America and most of Europe, even though Gurr's view is based on a careful scientific analysis of data produced over the past 25 years. Nevertheless, a recent prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins (which reduce LDL cholesterol) showed a lowering (20%) of major coronary events and stroke (Cholesterol Treatment Trialists (2005)).

The reader is also referred to Tholstrup et al. (1994) for information on saturated fatty acids, to Riemersma (1994) for a review of antioxidants in coronary heart disease prevention and to Wald (1994) for lipoprotein: CHD relationships.

11.1.4 Specific fat requirements

There is no doubt that PUFAs are necessary for good health, and both α-linolenic (18:3*n*-3) and linolenic (18:2*n*-6) are regarded generally as essential. However, in a thought-provoking article, Cunnane (2003) has argued that PUFAs should be regarded as conditionally indispensable or dispensable, depending on the development stage. Thus, during pregnancy, lactation, infancy, and childhood, he regards linoleate, α-linolenate, arachidonate, and docosahexaenoiate (22:6*n*-3) as conditionally indispensable. During adulthood, α-linolenate is described as conditionally indispensable, but eicosapentaenoic (20:5*n*-3) and 22:6*n*-3 could be described thus, depending on the geographical area and lifestyle of individuals (Cunnane, 2003).

Current recommendations for PUFAs in the diet are an *adequate* intake of linolenic acid at 2% energy, a *healthy* intake of α-linolenic acid at 0.7% energy and, for cardiovascular health, a *minimum* intake of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) *combined* of 500 mg/day. It was also recognised that there *may* be a *healthy upper limit* for linolenic acid (ISSFAL, 2004). Full references and justification will be found in this publication.

The role of PUFAs in human nutrition and metabolism has been reviewed (Neuringer et al., 1988; Galli and Simpoulos, 1989; Heird and Lapillonne, 2005). Polyunsaturated fatty acids may also be involved in a number of other disease states. These include acrodermatis enteropathica, biliary tract disease, kinky hair disease, hepatoma, kwashiorkor, mycobacterial infections and multiple sclerosis (Soderhjelm et al.,1971).

Essential fatty acid deficiency leads to problems in practically every tissue of the body (Holman, 1977). Classic symptoms include dermatitis, growth retardation, and infertility (Table 11.3). There are also biochemical changes, such as in mitochondrial efficiency, in various tissues (Holman, 1977; Gurr et al., 2002). Essential fatty acid deficiency is easily recognised because tissue fatty acids of the *n*-6 group are partly replaced by *n*-9 unsaturated fatty acids. In particular, arachidonic acid is reduced and eicosatrienoic acid (20:3*n*-9, the "Mead" acid) increased (Gurr et al., 2002) (Figure 11.1).

Many of the effects of essential fatty acids are due to their conversion to eicosanoids. There are three types of enzyme-catalysed conversions — cyclooxygenase, lipooxygenase and oxidations involving cytochrome P_{450}. The reactions give rise to prostaglandins, thromboxanes, prostacyclin, lipoxins, leukotrienes and other important biologically active molecules (Gurr et al., 2002). The *n*-3 and *n*-6 PUFAs compete with each other at a number of levels (Table 11.4). First, the main dietary PUFAs, linoleate and α-linolenate, are converted to the 20C eicosanoid precursors (arachidonate, eicosapentaenoate) using the same enzymes (see Figure 11.1). Secondly, the eicosanoids produced from arachidonate are proinflammatory, whereas those from eicosapentaenoate are mildly or

TABLE 11.3 Major effects of *n*-6 essential fatty acid deficiency in rats

Skin	Dermatosis, water permeability increased Sebum secretion decreased Epithelial hyperplasia
Body weight	Decreased
Circulation	Heart enlargement Capillary resistance decreased
Kidney	Enlargement, intertubular haemorrhage
Lung	Cholesterol accumulation
Endocrine glands	Adrenals: weight decreased in females, increased in males Thyroid: weight increased
Reproduction	Females: irregular oestrus; impaired lactation, reproduction Males: degeneration of the seminiferous tubules
Metabolism	Changes in tissue fatty acid composition Reduced cholesterol concentration in plasma Increased cholesterol concentration in liver, adrenals and skin Mitochondrial swelling and uncoupled oxidative phosphorylation Increased triacylglycerol output by liver

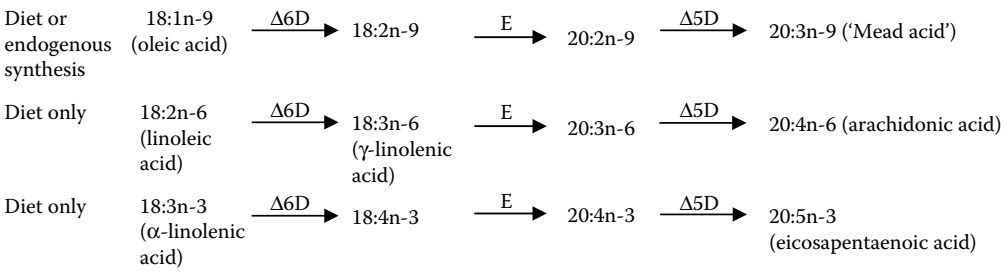


FIGURE 11.1 Competition between different fatty acids for production of 20 carbon PUFAs. Abbreviations: Δ5D, delta5-desaturase; E, elongase; Δ6D, delta6-desaturase.

noninflammatory. Thirdly, the *n*-3 and *n*-6 PUFAs have effects on the expression of many different proteins (Jump, 2002; Sampath and Ntambi, 2005) and their actions are often different (Harwood and Caterson, 2006).

It must also be noted that it has been recently discovered that the *n*-3 PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), can be converted to new types of biologically active and mainly anti-inflammatory metabolites. Thus, EPA can yield resolvins of the E series, while DHA can produce resolvins of the D series and neuroprotectins (Serhan et al., 2004).

While small amounts of essential fatty acids are needed to prevent deficiency syndromes, in practice, EFA-deficiency is seldom seen unless very unusual dietary conditions prevail. However, there is special interest now in elucidating the health benefits of substantial intakes of *n*-3 and *n*-6 PUFAs such as recommended in a healthy diet (see above). In particular, *n*-3 PUFAs are needed for the development and proper function of brain and retina (Lauritzen et al., 2001) and there is increasing evidence that they are of benefit in reducing senile dementia (Morris, et al., 2003; Lim et al., 2005) and other cognitive problems (Harwood and Caterson, 2006). There is also a good deal of interest in the role of PUFAs in cancer (Guthrie and Carroll, 1999; Diggle, 2002) and in cardiovascular disease (NHFA, 1999;

TABLE 11.4 How *n*-3 and *n*-6 PUFAs can compete with each other

Effect on enzymes during their metabolism	Δ5 and Δ6-desaturases and the 18C PUFA elongase show substrate competion.
Derived eicosanoids have opposing effects	In general, metabolites from arachidonic acid (AA) are proinflammatory (e.g., PGE_2, LTB_4), whereas those from eicosapentadecanoic acid (EPA, 20:5 n-3) are non- or anti-inflammatory (e.g., PGE_3, LTB_5)
The n-3 and n-6 PUFAs affect gene expression	The effects of the two sets of PUFAs are often opposite. For example, COX-2 expression and activity are reduced by EPA, whereas AA has no effect or increases activity in a variety of tissues

Wijendran and Hayes, 2004). Many of these important diseases have chronic inflammation as a major causative factor (Harwood and Caterson, 2006) and the relative roles of *n*-3 and *n*-6 PUFAs in this regard is fundamental (Calder, 1997; Calder et al., 1998).

Some further sources of information on dietary PUFAs are British Nutrition Foundation (1992), Chow (1992), Forsyth (1998), Garrow et al. (2000), Innes (1991), Lermer and Mattes (1999) and Lands (2005).

11.1.5 Vitamins

Although somewhat outside the scope of this book, it would be wrong to describe the role of lipids in diets without mentioning fat-soluble vitamins. Vitamin A is all-*trans*-retinol, which is only found in animal fats. However, plant materials often contain abundant quantities of β-carotene (provitamin A). This can be easily converted to all-*trans*-retinol in the body. Ritinyl esters, mainly retinol palmitate, are stored principally in the liver from where the latter is released by hydrolysis and transported to target tissues bound to retinal-binding protein.

Vitamin A has a number of important functions of which its role in vision is the best understood at the molecular level. An early sign of vitamin A deficiency is "night blindness." When severe deficiency occurs, it can lead to blindness in young children. This tragic disease, xerophthalmia, is one of the four most common preventable diseases in the world (Gurr et al., 2002; see also Section 11.8.2.5 for efforts to prevent this with "golden" rice).

The 9-*cis*-retinoic acid analog is involved importantly in differentiation. It can bind to two high-affinity receptor proteins, called RAR and RXR. Each of these may be present in one of three isoforms. Retinoic acid isomers are known to have extensive effects on gene expression and to interact (via RXR) with the vitamin D receptor or the PPAR (peroxisomal proliferator activated receptor) system. By these means, they have important effects on cellular differentiation (Gurr et al., 2002).

Vitamin A is important in preventing degenerative changes in epithelial surfaces, such as keratinization of skin. It is needed for normal bone development and a deficiency in young animals can lead to secondary nervous problems due to compression of the brain and spinal cord (de Luca, 1978). Vitamin A is involved in the immune response, mainly through the T-helper cell.

About 750 μg of vitamin A is needed for the average person daily but, like other fat-soluble vitamins, excessive intakes lead to accumulation, particularly in the liver. Chronic overconsumption may cause not only liver necrosis, but also permanent damage to bones, joints, muscles, and vision.

Vitamin D is needed for calcium homeostatis and has various other functions for tissue development (Gurr et al., 2002). It is needed to prevent rickets and its deficiency is involved in other pathological states (de Luca, 1978). A number of different structures have activity, the two most important of which are vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). Again, as with vitamin A, the diet is often able to supply provitamins (e.g., ergosterol), which are converted to the active compounds. All of the provitamins require ultraviolet light for their conversion. Like vitamin A, vitamin D is toxic in high doses. Even amounts only five times normal intake can be toxic and cause more calcium to be absorbed than can be excreted, resulting in excessive deposition in and damage to the kidneys (Gurr et al., 2002).

Vitamin E activity is possessed by eight tocopherols and tocotrienols (Gurr et al., 2002). The most potent and abundant form is α-tocopherol. Vitamin E is a natural antioxidant, although it may have other roles, such as structural functions in membranes (Wang and Quinn, 1999). The vitamin is needed for mitochondrial electron-transport function and it prevents oxidation of various compounds, including polyunsaturated fatty acids and vitamin A. The dependence of vitamin E requirement on the amount of dietary polyunsaturated fatty acids has been fully discussed by Jager (1975). Traditionally, vitamin E has been known as the "fertility" vitamin. However, deficiency leads to serious changes in skeletal muscle, the blood system and other tissues before reproduction is impaired. The effects on different animals are described by Jager (1975). Vitamin E is discussed by Scott (1978) and Packer and Fuchs (1993).

Vitamin K is the generic name given to a group of compounds, having in common a naphthoquinone ring system (menadione) with different side chains (Gurr et al., 2002). Vitamin K_1 is made by plants and found in their green tissues, while K_2 is synthesised by microorganisms. The best-studied role for vitamin K is in relation to blood clotting where four of the procoagulant proteins of the clotting cascade depend on vitamin K. It also functions as an enzyme cofactor and plays a role in bone metabolism. It is rare to see vitamin K deficiency in adults except where fat absorption is impaired (see Section 11.1.1 and Sickinger, 1975). Vitamin K is discussed by Suttie (1978). Unlike some other fat-soluble vitamins, there is little evidence of harmful effects from high doses of vitamin K.

For a general account of vitamin requirements and overdose symptoms, refer to Wilson (1994) and references therein.

References

Akoh, C.C. and Lai, O.M. Eds. (2005). *Healthful Lipids.* ACOS, Champaign, IL.

Babayan, V.K. (1974). Modification of food to control fat intake. *J. Am. Oil Chem. Soc.* **51**, 260–264.

Boyd, G.S. (1975). Cholesterol absorption. In *The Role of Fats in Human Nutrition*, Ed. A.J. Vergroesen, Academic Press, London, pp. 353–380.

British Nutrition Foundation (1987). Report of the Task Force on *Trans* Fatty Acids, Brit. Nutr. Foundation, London.

British Nutrition Foundation (1992). Task force report on unsaturated fatty acids: nutritional and physiological significance. Chapman and Hall, London.

Calder, P.C. (1997). n-3 Polyunsaturated fatty acids and immune cell function. *Adv. Enzyme Regul.* **37**, 197–237.

Calder, P.C. et al. (1998). Symposium of the Nutrition Society comprising twelve reviews on 'Lipids and the Immune System.' *Proc. Nutr. Soc.* **57**, 487–585.

Cholesterol Treatment Trialists (2005). Efficiency and safety of cholesterol-lowering treatment: prospective meta-analysis of data from participants in 14 randomised trials of statins. *Lancet*, **336**, 1267–1278.

Chow, C.K. Ed. (1992). *Fatty Acids in Foods and Their Health Implications.* Marcel Dekker, New York.

Cunnane, S.C. (2003). Problems with essential fatty acids: time for a new paradigm? *Prog. Lipid Res.* **42**, 544–568.

De Luca, H.F. (1978a). Vitamin D. In *Handbook of Lipid Research*, vol. 1, *The Fat-Soluble Vitamins*, Ed. H.F. De Luca, Plenum, New York, pp. 69–132.

De Luca, L.M. (1978b). Historical developments in vitamin A research. In *Handbook of Lipid Research*, vol. 2, *The Fat Soluble Vitamins,* Ed. H.F. De Luca, Plenum, New York, pp. 1–67.

Diggle, C.P. (2002). *In vitro* studies on the relationship between polyunsaturated fatty acids and cancer: tumour or tissue specific effects? *Prog. Lipid Res.* **41**, 240–253.

Forsyth, J.S. (1998). Lipids and infant formulas. *Nutr. Res. Rev.* **11**, 255–278.

Galli, C. and Simpoulos, A.P. Eds. (1989). Dietary ω3 and ω6 fatty acids. In *Biological Effects and Nutritional Essentiality.* Plenum Press, New York.

Garrow, J.S. et al. Eds. (2000). *Human Nutrition and Dietetics,* Churchill Livingston, Edinburgh, U.K.

Gold, P. et al. (1992). *Cholesterol and Coronary Heart Disease – The Great Debate*, Panthenon, Carnforth and Park Ridge.

Gregory, J. et al. (1990). *The Dietary and Nutritional Survey of British Adults*, HMSO, London.

Gunstone, F.D. (1998). Movements towards tailor-made fats. *Prog. Lipid Res.* **37**, 277–305.

Gurr, M.I. (1988). Lipids: products of industrial hydrogenation, oxidation and heating. In *Nutritional and Toxicological Aspects of Food Processing*, Eds. R. Walker and E. Quattrucci, Taylor & Francis, London, pp. 133–155.

Gurr, M.I. (1992a). Dietary lipids and coronary heart disease: old evidence, new perspective. *Prog. Lipid Res.* **31**, 195–243.

Gurr, M.I. (1992b). *The Role of Fat in Food and Nutrition*, Elsevier, London.

Gurr, M.I. (1996). Dietary fatty acids with *trans* unsaturation. *Nutr. Res. Rev.* **9**, 259–279.

Gurr, M.I. (1999). *Lipids in Health and Nutrition: A Reappraisal*. The Oily Press, Bridgwater, U.K.

Gurr, M.I. and Harwood, J.L. (1991). *Lipid Biochemistry*, 4th ed., Chapman & Hall, London.

Gurr, M.I., Harwood, J.L. and Frayn, K.N. (2002). *Lipid Biochemistry.* 5th ed., Blackwell, Oxford, U.K.

Guthrie, N. and Carroll, K.K. (1999). Specific versus non-specific effects of dietary fat on carcinogenesis. *Prog. Lipid Res.* **38**, 261–271.

Harwood, J.L. and Caterson, B. (2006). Dietary omega-3 polyunsaturated fatty acids and inflammation. *Lipid Technol.* **18**, 7–10.

Heird, W.C. and Lapillonne, A. (2005). The role of essential fatty acids in development. *Annu. Rev. Nutr.* **25**, 549–571.

Holman, R.T. (1977). The deficiency of essential fatty acids. In *Polyunsaturated Fatty Acids*, Eds. W.H. Kunau and R.T. Holman, American Oil Chemists' Society, Champaign, IL, pp. 163–182.

Hunter, J.E. (2004). Alternatives to *trans* fatty acids in foods. *INFORM,* **15**, 510–512.

Hwang, D. (2000). Fatty acids and immune responses — a new perspective in searching for clues to mechanism. *Annu. Rev. Nutr.* **20**, 431–456.

Innes, S.M. (1991). Essential fatty acids in growth and development. *Prog. Lipid Res.* **30**, 39–103.

ISSFAL (2004). Recommendations for intake of polyunsaturated fatty acids in healthy adults. *ISSFAL Newsl.* **11**, 12–25.

Jager, F.C. (1975). Linoleic acid intake and vitamin E requirement. In *The Role of Fats in Human Nutrition*, Ed. A.J. Vergroesen, Academic Press, London, pp. 381–432.

Jones, R.J. (1974). Role of dietary fat in health. *J. Am. Oil Chem. Soc.* **51**, 251–254.

Jump, D.B. (2002). Dietary polyunsaturated fatty acids and regulation of gene transcription. *Curr. Opin. Lipidol.* **13**, 155–164.

Kamal-Eldin, A. and Pokorny, J. (Eds.) (2005). *Analysis of Lipid Oxidation.* AOCS, Champaign, IL.

Keys, A., Anderson, J.T. and Grande, F. (1957). Prediction of serum cholesterol responses in man to changes in fats in the diet. *Lancet* (ii), 959–966.

Kodali, D.R. and List, G.R. Eds. (2005) *Trans Fats Alternatives.* AOCS, Champaign, IL.

Lands, W.E.M. (2005). *Fish, Omega-3 and Human Health,* 2nd ed., AOCS, Champaign, IL.

Lauritzen, L., Hansen, H.S., Jorgensen, M.H. and Michaelsen, K.F. (2001). The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina. *Prog. Lipid Res.* **40**, 1–94.

Lermer, C.M. and Mattes, R.D. (1999). Perception of dietary fat: ingestive and metabolic implications. *Prog. Lipid Res.* **38**, 117–128.

Lim, G.P. et al. (2005). A diet enriched with the omega-3 fatty acid docosahexaenoic acid reduces amyloid burden in an aged Alzheimer mouse model. *J. Neurosci.* **25**, 3032–3040.

Morris, M.C. et al. (2003). Consumption of fish and n-3 fatty acids and risk of incident Alzheimer disease. *Arch. Neurol.* **60**, 940–946.

Neuringer, M. et al. (1988). The essentiality of n-3 fatty acids for the development and function of the retina and brain. *Annu. Rev. Nutr.* **8**, 517–541.

NHFA (National Heart Foundation of Australia) (1999). A review of the relationship between dietary fat and cardiovascular disease. *Aust. J. Nut. Diet.* **56**, 55–522.

Packer, L. and Fuchs, J. (1993). *Vitamin E in Health and Disease*, Marcel Dekker, New York.

Pearson, A.M. and Dutson, T.R. (1990). Meat and health. *Advances in Meat Research*, vol. 6, Elsevier, New York.

Petersdorf, R.G. et al. (Eds.) (1983). *Principles of Internal Medicine,* 10th ed., McGraw-Hill, New York.

Recommendations of the European Atherosclerosis Society (1992) Prevention of coronary heart disease: scientific background and new clinical guidelines. *Nutri. Metab. Cardiovasc. Dis.* **2**, 113–156.

Riemersma, R.A. (1994). Epidemiology and the role of antioxidants in preventing coronary heart disease: a brief overview. *Proc. Nutr. Soc.* **53**, 59–65.

Rizek, R.L., Friend, B. and Page, L. (1974). Fat in todays food supply — level of use and sources. *J. Am. Oil Chem. Soc.* **51**, 244–250.

Sampath, H. and Ntambi, J.M. (2005). Polyunsaturated fatty acid regulation of genes of lipid metabolism. *Annu. Rev. Nutr.,* **25**, 317–340.

Scott, M.L. (1978) Vitamin E2. In *Handbook of Lipid Research*, *The Fat-Soluble Vitamins,* Ed. H.F. De Luca, Plenum, New York, pp. 133–210.

Serhan, C.N. et al. (2004). Resolvins, decosatrienes and neuroprotectins, novel omega-3-derived mediators and their aspirin-triggered epimers. *Lipids,* **39**, 1125–1132.

Sickinger, K. (1975). Clinical aspects and therapy of fat malassimilation with particular reference to the use of medium-chain triglycerides. In *The Role of Fats in Human Nutrition*, Ed. A.J. Vergroesen, Academic Press, London, pp. 116–209.

Söderhjelm, L. et al. (1971). The role of polyunsaturated acids in human nutrition and metabolism. *Prog. Chem. Fats Lipids*, **9**, 555–585.

Suttie, J.W. (1978). Current advances in vitamin K research. In *The Fat-Soluble Vitamins*, Ed. J.W. Suttie, William Heinemann, Ltd., London, pp. 211–220.

Varela, G. and Ruiz-Rosa, B. (2000). Some nutritional aspects of olive oil. In *Handbook of Olive Oil*, Eds. J.L. Harwood and R. Aparcio, Aspen Publishers, Gaithersburg, MD, pp. 565–582.

Vergroesen, A.J. (Ed.) (1975). *The Role of Fats in Human Nutrition*, Academic Press, London.

Vergroesen, A.J. and Crawford, M. (Eds.) (1989). *The Role of Fats in Human Nutrition*, Academic Press, London.

Wahle, K.W.J. et al. (2004). Conjugated linolenic acids: are they beneficial or detrimental to health? *Prog. Lipid Res.* **43**, 553–587.

Wald, N.J. et al. (1994). Apolipoproteins and ischemic-heart-disease — implications for screening. *Lancet*, **343**, 75–79.

Wang, X. and Quinn, P.J. (1999). Vitamin E and its function in membranes. *Prog. Lipid Res.* **38**, 225–248.

Wijenderan, V. and Hayes, K.C. (2004). Dietary n-6 and n-3 fatty acid balance and cardiovascular health. *Annu. Rev. Nutri.* **24**, 597–615.

Willett, W.C. et al. (1993). Intake of trans fatty acids and risk of coronary heart disease among women. *Lancet*, **341**, 581–585.

Wilson, J.D. (1994). Vitamin deficiency and excess. In *Principles of Internal Medicine*, Eds. K.J. Isselbacher, E. Braunwald, J.D. Wilson, J.B. Martin, A.S. Fanci and D.L. Kasper, 13th ed., McGraw-Hill, New York, pp. 472–480.

11.2 Lipids and cardiovascular disease

11.2.1 Cardiovascular disease

Cardiovascular disease (CVD) is a major cause of morbidity and mortality in the western world, with the number of individuals with the disease in developing countries increasing all the time. The clinical manifestations of CVD include heart attacks, stroke, and gangrene of the extremities. According to the statistics from the World Health Organisation (WHO), an estimated 17 million people die from CVD each year, with heart attacks and stroke responsible for the majority of deaths. It has been predicted that the total number of deaths from CVD may rise to 20.5 million by 2020 and 24.2 million by 2030 as developing countries acquire westernised habits. CVD is clearly a major economic burden due to expenses incurred for hospital care and medication for patients and days lost from work because of illness, death and looking after relations with the disease. According to the American Heart Association and the National Heart, Lung and Blood Institute, the economic cost of CVD in 2005 alone is estimated to be 393.5 billion dollars.

11.2.2 Atherosclerosis is the underlying cause of cardiovascular disease

Atherosclerosis, which comes from the Greek words "athero" (meaning gruel or paste) and "sclerosis" (hardness), is the principal cause of CVD. A normal artery consists of three layers, the intima lining the lumen, the middle layer called the media and an outermost layer termed the adventitia. The intima consists of a single layer of endothelial cells that regulate vascular tone. The media consists predominantly of smooth muscle cells, whereas the adventia contains smooth muscle cells, fibroblasts, and a looser connective tissue. Atherosclerosis, which develops during the life span of an individual, causes a buildup of plaques, consisting of cholesterol, other lipids, and debris from cellular death, in the inner lining of the arteries. Although continued growth of such plaques may impede blood flow, the major problem arises when it becomes fragile and ruptures. This leads to a clot that can block blood flow or can break off and get trapped in another part of the body. Heart attack (also called myocardial infarction, coronary occlusion, or coronary thrombosis) occurs when the clot blocks a coronary artery and, thus, deprives the heart of oxygen and nutrients. On the other hand, blockage of a blood vessel to the brain leads to stroke.

Atherosclerosis is initiated by damage to the endothelial cells by a number of risk factors (see Section 11.2.3 below), which then triggers a series of changes in the arterial wall (see Figure 11.2 for a summary; and Ross, 1999; Lusis, 2000; Glass and Witztum, 2001; and Lusis et al., 2004 for a detailed description of these changes). First of all, the permeability of the vascular wall is increased because of the synthesis of cell surface adhesion molecules, such as intercellular adhesion molecule-1, E-selectin, P-selectin and vascular cell-adhesion molecule-1. In addition, the damaged endothelial cells secrete chemo-attracting cytokines (chemokines), such as monocyte-chemoattractant protein-1, which in turn attracts monocytes and T-lymphocytes from circulation and stimulates their migration into the intima of the arterial wall. These monocytes then differentiate into macrophages, a process that is associated with the

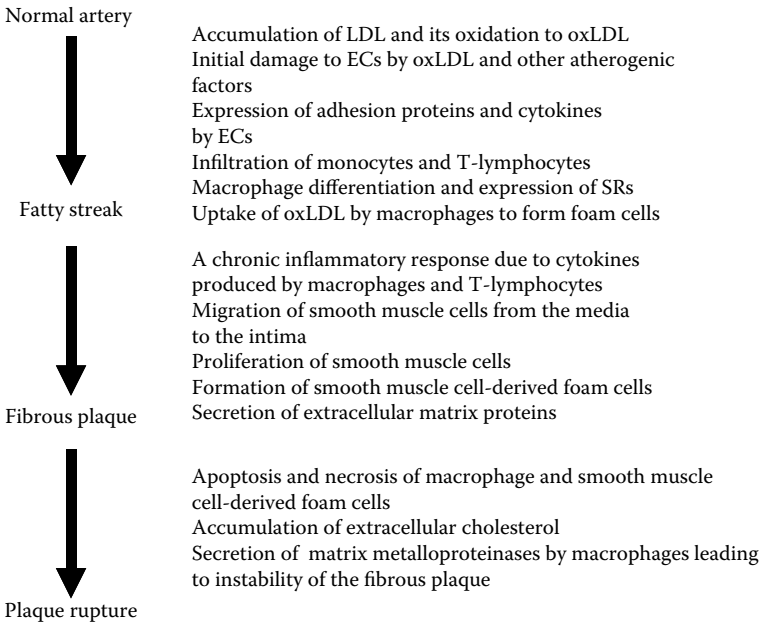


FIGURE 11.2 A schematic representation of the major steps during the pathogenesis of atherosclerosis (see text for more details). Abbreviations: ECs, endothelial cells; LDL, low density lipoproteins; oxLDL, oxidized low density lipoproteins; SRs, scavenger receptors.

expression of so-called scavenger receptors (SRs), such as SR-A and CD36. Although the normal function of these SRs is believed to be in the uptake of pathogens and apoptotic cells, they also take up modified lipoproteins (see Section 11.2.7 below). This latter property causes macrophages to transform into lipid-loaded foam cells. Such accumulation of foam cells in the vascular wall is a critical early step in the pathogenesis of atherosclerosis and is responsible for the formation of a so-called fatty streak. The macrophages also secrete cytokines, which lead to a local inflammatory response that recruits further monocytes and T-lymphocytes and, thereby, amplify foam cell formation. The importance of macrophages in atherogenesis can be gauged by the finding that diet-induced atherosclerosis in a murine model of the disease is significantly reduced in mice bred to have severely reduced monocyte levels (Smith et al., 1995; see Osterud and Bjorklid, 2003 for an in-depth review on the role of monocytes/macrophages in atherogenesis). This fatty streak then progresses to form a fibrous plaque via a number of cellular and biochemical changes.

First, an intermediate fibro-fatty lesion is formed, which consists of foam cells, smooth muscle cells (SMC), T-lymphocytes and a relatively poorly developed extracellular matrix. The transition from such fibro-fatty lesions to more complex lesions is associated with the migration of SMC from the media to the intima, where they proliferate and also take up lipoproteins, thereby contributing to further foam cell formation. In addition, such SMC synthesise extracellular matrix proteins leading to the development of a fibrous cap. More advanced lesions contain a dense fibrous cap, which protrudes into the lumen of the artery and covers a core of macrophages, SMC, T-lymphocytes, extracellular matrix and debris from dying foam cells. Instability of such advanced lesions may lead to plaque rupture and thrombus formation, which may ultimately result in the occlusion of the artery. An inflammatory response is a major contributor to plaque instability and the development of acute CVD has been found to be associated with elevated circulating levels of markers of inflammation, such as C-reactive protein.

11.2.3 Risk factors for CVD

More than 300 risk factors for CVD have been identified from laboratory- and clinical-based research. The major risk factors that have a high prevalence in many different populations are age, gender, hypertension, smoking, physical inactivity, obesity, diabetes, socioeconomic status, *Chlamydia pneumoniae* infection, hyperhomocysteinemia and high levels of circulating lipids (see Stoker and Keaney, 2004 for a more detailed discussion of the various risk factors). A number of such risk factors often co-exist in atherosclerotic patients where they may act in a synergistic manner. For example, hypercholesterolemia, obesity, hypertension and physical inactivity are often associated in a number of male patients.

The risk for the development of CVD increases with age, with the average risk in males aged 65 being about sevenfold greater than those who are 35. In addition, males have a much higher risk for CVD compared to age-matched women (Barrett-Conner and Bush, 1991). It has been suggested that estrogen provides protection in premenopausal women because such gender-specific effects are not seen in postmenopausal women. However, this speculation has not been substantiated further as estrogen treatment does not decrease

the incidence of the disease in postmenopausal women. Other factors are thus likely to contribute for such gender differences (see Mendelsohn and Karas, 2005); for example, women generally have higher levels of the protective high-density lipoprotein (HDL) (see Section 11.2.4) than age-matched males.

Hypertension, obesity and diabetes often coexist; for example, hypertension and diabetes are relatively common in obese individuals. According to figures from the WHO, 6.3% of individuals aged 20 or above in developed countries and 4.1% in developing countries suffer from diabetes. This figure is expected to increase in the future because sedentary lifestyle, intake of "convenience food" containing high levels of fat and salt, and obesity are increasing at an alarming rate. More than 60% of adults in the U.S. are overweight or obese and even in China, a population that has been previously classed as slim and physically active, there are 70 million overweight individuals.

A risk between smoking and CVD was first suggested in 1940 and this has been substantiated by numerous studies. Smoking promotes CVD via several mechanisms, including damage to the endothelial lining of the arterial wall, increase in the circulating levels of the proatherogenic low-density lipoproteins (LDL), decrease in the concentration of HDL (see Section 11.2.4) and stimulation of blood clotting. Cigarette smoking also raises the concentration of plasma carbon monoxide, which has a number of additional detrimental effects, such as promotion of both endothelial hypoxia and thrombus formation. The nicotine in cigarettes also accelerates heart rate and raises blood pressure.

From the different risk factors identified, hyperlipidemia, particularly high levels of serum cholesterol, has been the subject of intense research for a number of decades and caused the most debate. A number of epidemiological studies have shown a direct correlation between high plasma cholesterol levels and CVD (Martin et al., 1986; Anderson et al., 1987; Gurr, 1992). Additional support for a proatherogenic role of plasma cholesterol is provided by studies on individuals with the autosomal dominant disorder familial hypercholesterolemia, which is characterised by a two- to five-fold increase in plasma LDL cholesterol (see Section 11.2.6). About 80% of patients that are heterozygous for this disorder experience CVD by the age of 60 and this is reduced to 15 years in homozygous individuals. Furthermore, randomised clinical trials of lipid lowering therapy have shown a greater than 30% reduction in CVD (Maron et al. 2000; Grundy et al. 2004). From the different classes of plasma lipids, high levels of fasting cholesterol and LDL and low levels of HDL have been identified as the most proatherogenic. High concentration of fasting serum triacylglycerols also represents an independent risk factor. The different classes of serum lipids are considered below in more detail.

11.2.4 Serum lipids: classification and metabolic roles

All lipids are carried in the plasma as complexes with proteins. Although long-chain fatty acids circulate bound to plasma albumin, the other lipids are carried by a number of lipoprotein particles, which are responsible for the transport of endogenously produced and dietary lipids. Such lipoprotein particles are spherical in shape, with diameter between 5 μm and 1200 μm, and comprised of a hydrophobic core, containing triacyglycerols and cholesterol esters, surrounded by a hydrophilic shell of phospholipids, unesterified (free) cholesterol and apolipoproteins.

There are five major classes of lipoproteins: chylomicrons, very low-density (also called pre-β) lipoproteins (VLDL), intermediate density lipoproteins (IDL), low-density (β-) lipoproteins, and high-density (α-) lipoproteins. The classification is based on the hydrated density of the lipoproteins. The different lipoproteins differ in size, electrophoretic mobility, and composition of lipids and apolipoproteins (see Table 11.5). Although each lipoprotein is synthesised with a characteristic set of apolipoproteins, considerable exchange of these apolipoproteins with other lipoprotein particles occur during their metabolism (see Table 11.5).

Chylomicrons are synthesised by the mucosal cells of the small intestine and act as a vehicle for the transport of dietary triacylglycerols and cholesterol. VLDL, which is synthesised and secreted by the liver, is also rich in triacylglycerols, but these are derived from endogenous sources. IDL is formed as triacylglycerols are removed from VLDL (see Section 11.2.5). LDL is the main carrier of cholesterol in the plasma and is derived primarily from the catabolism of VLDL. HDL, on the other hand, is formed mainly in the liver as a lipid-poor particle that becomes modified

TABLE 11.5 The composition of plasma lipoproteins

			Composition (% total)				
Lipoprotein	**Density (g/ml)**	**Diameter (μm)**	**TAG**	**Cholesterol**	**Phospholipid**	**Protein**	**Apolipoproteins[a]**
Chylomicrons	< 0.95	75–1200	90	5	3	2	B48 (A, C, E)
VLDL	0.95–1.006	30–80	60	12	18	10	B100 (A, C, E)
IDL	1.006–1.019	25–35	27	34	27	12	B100, E
LDL	1.019–1.036	18–25	10	50	15	25	B100
HDL	1.063–1.210	5–12	5	20	25	50	AI, AII (C, E)

[a] The main apolipoproteins in each of the lipoproteins are shown first, with those that are exchanged with other lipoprotein particles indicated in parenthesis (see text for more details). TAG, triacylglycerol.

during the catabolism of VLDL. The major function of HDL is to aid the transport of cholesterol from peripheral tissues to the liver where it can be excreted as bile acids (a process called reverse cholesterol transport).

In addition to the lipoprotein classes detailed above, Lipoprotein (a) [Lp (a)] is also found in the plasma where its concentration may range from 0.2 mg/dl to 120 mg/dl. LP (a), which is synthesised in the liver, has generated substantial interest because of its presence in atherosclerotic lesions and its identification as a major risk factor for CVD (see Berglund and Ramakrishnan, 2004; Boffa et al., 2004). LP (a) contains an LDL particle with apoB100 to which a genetically polymorphic form of apo (a) is attached by a disulfide bond. The precise mechanism(s) for a proatherogenic role of LP (a) remain to be determined. However, LP (a) is known to be taken up by macrophages and, thus, contributes to foam cell formation. Apo (a) also shows striking homology with the fibrinolytic proenzyme plasminogen. Although LP (a) does not possess the protease activity associated with plasminogen, it may still interfere with its action and, thereby, impair fibrinolysis (clot resolution).

11.2.5 Metabolic fates of circulating lipoproteins

At least four enzymes play crucial roles in the metabolism of lipoproteins: lipoprotein lipase (LPL), hepatic lipase (HL), lecithin:cholesterol acyltransferase (LCAT), and cholesteryl ester transfer protein (CETP). LPL and HL are involved in the hydrolysis of triacylglycerol-rich lipoproteins. LPL, which requires apoCII as a specific co-activator, interacts with heparin sulfate proteoglycans (HSPG) on the surface of vascular endothelial cells, whereas HL is associated with the plasma membrane in the liver. LPL hydrolyses triacylglycerols in chylomicrons and VLDL to produce nonesterified fatty acids and 2-monoacylglycerol, which are either re-esterified for storage in the adipose tissue or used as a source of energy in the muscle. On the other hand, HL acts on particles that have already been partially digested by LPL and facilitates the conversion of IDL to HDL. LCAT, which is activated by apoAI, esterifies cholesterol acquired by HDL, whereas CETP catalyses the transfer of cholesterol esters from HDL to triacylglycerol-rich lipoproteins.

The apolipoproteins play a crucial role in the regulation of lipoprotein metabolism and the stabilisation of the lipoprotein particles. Additionally, they modulate the activity of several key enzymes in lipoprotein metabolism and some of them also bind to specific cell surface receptors and, thereby, regulate the metabolic fates of lipoproteins (Table 11.6). There are at least four receptors for lipoproteins and remnant particles: the VLDL receptor, the remnant receptor(s), the LDL receptor (LDL-R) (also called the apo B/E receptor), and the LDL receptor-related protein (LRP).

Chylomicrons synthesised by the intestine are secreted into the lymphatic system and reach the plasma through the thoracic duct. Chylomicrons contain apoB48, which is synthesised in the intestine, along with apolipoproteins-A, -C, and -E. ApoB48 is found exclusively in chylomicrons and is derived from the apoB100 gene by RNA editing in the intestinal epithelium. ApoB48 lacks the LDL-R-binding domain present in apoB100. The triacylglycerol core of chylomicrons is hydrolysed by LPL present on the surface of capillary endothelial cells. This is accompanied by transfer of phospholipids, via phospholipid transfer protein (PLTP), and apolipoproteins-A and -C to HDL. The loss of apoCII, an activator of LPL, prevents further hydrolyses of the smaller, chylomicron remnant particles. These chylomicron remnants, which contain primarily cholesterol, apoE and apoB48, are then taken up by the liver principally via a chylomicron remnant receptor.

The VLDL synthesised and secreted by the liver contains apoB100 and acquires cholesteryl esters and apolipoproteins-A, -C, and -E from circulating HDLs. Similar to chylomicrons, the triacylglycerol component of VLDL is subjected to LPL-mediated hydrolysis. The action of LPL, along with the transfer of phospholipids and apolipoproteins-A and -C to circulating HDLs, converts VLDLs to IDLs (also called VLDL remnants). Approximately half of these VLDL remnants are removed from the circulation by high affinity binding to the LDL-R of liver cells due to the presence of both apoB100 and apoE. The remaining remnants transform into cholesterol-rich LDL particles by losing more triacylglycerols, via hydrolysis by HL, and shedding all of their lipoproteins except for apoB100.

TABLE 11.6 Properties of major apoliproteins

Apoliprotein	Major lipoprotein	Effect on enzyme activity	Putative receptor
AI	HDL	Activates LCAT	SR-BI
AII	HDL	Activates HL LCAT cofactor	?
B48	Chylomicrons	–	LRP
B100	VLDL, IDL and LDL	–	LDL-R
CI	Chylomicrons, VLDL and HDL	LCAT?	–
CII	Chylomicrons, VLDL and HDL	Activates LPL	–
CIII	Chylomicrons, VLDL and HDL	Inhibits LPL	–
E	Remnants	–	LDL-R

Note: HL, hepatic lipase; LCAT, lechithin:cholesterol acyltransferase; LDL-R, LDL receptor; LPL, lipoprotein lipase; LRP, LDL receptor-related protein; SR-BI, scavenger receptor-BI.

LDL particles are taken up by the liver or peripheral tissues via LDL-R-mediated endocytosis. The endocytosed membrane vesicles fuse with lysosomes where the apolipoproteins are digested and the cholesterol esters are hydrolysed to yield free cholesterol. The excess intracellular cholesterol is then re-esterified for storage by the action of the enzyme acyl-CoA-cholesterol acyltransferase (ACAT), whose activity is enhanced by free cholesterol.

The cellular uptake of LDL via LDL-R is under negative feedback inhibition by the concentration of intracellular cholesterol. This regulation occurs through a family of membrane-bound transcription factors called sterol regulatory element-binding proteins (SREBPs). A low level of intracellular cholesterol leads to the activation of SREBPs, which then stimulate the transcription of the LDL-R gene along with a number of other genes implicated in cholesterol biosynthesis (see Brown and Goldstein, 1999; Eberle et al., 2004). SREBPs are produced as an integral membrane-bound precursor form in the endoplasmic reticulum and are activated by a SREBP cleavage-activating protein (SCAP), which contains a sterol-sensing domain. When the intracellular concentration of cholesterol is low, SCAP and the SREBP precursors move from the endoplasmic reticulum to serine proteases located in the Golgi apparatus. The proteases cleave the SREBP precursors and produce the active transcription factor, which can translocate to the nucleus and bind to its recognition sequences in the regulatory regions of target genes.

As discussed in detail in Section 11.2.7 below, lipoproteins, such as LDL, are subject to several types of modifications, particularly oxidation. Such modified lipoproteins are readily taken up by scavenger receptors (SRs). Macrophages express at least six different forms of SRs for modified LDL. Unlike the LDL-R, such SRs are not subject to feedback inhibition by intracellular cholesterol concentration and can, therefore, take up cholesterol in an uncontrolled manner, thereby contributing to foam cell formation (see Greaves and Gordon, 2005, for details on SRs).

HDL plays a crucial role in reverse cholesterol transport (Figure 11.3). Nascent HDL is formed in the liver and the intestine as a disk-like particle containing apoAI and some phospholipids. This HDL particle acts as a potent acceptor of cholesterol derived from peripheral cells. The ATP-binding cassette transporter (ABC)-A1, along with a number of other such transporters, play a key role in the efflux of cholesterol from cells using ATP as a source of energy. The importance of ABCA1 is shown by patients with Tangier disease, which lack this transporter and suffer premature CVD because of a massive accumulation of cholesteryl esters in a number of tissues. The cholesterol taken up by HDL becomes esterified to cholesteryl esters through the action of LCAT. The cholesteryl esters then move deeper into the HDL particle, which now assumes a small, spherical shape and is called HDL_3. HDL transfers part of its cholesteryl esters to triacylglycerol-rich lipoproteins and acquires phospholipids and apolipoproteins from them to form larger HDL_2 particles.

The return of HDL cholesterol to the liver occurs via three pathways (see Figure 11.3). First, the entire HDL particle is taken up by the liver through the LDL-R. Secondly, the cholesterol ester in HDL is transferred to other lipoproteins, via the action of CETP, and these lipoproteins are then taken up by the liver through the LDL-R. The importance of this pathway is supported by several lines of evidence. For example, premature CVD is common in individuals with CETP-deficiency despite the presence of high HDL levels (Bruce et al., 1998). In addition, overexpression of CETP in mice, which normally lack this enzyme, is anti-atherogenic (Bruce et al., 1998). Thirdly, selective delivery of cholesteryl esters in HDL to the liver can occur via scavenger receptor class BI (SR-BI) (Acton et al., 1996). SR-BI binds HDL avidly and mediates the selective delivery of cholesteryl esters to the cell membrane in the liver without internalisation and degradation of the HDL particle. This direct uptake of cholesteryl ester from HDL is facilitated by the binding of HDL to cell surface HSPG because of the presence of apoE in the lipoprotein

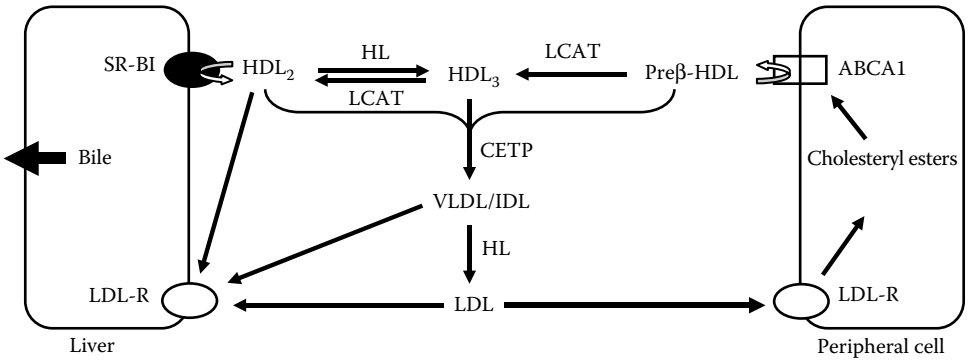


FIGURE 11.3 A schematic representation of reverse cholesterol transport (see text for more details). Abbreviations: ABCA1, ATP-binding cassette transporter 1; CETP, cholesteryl ester transfer protein; HDL, high density lipoproteins; HL, hepatic lipase; IDL, intermediate density lipoproteins; LCAT, lecithin-cholesterol acyltransferase; LDL-R, low density lipoprotein receptor; SR-B1, scavenger receptor B1; VLDL, very low density lipoproteins.

(Arai et al., 1999). The importance of SR-BI can be gauged by the finding that its inactivation in mice is associated with a dramatic increase in serum cholesterol levels and the size of HDL particles without any significant effect on the HDL protein concentration (Rigotti et al., 1997). In addition, liver-specific expression of SR-BI in mice results in reduced HDL levels and an increase in reverse cholesterol transport (Wang et al., 1998).

Cholesterol delivered to the liver by HDL is excreted as a free sterol or as bile acids. The HDL particle shrinks as a result of the transfer of cholesteryl ester to the liver and some of the particles become nascent HDL for another round of transport of cholesterol.

11.2.6 Factors affecting the composition and metabolism of circulating lipids

When factors that affect the composition of circulating lipids in humans are considered, the nature of the "normal" subject must be defined as genetic prepositions, age, sex, diet, exercise, and overt or hidden disease may all contribute to discernible differences. Where there is no evidence of malnutrition, the dietary components that most affect the lipid profiles are fats and carbohydrates. In relation to fats, the nature of the fatty acid components has attracted the most attention. When conventional diets are changed by an increased intake of polyunsaturated fats, significant reductions in total plasma cholesterol concen-trations are seen, with a major reduction in the LDL fraction. The beneficial effects of polyunsaturated fatty acids are seen at virtually all stages of the disease, including the control of overall lipid metabolism and transport, regulation of nuclear receptors (see Section 11.2.8), modulation of adhesion protein and cytokine expression by endothelial cells, and control of platelet function (De Caterina et al., 2004; Mori and Beilin, 2004; Vanden Heuvel, 2004; Mori, 2004; Jump, 2004).

There are a number of rare inborn errors of lipid metabolism. Those that comprise the familial hyperlipoproteinemias are probably the most widely studied (Table 11.7; Levy and Fredrickson, 1968; Fredrickson and Breslow, 1973; Genest, 2003). These are divided into five main types according to the major changes in plasma lipoprotein profiles.

Type I hyperlipoproteinemia, also known as familial hyperchylomicronemia, familial exogenous hypertriglyceridemia, or familial fat-induced lipemia, is a rare recessive condition due to a deficiency of the enzyme LPL or, more rarely, by the absence of its activator apoCII. Chylomicron and VLDL metabolism, therefore, is defective and their accumulation results in very high plasma concentration of triacylglycerols. Clinical symptoms include eruptive xanthomas, hepatosplenomegaly, lipemia retinalis, abdominal pain and pancreatitis. Indeed, pancreatitis rather than atherosclerosis is the major reason for early deaths in these patients.

Type II hyperlipoproteinemia includes several genetic conditions, including familial hypercholesterolemia, familial combined hyperlipidemia, familial defective apolipoprotein B and polygenic hypercholesterolemia. These disorders are associated with a very high concentration of plasma LDL and, in certain cases, VLDL. Some classify type II hyperlipoproteinemias into type IIA and type IIB depending on whether hypertriglyceridemia is absent or present, respectively (see Table 11.7). The other major symptoms include xanthomas, particularly on the hand and knee tendons.

Familial hypercholesterolemia is caused by the absence of fully functional LDL-R, resulting in delayed clearance of LDL. Homozygous individuals have extremely high levels of plasma LDL-cholesterol irrespective of diet or life style and exhibit severe CVD, usually resulting in death at an early age. Heterozygous individuals are a more diverse group with a 50% probability of death from CVD before the age of 50. A form of familial hypercholesterolemia with

TABLE 11.7 Hyperlipoproteinemias

Type[a]	Other designations	Elevated lipoprotein class	[Cholesterol][b]	[Triacylglycerols][b]
I	Familial Hyperchylomicronemia Familial Exogenous Hypertriglyceridemia Familial Fat-Induced Lipemia	Chylomicrons	↑	↑
II-a	Familial Hypercholesterolemia Familial Defective Apolipoprotein B Polygenic Hypercholesterolemia	LDL	↑	–
II-b	Familial Combined Hyperlipidemia	LDL, VLDL	↑	↑
III	Broad Beta Disease Familial Dysbetalipoproteinemia	β-VLDL	↑	↑
IV	Endogenous Hypertriglyceridemia Hyperprebetalipoproteinemia	VLDL	–	↑
V	Mixed Hyperglyceridemia Mixed Hyperlipidemia Hyperprebetalipoproteinemia with Chylomicronemia	VLDL Chylomicrons	↑	↑

[a] The designation is as proposed by Fredrickson and adopted by the WHO.
[b] ↑, increase; ↓, decrease; –, no change.

a similar clinical phenotype, called familial defective apolipoprotein B, arises because of mutations in apoB100 in regions that represent binding sites for the LDL-R. Familial combined hyperlipidemia is characterised by excess circulating levels of LDL, VLDL, or both. Excessive hepatic production of apoB100, a major protein constituent of VLDL and LDL, is common. It is transmitted in a dominant manner, but does not often manifest until after adolescence. Defects in a number of genes implicated in lipoprotein metabolism and transport is the most frequent cause for this disorder. Finally, polygenic hypercholesterolemia is a heterogenous group of disorders that accounts for the largest number of patients in type II hyperlipoproteinemias. Most patients have elevated levels of LDL due to its impaired clearance.

Type III hyperlipoproteinemia, also known as broad beta disease or familial dysbetalipoproteinemia, is associated with elevated levels of triacyglycerols and cholesterol because of abnormalities in VLDL (i.e., presence of an abnormal form of β-migrating VLDL), xanthomas, and premature CVD (Mahley et al., 1999). The major defect is the presence of an abnormal apoE, which does not bind efficiently to hepatic receptors that require this apolipoprotein for interaction with lipoproteins. The patients have the so-called apoE-2/2 phenotype or genotype. There are three common alleles for apoE, apoE-2, -E3, and E-4, with the apoE-2 allele associated with a marked decrease in binding to the LDL-R and premature CVD (Greenow et al., 2005).

Type IV hyperlipoproteinemia, also called endogenous hypertriglyceridemia or hyperprebetalipoproteinemia, is a common disorder characterised by elevated levels of circulating VLDL and increased predisposition to CVD. This disorder is frequently associated with insulin resistance and obesity and is particularly common in American middle-aged men.

Type V hyperlipoproteinemia, also called mixed hypertriglyceridemia, mixed hyperlipidemia, or hyperprebetalipoproteinemia with chylomicronemia, is a relatively uncommon disorder associated with defective clearance of exogenous and endogenous triacylglycerols. Symptoms include eruptive xanthomas, lipemia retinalis, hepatosplenomegaly, and abdominal pain. Similar to type I hyperlipoproteinemia, acute pancreatitis rather than CVD is the major reason for early deaths.

In addition to these relatively common forms of hyperlipoproteinemias, a number of other rare genetic disorders exist that are characterised by marked hypercholesterolemia and/or abnormal lipid and lipoprotein profile (Rader et al., 2003). For example, autosomal recessive hypercholesterolemia (ARH) is caused by mutations of the ARH gene, which codes for a novel adapter protein involved in the internalisation of the LDL-R:LDL complex. Homozygous ApoA1 deficiency results in the virtual absence of HDL and early CVD. Individuals with LCAT deficiency also exhibit extremely low levels of HDL. Sitosterolemia, which is also associated with premature CVD and caused by mutations of ABCG5 and ABCG8, is characterised by an accumulation of both animal and plant sterols in the body. This is thought to be due to abnormal absorption of plant sterols, cholesterol hyperabsorption and reduced secretion of sterols into bile. Another recessive form of hypercholesterolemia is associated with deficiency of cholesterol 7α-hydroxylase, a key enzyme in the synthesis of bile acids. Finally, Tangier disease, first identified in the island of Tangier in the Chesapeake Bay in the U.S., is an autosomal recessive disorder because of mutations in the ABCA1 gene that results in hypertriglyceridemia and extremely low levels of HDL and apoA1.

The most common form of dyslipidemias, however, has multifactorial origins, such as defects in several genes implicated in cardiovascular disease, environmental and life-style influences and other pathological conditions. Hyperlipidemia is known to arise because of medication (e.g., estrogen, oral contraceptives, steroids), excessive alcohol consumption, chronic and uncontrolled diabetes, nephrosis and endocrine disorders (e.g., hyperthyroidism). Infection and inflammatory responses associated with certain pathological conditions can also cause hyperlipidemias, changes in lipoprotein profile and premature CVD. The elevated levels of circulating cytokines are mainly responsible for such changes (see Mead and Ramji, 2002; Mead et al., 2002; Daugherty et al., 2005; Greenow et al., 2005; Harvey and Ramji, 2005). For example, pro-inflammatory cytokines reduce the expression of LPL in the adipose tissue, thereby causing an accumulation of chylomicrons and VLDL because of their defective clearance. In addition, pro-inflammatory cytokines inhibit the expression of apoE and ABCA1 by macrophages, thereby suppressing cholesterol efflux and accelerating foam cell formation. The most frequent cause of hyperlipidemia is the Metabolic Syndrome (Section 11.3.7) seen in obese individuals. This is clearly a major problem at the moment as the number of obese individuals, including young children, is increasing throughout the world. Lack of physical activity, a diet rich in saturated fats and refined sugars, high intake of calories compared to expenditure, and a sedentary life style all contribute to the proatherogenic lipid and lipoprotein profile in these individuals. In addition, they frequently have elevated blood pressure, peripheral insulin resistance, reduced HDL-cholesterol levels, increased plasma triacylglycerols, and onset of type II diabetes. The combination of these factors often acts in a synergistic manner to promote premature CVD.

11.2.7 Circulating lipids and the pathogenesis of atherosclerosis

The transformation of macrophages into foam cells is clearly a key step in the pathogenesis of atherosclerosis and a major target for therapeutic intervention of the disease (Li and Glass, 2002). Native LDL is not taken up by macrophages rapidly enough to form foam cells and numerous studies have shown that modification of the lipid

and apoB100 moiety drives the formation of fatty streaks (Navab et al., 1996). Oxidation of LDL occurs in the arterial wall and becomes prevalent when levels of circulating LDL are raised. LDL diffuses through the endothelial cell junctions to the subendothelial matrix and its retention in the vessel wall may involve interaction with matrix proteoglycans (Stoker and Keaney, 2004). Although the precise mechanisms responsible for the oxidation of LDL remain to be fully elucidated, lipooxygenases, myelo-peroxidases, NADPH oxidases, and inducible nitric oxide synthase are major contributing enzymes (Stoker and Keaney, 2004). For example, there is diminished atherosclerosis in mice lacking 12/15 lipoxygenase. The inducible nitric oxide synthase also contributes to LDL oxidation *in vivo* and inhibitors of this enzyme have been shown to decrease atherosclerosis in rabbits.

The precise action of oxidised LDL depends on the extent of its modification, which ranges from minimal to extensive. Minimal modification allows LDL to be recognised by the LDL receptor as normal, whereas extensive modification results in the particle being bound by SRs expressed on the surface of macrophages and SMCs, primarily SR-A and CD36. The importance of scavenger receptors in atherogenesis can be gauged by the observation that inactivation of SR-A or CD36 leads to reduced atherosclerosis in murine models of the disease (Greaves and Gordon, 2005).

OxLDL taken up by macrophage SRs is delivered to lysosomes where its cholesteryl ester content is hydrolysed to free cholesterol and fatty acids. This free cholesterol has a number of potential metabolic fates, including esterification and storage as lipid droplets in foam cells (see Li and Glass, 2002). The cholesteryl ester stores of macrophages have been shown to undergo a continuous cycle of hydrolysis and reesterification. The hydrolytic step of this cycle is carried out by a neutral cytoplasmic cholesteryl ester hydrolase and the reesterification step is mediated by ACAT-1. As intracellular cholesterol levels increase, the proteolytic activation of SREBPs required for cholesterol biosynthesis and LDL-R expression is inhibited. Although this prevents the further accumulation of cholesterol via these pathways, cholesterol is still taken up via the SRs and, therefore, cholesterol homeostasis cannot be maintained.

The macrophage can dispose of excess cholesterol by either enzymatic modification to more soluble forms or efflux to acceptors, such as HDL. The enzyme 27-hydroxylase is expressed in macrophages at relatively high levels and may play a role in cholesterol excretion by converting it to the more soluble 27-oxygenated steroid, which can be readily accepted by albumin (Babiker et al., 1997). The efflux of cholesterol involves the ABC family of membrane transporters, particularly ABCA1, and apoA1 and apoE in HDL as major acceptors of the steroid.

LDL also undergoes other types of modifications, such as nonenzymatic glycation, enzymatic degradation, and aggregation. All such modifications generate a wide range of epitopes, resulting in not only a cellular immune response, but also a humoral response (Horkko et al., 2000). For example, oxLDL is known to stimulate endothelial cells to secrete a range of pro-atherogenic cytokines. In addition, modified LDL is able to activate nuclear factor-κB (NF-κB), a master transcription factor implicated in the induced expression of a battery of genes associated with an inflammatory response. Modified LDL has also been shown to act as a chemoattractant for circulating monocytes, modulate vascular tone and cause aggregation of platelets (Stoker and Keaney, 2004).

11.2.8 Therapeutic approaches in cardiovascular disease based on the control of lipid metabolism

Various health organisations are strongly recommending dietary and other lifestyle changes to help to slow down the development of CVD and decrease the pro-atherogenic parameters. These include cessation of smoking; reduced intake of diets rich in fats, particularly saturated fats, and salt; substituting saturated fatty acids in the diet with polyunsaturated fatty acids; eating the recommended daily five servings of fruit and vegetables; at least 30 minutes of moderate exercise on 5 or more days of the week by adults; moderate alcohol consumption (one or two drinks per day); and combating detrimental psychosocial factors, such as stress, depression and anxiety. A number of other dietary supplements, such as antioxidants, which inhibit oxidation of LDL at least *in vitro*, have also been recommended for the prevention of atherosclerosis. However, the results from clinical studies have not always identified a positive anti-atherogenic effect.

Dietary and life-style changes are clearly not sufficient when a "clinical horizon" has been reached. Intake of drug(s) that limit the levels of the pro-atherogenic agent(s) in patients is necessary to prevent premature death. Because atherosclerosis is associated with dramatic changes in lipid metabolism and transport, it is not surprising that several drugs, that are either in current use or being developed, target key proteins or enzymes implicated in the maintenance of lipid homeostasis (Choy, 2004; Wierzbicki, 2004).

As detailed above, numerous studies in the past 4 decades have supported a strong link between high circulating levels of LDL-cholesterol and atherosclerosis. About 75% of the total cholesterol pool in the body is derived from *de novo* synthesis with the remainder obtained from dietary intake. Inhibition of cholesterol synthesis, therefore, represents the main approach for reducing levels of circulating LDL-cholesterol. The conversion of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) to mevalonate, which is catalysed by the enzyme HMG-CoA reductase, is the rate-limiting step in the biosynthesis of cholesterol. The activity of this enzyme is inhibited by the statin class of drugs, such as lovastatin,

rosuvastatin, atorvastatin, pravastatin and simvastatin (Maron et al., 2000; Grundy et al., 2004). By decreasing cholesterol synthesis, the statins also increase the expression of the LDL-R by relieving the feedback inhibition, thereby further decreasing circulating LDL-cholesterol levels. Statins also cause a moderate decrease in serum triacylglycerol levels, a slight increase in HDL-cholesterol and inhibit the inflammatory response (Linsel-Nitschke and Tall, 2005; Elrod and Lefer, 2005). Statins are generally well tolerated except when taken at high doses where they may affect liver function. Although a reduction of LDL-cholesterol of 50 to 60% can be achieved by statins, doubling of doses produces only a marginal added decrease in LDL-cholesterol levels, but at the expense of increased side-effects. The inhibition of intestinal uptake of dietary cholesterol, which accounts for approximately 25% of this lipid in the body, is necessary to achieve further reduction of circulating LDL-cholesterol. Consumption of products rich in plant sterols and stanols, such as the Pro-Activ^{TM} range of products from Flora^{R} and similar products from Benecol^{R}, which act as inhibitors of intestinal cholesterol uptake, can lower serum LDL cholesterol levels by 10% to 14% (Plat and Mensink, 2005; Cater et al., 2005). Bile acid sequestrants (e.g., colesevelam), which bind to bile acids in the intestine, also cause a 10 to 15% decrease in plasma LDL-cholesterol levels. Such agents interrupt the enterohepatic circulation of bile acids through which most of them are recycled back to the liver and reabsorbed and, thereby, promote their excretion in the faeces (Norata and Catapano, 2004). The use of such sequestrants is, however, limited because of their severe side effects. Direct inhibition of intestinal cholesterol absorption, therefore, offers the most promise. Ezetimibe is a new drug that acts in this manner and can often be taken with statins to achieve maximal reduction of LDL-cholesterol (Gagne et al., 2002; Clader, 2005).

Increasing circulating levels of HDL represents another, important avenue to limit atherosclerosis and its complications. Low circulating levels of HDL are common in obese individuals and this is likely to be a major problem in the future because of a dramatic worldwide increase in obesity. Nicotonic acid has been used to raise HDL levels indirectly by inhibiting hepatic VLDL synthesis and peripheral lipolysis and stimulating ABCA1 expression (Carlson, 2005). More recently, major advances in devising potential approaches for increasing circulating HDL levels and reducing other pro-atherogenic changes have been made from studies on nuclear receptors, which represent "hot" therapeutic targets for CVD at the moment. Such nuclear receptors are transcription factors that regulate the expression of a battery of genes implicated in the control of triacylglycerol and cholesterol homeostasis and, additionally, have potent antiinflammatory properties. A number of such receptors were originally identified from studies on the action of antidiabetic and lipid-lowering drugs. The major nuclear receptors that are currently being studied intensely for therapeutic intervention of CVD are peroxisome proliferators-activated receptors (PPARs), liver-X-receptors (LXRs), and the farnesoid X receptor (FXR) (see Li and Glass, 2004; Marx et al., 2004; Ory, 2004; Barish and Evans, 2004; Berger et al., 2005; Claudel et al., 2005; Lehrke et al., 2005). These receptors form obligate heterodimers with retinoid X receptor, another nuclear receptor, and interact with recognition sequences in the regulatory regions of target genes.

The PPAR family contains three members; PPAR-α, -γ, and -δ (also called PPAR-β). Fibrates, such as fenofibrate and gemfibrozil, are PPAR-α agonists, which lower concentration of circulating triacylglycerols by stimulating the expression of numerous genes implicated in the cellular uptake and catabolism of lipids. Many natural compounds have also been shown to act as PPAR-α agonists, including polyunsaturated fatty acids, such as eicosapentanoic acid, dodecahexanoic acid and linoleic acid. The action of PPAR-α on lipid homeostasis is mediated, at least in part, through the activation of genes implicated in the uptake, metabolism and β-oxidation of fatty acids. The channeling of fatty acids to the β-oxidative pathway reduces the availability of substrate for the synthesis of triacylglycerols and, thereby, ultimately leads to a decrease in the synthesis and secretion of VLDL by the liver. In addition, PPAR-α agonists stimulate the hydrolysis of circulating lipoproteins by increasing the synthesis of LPL and decreasing the levels of apoCIII, an inhibitor of LPL. Furthermore, PPAR-α agonists elevate HDL levels because of their ability to increase the synthesis of apoA1, thereby enhancing the formation of new HDL particles and inhibiting the expression of SR-BI, thus decreasing the clearance of HDL. Moreover, PPAR-α agonists have been shown to stimulate cholesterol efflux from foam cells.

The PPAR-γ gene gives rise to two isoforms, PPAR-γ1 and γ2, by alternative use of promoters. PPAR-γ2 is expressed specifically in the adipose tissue, whereas PPAR-γ1 is the predominant isoform in other tissues, such as the liver and muscle. PPAR-γ2 is essential for the differentiation of adipocytes and the maintenance of normal glucose metabolism and promotes lipid accumulation by these cells. A number of naturally occurring fatty acid metabolites can activate PPAR-γ, including 15-deoxy$\Delta_{12,14}$-prostaglandin J_2 and oxidised linoleic acid (9- and 13-hydroxyoctadecadienoic acids). Pharmacological activators of PPAR-γ, such as glitazones (e.g., rosiglitazone, pioglitazone), have been used widely to improve insulin sensitivity in type II diabetes. Several actions of PPAR-γ are likely to contribute to improved insulin sensitivity, including induced expression of insulin-dependent glucose transporter GLUT4, stimulation of insulin signalling, inhibition of lipolysis and increased uptake of fatty acids and synthesis of triacylglycerols. In addition, PPAR-γ agonists regulate the secretion of several proteins by the adipose tissue, such as adiponectin, which then affects insulin signalling

in the liver and muscles. PPARγ activators have been shown to inhibit atherosclerosis in murine models of the disease.

Activators of both PPAR-α and PPAR-γ are also known to inhibit the inflammatory response indirectly by antagonising the actions of key transcription factors, such as NF-κB. As both PPAR-α and -γ have beneficial effects on lipid metabolism, inflammation, and insulin sensitivity, combined use of agonists for both these nuclear receptors promises to be a more effective approach at limiting atherosclerosis than agonists for individual receptors.

Studies on the third PPAR member, PPAR-δ (previously called PPAR-β), have generally lagged behind the other two family members, but recent studies have suggested that it also represents another promising therapeutic target for limiting atherosclerosis. PPAR-δ is expressed in a ubiquitous manner and regulates energy homeostasis and fatty acid catabolism. PPAR-δ-deficient mice are susceptible to obesity, whereas transgenic mice that overexpress an activated form of this transcription factor are resistant to genetically or diet-induced hyperlipidemia and obesity.

There are two LXRs, LXR-α and LXR-β, with the latter being expressed ubiquitously and the former present at high levels in the liver, intestine, adipose tissue, and macrophages. Both LXRs are activated by oxidised derivatives of cholesterol (e.g., 22(R)-hydroxycholesterol, 24(S)-hydroxycholesterol, 24(S), 25-epoxycholesterol, 27-hydroxycholesterol) and, therefore, act as intracellular sensors of cholesterol. Target genes for the action of LXRs include those implicated in the efflux of cellular cholesterol (e.g., apoE, ABCA1, ABCG1, ABCG4), modification of HDL (e.g., CETP, PLTP), secretion of cholesterol into bile (cholesterol 7α-hydroxylase, ABCG5, ABCG8), and fatty acid metabolism (e.g., sterol response element binding protein SREBP1c, fatty acid synthase, sterol-CoA desaturase-1). The expression of the LXR-α gene is also subject to autoregulation. Overall, LXRs restore cellular cholesterol balance and prevent lipotoxicity by activating cholesterol catabolic and efflux pathways along with those involved in *de novo* lipogenesis. In addition, LXR activators inhibit the expression of genes implicated in inflammation by antagonising the actions of key transcription factors, such as NF-κB and activator protein-1 (AP-1), largely via a mechanism that does not require sequence-specific DNA binding by the LXRs.

A number of *in vitro* and *in vivo* studies have revealed a potent anti-atherogenic action of LXRs. However, a major current limitation of employing LXRs as therapeutic targets for atherosclerosis is that they increase the synthesis of fatty acids and cause the accumulation of triacylglycerols. Development of synthetic agonists that increase HDL levels without causing hypertriglyceridemia will clearly be necessary. This is a possibility since ongoing research suggests differences in the action of LXR-α and LXR-β, with the former having a dominant effect on hepatic lipogenesis. Thus, selective LXR-β agonists could represent potential therapeutic agents for limiting atherosclerosis.

FXR was initially identified as a nuclear receptor that is activated by bile acids and products of cholesterol metabolism. FXR regulates the expression of a number of genes implicated in the synthesis, transport and detoxification of bile acids. In addition, recent studies have suggested a role for FXR in the control of lipid metabolism and implicate its activation as a new means for limiting atherosclerosis. For example, polyunsaturated fatty acids, such as arachidonic, linolenic and docosahexaenoic acid, have all been shown to act as FXR ligands *in vitro*. Additionally, FXR-deficient mice have increased circulating levels of total triacylglycerols and cholesterol and decreased expression of SR-BI in the liver.

Other targets for preventing atherosclerosis by modulating circulating lipid levels include inhibitors of CETP and ACAT and infusion of HDL particles or apolipoproteins that act as acceptors of cholesterol (Choy, 2004). CETP deficiency in humans is characterised by increased HDL levels and slightly reduced LDL levels. Moderate consumption of alcohol can increase circulating HDL levels and reduce atherosclerosis, at least in part, by inhibiting CETP. Inhibitors of CETP (e.g., CP-529/414 (torcetrapib), JTT-705) have been developed and are undergoing advanced clinical trials. The esterification of cholesterol by ACAT is critical for macrophage foam cell formation and lipoprotein synthesis in the liver and the intestine. Inhibitors of ACAT, such as avasimibe and CS-505, offer a further avenue for limiting atherosclerosis. Interest in apoA1 as a therapeutic target has emerged from the finding that its deficiency in humans is associated with premature atherosclerosis and extremely low levels of circulating HDL. Although a search of synthetic molecules that specifically induce apoA1 expression has not been fruitful, some success has been achieved, at least in murine models of atherosclerosis, by infusion of apoAI or synthetic peptides that mimic the actions of this lipoprotein.

Overall, it appears that patients with CVD will be treated in the future with combination of drugs that lower cholesterol synthesis and induce the expression of the LDL-R (statins), inhibit the absorption of dietary cholesterol (e.g., ezetimibe) and enhance cholesterol efflux from foam cells and raise circulating HDL levels (e.g., agonists of nuclear receptors).

References

Acton, S. et al. (1996). Identification of scavenger receptor SR-BI as a high-density lipoprotein receptor. *Science*, **271**, 518–520.

Anderson, K.M. et al. (1987). Cholesterol and mortality: 30 years of follow-up from the Framingham study. *JAMA*, **257**, 2176–2180.

Arai, T. et al. (1999). Decreased selective uptake of high-density lipoprotein cholesteryl esters in apolipoprotein

E knock-out mice. *Proc. Natl. Acad. Sci.* U.S.A. **96**, 12050–12055.

Babiker, A. et al. (1997). Elimination of cholesterol in macrophages and endothelial cells by the sterol 27-hydroxylase mechanism. Comparison with high density lipoprotein-mediated reverse cholesterol transport. *J. Biol. Chem*. **272**, 26253–26261.

Barish, G.D. and Evans, R.M. (2004). PPARs and LXRs: atherosclerosis goes nuclear. *Trends Endocrinol. Metab.* **15**, 158–165.

Barrett-Connor, E. and Bush, T.L. (1991). Estrogen and coronary heart disease in women. *JAMA* **265**, 1861–1867.

Berger, J.P. et al. (2005). PPARs: therapeutic targets for metabolic disease. *Trends Pharmacol. Sci.* **26**, 244–251.

Berglund, L. and Ramakrishnan, R. (2004). Lipoprotein (a): an elusive cardiovascular risk factor. *Arterioscler. Thromb. Vasc. Biol.* **24**, 2219–2226.

Boffa, M.B. et al. (2004). Lipoprotein (a) as a risk factor for atherosclerosis and thrombosis: mechanistic insights from animal models. *Clin. Biochem.* **37**, 333–343.

Brown, M.S. and Goldstein, J.L. (1999). A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood. *Proc. Natl. Acad. Sci.* U.S.A. **96**, 11041–11048.

Bruce, C. et al. (1998). Plasma lipid transfer proteins, high-density lipoproteins, and reverse cholesterol transport. *Annu. Rev. Nutr.* **18**, 297–330.

Carlson, L.A. (2005). Nicotinic acid: the broad-spectrum lipid drug. A 50th anniversary review. *J. Intern. Med.* **258**, 94–114.

Cater, N.B. et al. (2005). Responsiveness of plasma lipids and lipoproteins to plant stanol esters. *Am. J. Cardiol*. **96**, 23D–28D.

Choy, P.C. (2004). Lipids and atherosclerosis. *Biochem. Cell Biol.* **82**, 212–224.

Clader, J.W. (2005). Ezetimibe and other azetidinine cholesterol absorption inhibitors. *Curr. Top. Med. Chem.* **5**, 243–256.

Claudel, T. et al. (2005). The farnesoid X receptor: a molecular link between bile acid and lipid and glucose metabolism. *Arterioscler. Thromb. Vasc. Biol.* **25**, 2020–2030.

Daugherty, A. et al. (2005). Thematic review series: the Immune system and atherogenesis. Cytokine regulation of macrophage functions in atherogenesis. *J. Lipid Res.* **46**, 1812–1822.

De Caterina, R. et al. (2004). Effects of omega-3 fatty acids on cytokines and adhesion molecules. *Curr. Atheroscler. Rep.* **6**, 485–491.

Eberle, D. et al. (2004). SREBP transcription factors: master regulators of lipid homeostasis. *Biochimie.* **86**, 839–848.

Elrod, J.W. and Lefer, D.J. (2005). The effects of statins on endothelium, inflammation and cardioprotection. *Timely Top. Med. Cardiovasc. Dis.* **9**, E20.

Fredrickson, D.S. and Breslow, J.L. (1973). Primary hyperlipoprotenemia in infants. *Annu. Rev. Med.* **24**, 315–324.

Gagne, C., Gaudet, D. and Bruckert, E. (2002). Efficacy and safety of ezetimibe added to ongoing statin therapy for treatment of patients with primary hypercholesterolemia. *Am. J. Cardiol.* **90**, 1084–1091.

Genest, J. (2003). Lipoprotein disorders and cardiovascular risk. *J. Inherit. Metab. Dis.* **26**, 267–287.

Glass, C.K. and Witztum, J.L. (2001). Atherosclerosis. The road ahead. *Cell*. **104**, 503–516.

Greaves, D.R. and Gordon, S. (2005). Recent insights into the biology of macrophage scavenger receptors. *J. Lipid Res.* **46**, 11–20.

Greenow, K. et al. (2005). The key role of apolipoprotein E in atherosclerosis. *J. Mol. Med.* **83**, 329–342.

Grundy, S.M. et al. (2004). Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III Guidelines. *J. Am. Coll. Cardiol.* **44**, 720–732.

Gurr, M.I. (1992). Dietary lipids and coronary heart disease: old evidence, new perspective. *Prog. Lipid Res.* **31**, 195–243.

Harvey, E.J. and Ramji, D.P. (2005). Interferon-γ and atherosclerosis: pro- or anti-atherogenic? *Cardiovasc. Res.* **67**, 11–20.

Horkko, S. et al. (2000). Immunological responses to oxidized LDL. *Free Radic. Biol. Med.* **28**, 1771–1779.

Jump, D.B. (2004). Fatty acid regulation of gene transcription. *Crit. Rev. Clin. Lab. Sci.* **41**, 41–78.

Lehrke, M. et al. (2005). Gaining weight: the Keystone symposium on PPAR and LXR. *Genes Dev.* **19**, 1737–1742.

Levy, R.I. and Fredrickson, D.S. (1968). Diagnosis and management of hyperproteinemia. *Am. J. Cardiol.* **22**, 576–583.

Li, A.C. and Glass, C.K. (2002). The macrophage foam cell as a target for therapeutic intervention. *Nat. Med.* **8**, 1235–1242.

Li, A.C. and Glass, C.K. (2004). PPAR- and LXR-dependent pathways controlling lipid metabolism and the development of atherosclerosis. *J. Lipid. Res.* **45**, 2161–2173.

Linsel-Nitschke, P. and Tall, A.R. (2005). HDL as a target in the treatment of atherosclerotic cardiovascular disease. *Nat. Rev. Drug Discov.* **4**, 193–206.

Lusis, A.J. (2000). Atherosclerosis. *Nature.* **407**, 233–241.

Lusis, A.J. et al. (2004). Genetics of atherosclerosis. *Annu. Rev. Genomics Hum. Genet*. **5**, 189–218.

Mahley, R.W. et al. (1999). Pathogenesis of type III hyperlipoproteinemia (dysbetalipoproteinemia). Questions, quandaries, and paradoxes. *J. Lipid. Res.* **40**, 1933–1949.

Maron, D.J. et al. (2000). Current perspectives on statins. *Circulation.* **101**, 207–213.

Martin, M.J. et al. (1986). Serum cholesterol, blood pressure and mortality: implications from cohort of 361,662 men. *Lancet,* **2**, 933–936.

Marx, N. et al. (2004). Peroxisome proliferators-activated receptors and atherogenesis: regulators of gene expression in vascular cells. *Circ. Res.* **94**, 1168–1178.

Mead, J.R. and Ramji, D.P. (2002). The pivotal role of lipoprotein lipase in atherosclerosis. *Cardiovasc. Res.* **55**, 261–269.

Mead, J.R. et al. (2002). Lipoprotein lipase: structure, function, regulation, and role in disease. *J. Mol. Med.* **80**, 753–769.

Mendelsohn, M.E. and Karas, R.H. (2005). Molecular and cellular basis of cardiovascular gender differences. *Science,* **308**, 1583–1587.

Mori, T.A. (2004). Effect of fish and fish oil-derived omega-3 fatty acids on lipid oxidation. *Redox Rep.* **9**, 193–197.

Mori, T.A. and Beilin, L.J. (2004). Omega-3 fatty acids and inflammation. *Curr. Atheroscler. Rep.* **6**, 461–467.

Navab, M. et al. (1996). The yin and yang of oxidation in the development of the fatty streak. A review based on the 1994 George Lyman Duff Memorial Lecture. *Arterioscler. Thromb. Vasc. Biol.* **16**, 831–842.

Norata, G.D. and Catapano, A.L. (2004). Lipid lowering activity of drugs affecting cholesterol absorption. *Nutr. Metab. Cardiovasc. Dis.* **14**, 42–51.

Ory, D.S. (2004). Nuclear receptor signalling in the control of cholesterol homeostasis: have the orphans found a home? *Circ. Res.* **95**, 660–670.

Osterud, B. and Bjorklid, E. (2003). Role of monocytes in atherogenesis. *Physiol. Rev.* **83**, 1069–1112.

Plat, J. and Mensink, R.P. (2005). Plant stanol and sterol esters in the control of blood cholesterol levels: mechanism and safety aspects. *Am. J. Cardiol.* **96**, 16D–22D.

Rader, D.J. et al. (2003). Monogenic hypercholesterolemia: new insights in pathogenesis and treatment. *J. Clin. Invest.* **111**, 1795–1803.

Rigotti, A. et al. (1997). A targeted mutation in the murine gene encoding the high density lipoprotein (HDL) receptor scavenger receptor class B type I reveals its key role in HDL metabolism. *Proc. Natl. Acad. Sci.* U.S.A. **94**, 12610–12615.

Ross, R. (1999). Atherosclerosis — an inflammatory disease. *N. Engl. J. Med.* **340**, 115–126.

Smith, J. D. et al. (1995). Decreased atherosclerosis in mice deficient in both macrophage colony stimulating factor (op) and apolipoprotein E. *Proc. Natl. Acad. Sci.* U.S.A. **92**, 8264–8268.

Stocker, R. and Keaney, J.F., Jr., (2004). Role of oxidative modifications in atherosclerosis. *Physiol. Rev.* **84**, 1381–1478.

Vanden Heuvel, J.P. (2004). Diet, fatty acids, and regulation of genes important for heart disease. *Curr. Atheroscler. Rep.* **6**, 432–440.

Wang, N. et al. (1998). Liver-specific overexpression of scavenger receptor BI decreases levels of very low-density lipoprotein apoB, low density lipoprotein apoB and high density lipoprotein in transgenic mice. *J. Biol. Chem.* **273**, 32920–32926.

Wierzbicki, A.S. (2004). Lipid-altering agents: the future. *Int. J. Clin. Prac.* **58**, 1063–1072.

11.3 Clinical aspects of lipids with emphasis on cardiovascular disease and dyslipaemia

11.3.1 Objectives of cardiovascular disease prevention in clinical practice

The specific objective of cardiovascular disease (CVD) prevention for all high-risk people in clinical practice is to reduce the risk of the disease and its associated complications. These include the need for percutaneous or surgical revascularisation procedures in any arterial territory, along with improvements in both quality of life and life expectancy. Cardiovascular disease risk reduction in practice involves a multifactorial approach involving appropriate lifestyle changes, such as weight loss, dietary modification, increasing exercise and smoking cessation. Pharmacological interventions aimed at reducing cardiovascular risk also revolve around a multifactorial approach including antiplatelet therapy, anticoagulation and a target-driven approach to blood pressure reduction. Although optimal cardiovascular disease risk reduction requires a multifactorial approach, prospective epidemiological data have consistently demonstrated the preeminence of abnormalities in lipid metabolism in the aetiology of atherosclerotic vascular disease, in particular, coronary heart disease. Hence, the management of dyslipidaemia in clinical practice represents a central pillar in the approach to cardiovascular disease risk reduction.

11.3.2 Dyslipidaemia and cardiovascular disease

As concentrations of total and LDL-cholesterol increase, so does the risk of cardiovascular disease (Stamler et al., 2000). The relationship between cholesterol and cardiovascular risk is continuous, while reduction of total and low density lipoprotein-cholesterol (LDL-C) is associated with numerous sequelae (which attenuate atherogenesis) including improved endothelial function, reduced oxidative stress and reduced inflammation (Ross, 1993; Vogel, 1997).

In the context of lipids, cardiovascular risk is principally determined by the concentrations of total, LDL- and high density lipoprotein-cholesterol (HDL-C) (inversely) and, to a lesser extent, plasma triacylglycerol concentrations. Indeed, data from various studies including Framingham and MRFIT (Kannel and Larson, 1993; Stamler et al., 1993) have demonstrated that low levels of HDL-C independently predict increased cardiovascular risk, irrespective of total or LDL-C levels (Stamler et al., 1993). The ratio of total or LDL-C to HDL-C has been consistently demonstrated as the strongest determinant of cardiovascular risk in prospective studies. This was further illustrated by the recent INTERHEART study, in which the ratio of apo B- to apo AI-containing lipoproteins was demonstrated to be the single most important determinant of risk for first myocardial infarction from over 10,000 subjects from varying geographical locations and ethnic origins (Yusuf et al., 2004). Meta-analysis of prospective studies has consistently demonstrated that a 1% decrease in HDL-C is associated with a 2 to 3% increase in cardiovascular risk, while the relationship between HDL-C and cardiovascular risk may be stronger in women (Expert Panel, 2001).

The reduction of total LDL and LDL-cholesterol is, however, currently the primary goal of lipid-lowering therapy with respect to cardiovascular risk reduction. The reduction of cholesterol whether by diet, drugs, or other means is associated with a reduced risk of CVD (Brady and Betteridge, 2003) and since lipoproteins are only one element of cardiovascular risk, which is determined overall by the presence of other risk factors, the absolute benefit of cholesterol reduction, is a function of baseline cardiovascular risk. The MRC/BHF Family Heart Protection Study (2002) demonstrated that the benefits of cholesterol lowering therapy extend into all forms of atherosclerotic vascular disease including peripheral vascular disease (PVD) and cerebrovascular disease. In a systematic review and meta-analysis evaluating the effects of cholesterol reduction on coronary heart disease (CHD) and stroke, 58 clinical trials of cholesterol reduction by any means were included (Law et al., 2003). Reduction in CHD death and nonfatal myocardial infarction for a 1.0 mmol/l reduction in cholesterol was 11% in the first year, 24% in the second, 33% in the third to fifth, and 36% in the sixth and subsequent years. After standardisation for reduction in LDL-C and duration of treatment, the reduction in risk of fatal and nonfatal events was similar for different

methods of reducing cholesterol and for people with and without CHD. The reduction in cholesterol and the duration of treatment were the primary determinants of risk reduction, with a reduction in LDL-C of 1.6 mmol/l or more after 2 years resulting in a 51% reduction in risk.

The most compelling evidence for cholesterol lowering comes from clinical trials using statins. The early major statin trials in people with established CVD using simvastatin and pravastatin and in people at risk of developing CVD using pravastatin and lovastatin, demonstrated reduction in coronary morbidity and mortality, and in all-cause mortality where statistical power was sufficient. A meta-analysis of these studies demonstrated reductions in coronary events of 31%, coronary mortality of 29%, and all-cause mortality of 21% (Baienet et al., 2005). More recent trials have extended the evidence base for this class of drug into many populations including women, the elderly, acute coronary disease patients, people with diabetes and renal transplantation, and people previously thought to be at low risk due to low baseline cholesterol levels.

In the recent cholesterol trialists collaboration (Baienet et al., 2005), a meta-analysis of data from 90,056 individuals in 14 randomised trials of statins, has shown that statin therapy can safely reduce the 5-year incidence of major coronary events, coronary revascularisation and stroke by about one-fifth per mmol/l reduction in cholesterol. During a mean of 5 years, there were 8186 deaths, 14,348 individuals had major vascular events, and 5103 developed cancer. Mean LDL cholesterol differences at 1 year ranged from 0.35 mmol/l to 1.77 mmol/l (mean 1.09) in these trials. There was a 12% proportional reduction in all-cause mortality per mmol/l reduction in LDL cholesterol. This reflected a 19% reduction in coronary mortality, and nonsignificant reductions in noncoronary vascular mortality and nonvascular mortality. There were corresponding reductions in myocardial infarction or coronary death, in the need for coronary revascularisation, in fatal or nonfatal stroke, and, combining these, of 21% in any such major vascular event. These benefits were significant within the first year, but were greater in subsequent years. Taking all years together, the overall reduction of about one-fifth per mmol/l, LDLC reduction translated into 48 fewer participants having major vascular events per 1000 among those with preexisting CHD at baseline, compared with 25 per 1000 among participants with no such history. A similar proportional reduction in risk is seen for all people with atherosclerotic disease regardless of the vascular territory. Women demonstrated a similar proportionate reduction in risk to men, with no age at which these benefits have not been demonstrated, with older people who are at higher absolute risk having similar risk reduction as younger people. The absolute benefit of statin therapy is related chiefly to an individual's absolute cardiovascular risk and to the absolute reduction in cholesterol achieved. These observations reinforce the need to consider prolonged statin treatment with substantial LDL-C reductions in all patients at high risk of any type of major vascular event. Furthermore, in a recent study of the safety and efficacy of intensive cholesterol reduction in 1825 patients with acute coronary syndrome, continuing outcome benefits were demonstrated in association with reductions in LDL-C as low as <1 mmol/l (Wiviott et al., 2005).

Although LDL-C is undoubtedly a causal risk factor for CHD, LDL-C alone is insufficient to fully evaluate cardiovascular risk. The role of triacylglycerol (TAG) and HDL-C levels in determining vascular risk has been demonstrated by various prospective studies including the PROCAM study (Assman et al., 1998). Within each LDL-C sub-group the risk of myocardial infarction appears to increase with increasing TAG levels and reduced HDL-C levels, an effect which was most pronounced in subjects with lower LDL-C levels. Thus, optimal lipid lowering therapy with respect to maximising cardiovascular outcome benefits may also include TAG reduction, raising HDL-C as well as reducing LDL-C.

Presently five classes of drugs are available for the treatment of dyslipidaemia:

1. Bile acid sequestrants (resins)
2. Fibric acid derivatives (fibrates)
3. Nicotinic acid (niacin)
4. Omega-3 fish oils
5. 3-HMG-CoA reductase inhibitors (statins)

The statins are the most potent LDL-C lowering agents, but have variable effects on HDL-C, which may represent a potential limitation with respect to optimal CHD risk reduction with statin therapy.

A meta-analysis of 53 trials (16,802 subjects) using fibrates (Brijmohun et al., 2005) and 30 trials using nicotinic acid (4749 subjects) reported a 25% reduction in risk of a major cononary event for fibrates and a 27% reduction for the same end-points for nicotinic acid. However, there was no reduction in total mortality. The largest study of fibrates to date, the FIELD (2005) trial of 9795 people with type 2 diabetes, showed that taking fenofibrate reduced the total CVD events significantly with a reduction of 11%. There were a variety of confounding issues in the FIELD trial, in particular, the high degree of non-trial lipid lowering medication, namely statins, in both fenofibrate and placebo groups.

Thus, the case of fibrates, particularly in people with type 2 diabetes, has not been firmly established, at least based on this particular trial evidence. The results of the ACCORD trail in diabetes comparing the effect of statin therapy both with and without fibrates will be available in the year 2010 and will provide more evidence regarding the utility of combination statin and fibrate therapy on cardiovascular disease risk reduction. Currently, however, statins and the reduction of total LDL levels may be the mainstay of lipid-lowering therapy of risk factor cardiovascular disease risk reduction.

11.3.3 Cholesterol: current evidence and guidelines

Guideline recommendations are useful benchmarks for evidence-based practice, applied in the context of clinical judgment, but they may not always reflect the most up-to-date evidence, as they are published intermittently.

Despite the clear epidemiological association between cholesterol and cardiovascular risk, many individuals who develop vascular disease do not have particularly elevated cholesterol levels. The evidence that incrementally lower cholesterol is better in terms of cardiovascular disease risk (Figure 11.4) comes both from epidemiological studies and randomised clinical trials. Epidemiological evidence supporting the notion that lower LDL-C levels are associated with lower CHD risk comes from studies of men in rural China, where participants in the lowest quartile of LDL-C (<3.0 mmol/l) had coronary event rates 75% lower than those in the highest quartile (Chen et al., 1991). Further evidence in support of this notion comes from the Seven Countries study, initiated in the 1960s, which assessed the relationship between serum cholesterol and CHD on both an intra- and interpopulation basis (Kannel and Larson, 1993), as well as prospective longitudinal studies, such as PROCAM (Prospective Cardiovascular Munster Study) and the Framingham study. Every major clinical endpoint trial of lipid lowering therapy has demonstrated that lower LDL-C levels are associated with a reduced atherosclerotic disease burden (see Figure 11.4).

Comparing early trials of statins and other treatment modalities (such as bile acid sequestrant resins and ileal-bypass) with more recent statin trials, it appears that the benefit of absolute LDL reduction is present across a wide range of baseline LDL cholesterol values (Figure 11.5). As quantified in such a meta-analysis, this illustrates an approximate linear relationship, with greater cardiovascular disease reductions associated with greater LDL reductions, from baseline LDL values up to 5 mmol/l to achieved LDL cholesterol values <2 mmol/l. These

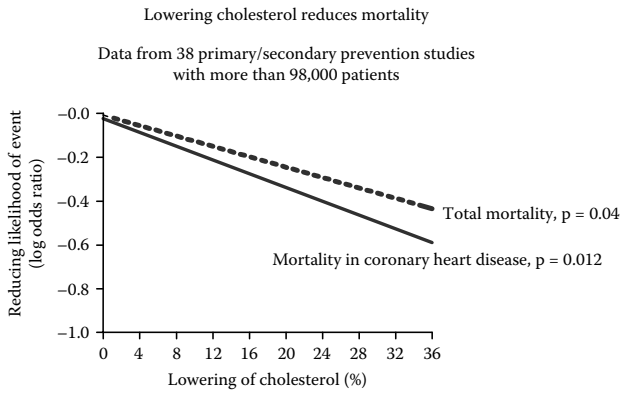


FIGURE 11.4 Cholesterol reduction and the risk of vascular events — lower is better. (Adapted from Gould, A.L., et al., 1998.)

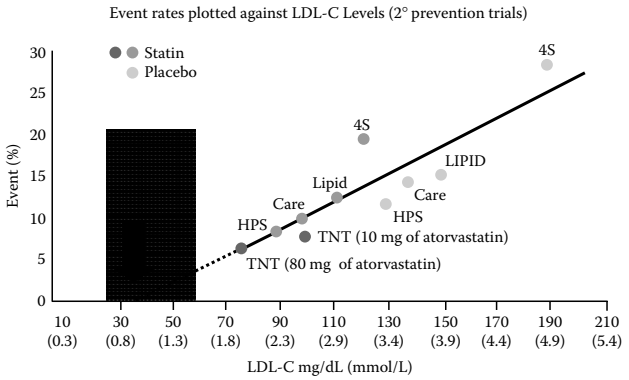


FIGURE 11.5 Linear relationship between achieved LDL-C levels in major statin trials and coronary event rates. The black block identifies a theoretical LDL-C range at which coronary events become negligible. (From La Rosa, J.C., et al., 2005.)

observations are not just true for statin trials, but also trials using dietary modification, anion exchange resins, or ileal-bypass surgery. The relation is present where individuals have either established cardiovascular disease or are at high risk of developing cardiovascular disease, or whether they had initially higher or lower LDL cholesterol values. In the more recent trials the baseline LDL cholesterol values were around 3 mmol/l and fell following treatment to <2 mmol/l.

Such observations suggest that there may be no lower threshold for LDL-C reduction, below which additional cardiovascular benefit may not be achieved. A potential optimum LDL-C range of between 1.3 to 1.8 mmol/l has been suggested, which was the typical LDL-C levels of our hunter-gatherer forefathers, where there was little evidence of atherosclerotic vascular disease, even in individuals living to their 7th or 8th decade of life (O'Keefe et al., 2004).

This LDL-C range is interestingly that range, hypothesised by Goldstein and Brown (1992), beyond which the LDL receptor becomes supersaturated. Thus, with the advent of increasingly potent cholesterol-reducing agents, it is conceivable that reductions in cholesterol to this range may result in free LDL-receptor capacity, which may result in enhanced clearance of all cholesterol containing lipoproteins (VLDL, IDL, etc.), which may potentiate atherosclerosis disease regression. Indeed the notion that intensive LDL-C reduction may result in atherosclerosis disease regression has been recently supported by the ASTEROID study in which LDL-C reductions below 1 mmol/l were associated with significant regression in coronary atherosclerosis as assessed by intravascular ultrasound (Nissen et al., 2006).

Historically, guideline base recommendations about initiation of cholesterol-lowering therapy primarily on an estimated risk of coronary events alone. The MRC/BHF Heart Protection Study (2002) demonstrated not just a reduction in CHD events but also in ischaemic strokes and peripheral revascularisations. In this study, the chief determinants of absolute risk were the type of preexisting

TABLE 11.8 Guidelines for lowering LDL-C

Guideline	Year published	LDL-C target (mmol/l)	Total cholesterol target (mmol/l)
JBS	1998	<3.0	<5.0
EAS	1998	<3.0	<5.0
NSF for CHD	2000	<3.0 and 30% reduction	<5.0 and 25% reduction
EAS	2003	<2.5 in high risk	<4.5 in high risk
BHS IV	2004	<2.0 in high risk	<4.0 in high risk
European Guidelines	2004	<3 mmol/l in nonhigh risk	<5 mmol/l in nonhigh risk
		<2.5 mmol/l in high risk, CVD and diabetes	<4.5 mmol/l in high risk, CVD and diabetes
NCEP ATP III	2004	<1.8 mmol/l in v. high risk	Not specified in 2004 update
		<2.6 mmol/l in mod. to high risk and 30–40% reduction	
JBS 2	2005	<2 mmol/l	<4 mmol/l

vascular disease, the presence or absence of type 2 diabetes, or some combination of these conditions, with significant reductions in relative risk produced by statin therapy irrespective of pretreatment LDL-C levels. Such a concept is further supported by data from the CARDS trial that demonstrated statin therapy resulted in significant reduction of cardiovascular events in patients with type 2 diabetes plus one other cardiovascular risk, who had pretreatment cholesterol levels at or near to previously accepted target levels (Colhoun et al., 2004).

11.3.3.1 Cholesterol reduction and stroke

The relation between cholesterol reduction and stroke in epidemiological studies is less clear than for coronary heart disease. Meta-analyses of observation studies have either not shown any relation between strokes and cholesterol levels or have reported a positive relation to ischaemic stroke and inverse relation to haemorrhagic stroke (Eastern Stroke Group, 1998; Zhang et al., 2003). The effects of lipid lowering therapy on the incidence and recurrence of stroke has been uncertain in individual trials, with some showing benefit and others not, but early meta-analysis of statin trials reported significant reductions in stroke events of between 15 to 30% (Crouse et al., 1998). In more recent meta-analyses, cholesterol reduction by any means was associated with a 20% reduction in risk of all stroke, primarily due to reduction in thromboembolic stroke (Baienet et al., 2005). More recent placebo controlled statin trials with specified stroke as a predefined end point have demonstrated a substantial reduction in ischaemic stroke.

In the MIRACL trial (Schwartz et al., 2001) stroke was a predefined secondary end point and despite the 16th week follow-up there was a 50% relative reduction in fatal and nonfatal stroke, which was also significant for nonfatal stroke alone. In the PROSPER trial, stroke was part of the composite primary end point and based on a total of 266 nonfatal and fatal strokes there was no difference for stroke alone (Shepard et al., 2002). In the ASCOT LLA trial, the combination of a fatal and nonfatal stroke together was reduced by 27% (Sever et al., 2003). In the CARDS trial, stroke was also a prespecified secondary end point; this was reduced by 48%. In the meta-analysis of statin trials, there was an average reduction of 1 mmol/l in LDL-cholesterol associated with a 21% reduction in stroke risk (Amarenco et al., 2004). In the cholesterol trialists collaboration report, a 17% proportional reduction in the incidence of first stroke of any type per mmol/l reduction in cholesterol was noted (Baienet et al., 2005).

Thus, the clinical trial evidence for statin therapy in the primary prevention of stroke or cerebrovascular ischaemia is now compelling and applies to both people with and without established cardiovascular disease, those with hypertension, diabetes, and all subjects at high risk of developing cerebrovascular disease. Therefore, the most recent treatment guidelines for the management of cholesterol advocate global cardiovascular disease reduction including both coronary heart disease and cerebrovascular disease prevention as an indication for initiation of lipid lowering therapy.

For those subjects who have already had a stroke there is currently no evidence that statin therapy will reduce the risk of recurrent stroke. Thus, the clinical trial evidence for lipid lowering therapy, in particular, statin treatment following the development of stroke, supports the use of these agents to reduce the risk of further major vascular events, but not at present for the reduction in risk of recurrent stroke.

11.3.3.2 Cholesterol: current clinical guidelines and practice

11.3.3.2.1 Lipid assessment

The most recent treatment guidelines (JBS 2, 2005) advocate that all adults over the age of 40 should have their total and HDL-C measured as part of opportunistic CVD risk assessment, with the total and LDL-C levels viewed in the context of total cardiovascular risk. For asymptomatic individuals with no history of CVD or diabetes, cardiovascular risk is assessed on the basis of standard risk predication charts utilising age, blood pressure, and total to HDL-C ratio to calculate risk. Since obesity, ethnicity, serum TAG, and smoking are all recognised cardiovascular risk factors, the risk score derived from the risk prediction charts should be multiplied by an

appropriate factor. Although total and HDL-C can be measured in the nonfasting state, all people at risk of CVD require a full fasting lipid profile, following an overnight fast enabling measurement of total, LDL-, and HDL-cholesterol as well as plasma TAG levels. When LDL-C levels cannot be measured directly, the Friedwald equation may be employed (LDL-C = total cholesterol – HDL-C – [TAG/ 2.2]). In asymptomatic individuals, lipid values should be repeated on several separate occasions prior to initiating therapy due to both biological and laboratory variation.

At the time of an acute coronary syndrome, including myocardial infarction, total cholesterol, LDL-cholesterol, and HDL-cholesterol levels decrease, while triacylglycerols may rise. Other acute vascular diseases, other severe diseases, and major surgery will also impact on blood lipids in a similar manner. The depression of total cholesterol following a myocardial infarction generally lasts for no longer than 6 to 8 weeks, but can be longer if there is a complicated recovery period (Mbewu et al., 1993). A lipid measurement, however, should be conducted as soon as possible and preferably within 24 hours of the onset of symptoms. This would give a reasonable indication of total cholesterol and HDL-cholesterol values before the acute event, although it usually will be an underestimation. It is important, therefore, to measure the full fasting lipid profile around 8 to 12 weeks following an acute cardiovascular event, although this will usually be in the context of ongoing lipid lowering therapy. The clinical reasons for repeated lipid measurements are (1) to determine whether the patient has familial dyslipidaemia, in particular familial hypercholesterolaemia, therefore, resulting in family screening; (2) to assess whether the person has achieved appropriate LDL and total cholesterol targets; and (3) as a guide to possible treatment changes in order to optimise the lipid profile. The commonest adverse events related to statin therapy in practice include derangement of liver function tests and muscle toxicity.

While the main treatment target for lipid management in people at risk of cardiovascular disease is LDL-cholesterol, many still exhibit a mixed dyslipidaemia with elevated triacylglycerols and low HDL. In such individuals, the primary treatment is still to treat LDL-C to target with a statin. This mixed dyslipidaemia represents an accumulation of atherogenic triacylglycerol-rich remnant lipoproteins including LDL, IDL and chylomicrons. Although there are no lipid targets for this proportion of the lipid profile, namely a non-HDL-cholesterol, the currently suggested desirable level of <3 mmol/l is advocated for high risk patients by current guidelines. Statins, the current mainstay of lipid lowering therapy, reduce LDL-cholesterol and non-HDL-cholesterol by approximately a similar order of magnitude, as their primary mode of action, mediated via the IDL receptor, results in clearance of all cholesterol-containing lipoprotein particles.

11.3.3.3 Impact on cardiovascular disease by high-density lipoprotein, triacylglycerol and other lipid subfractions

The role of triacylglycerol (TAG) and HDL-C levels in determining vascular risk has been demonstrated by studies, such as the PROCAM study (Assmann et al., 1998). Within each LDL-C subgroup, the risk of myocardial infarction increased with increasing TAG levels and reduced HDL-C levels, an effect which was most pronounced in subjects with lower LDL-C levels. Another study further illustrated the importance of HDL-C in predicting CHD risk in subjects with average LDL-C levels. In this study subjects with low HDL-C and average LDL-C levels benefited disproportionately from statin therapy (Gotto et al., 2000).

In a recent study of patients with unstable angina, elevated plasma TAG and reduced HDL-C, and apo A-I levels were highly significant predictors of progression to coronary events (Bolibar et al., 2000). LDL-C was of marginal significance and the risk of events increased substantially with increasing tertiles of TAG and decreasing apo A-I levels. Indeed apo-A-I is increasingly recognised as an important factor in modulating atherogenesis (Nicholls et al., 2006; Nissen et al., 2003).

Despite the considerable data describing the role of HDL-C in attenuating atherogenesis and the preeminence of non-HDL-C levels in predicting vascular risk, there are no current treatment targets for HDL cholesterol.

Plasma TAG is becoming increasingly recognized as a CHD risk factor. Fasting hypertriglyceridaemia (>1.7 mmol/l) is associated with the generation of TAG-rich lipoprotein particles with enhanced atherogenic properties, with TAG enrichment of VLDL and IDL particles in the Monitored Atherosclerosis Regression Study being shown to strongly associate with atherosclerotic disease progression. Furthermore, in a meta-analysis of lipid-lowering studies, plasma TAG was identified as an independent marker of CHD risk.

Although evidence accumulates to support plasma TAG as a CHD risk factor, the effects of TAG reduction on CHD risk are unclear (see Scandanavian Simvastatin Trial, 1994; Sacks et al., 2000; Expert Panel on Detection Evaluation, 2001).

The thizolidinedione (TZD) class of insulin sensitizing drugs is increasingly used in the management of type 2 diabetes. However, there is conflicting data on the effects of the individual TZDs on LDL-C levels (Vosper et al., 2002) and there is no long-term data on the clinical relevance of the lipid changes associated with individual TZDs. In view of the potential complementary lipid modifying effects of the TZD and statin classes of drugs, combination TZD and statin therapy offers a potential therapeutic advance in the management of diabetic dyslipidaemia and, thus, vascular risk in patients with type 2 diabetes, which requires further evaluation.

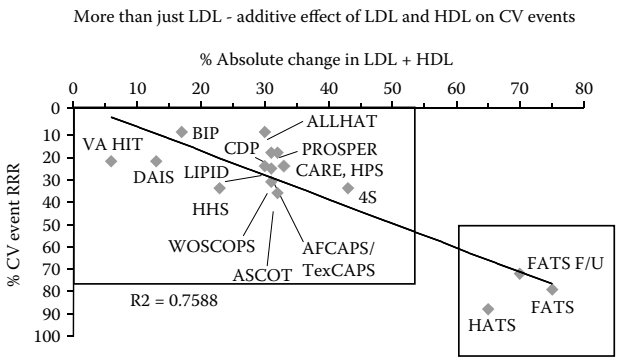


FIGURE 11.6 Additive effects of LDL-C reduction and increasing HDL-C on cardiovascular risk.

The results of the Strong Heart Study (Wood et al., 1998) indicate that non-HDL-C may be a superior predictor of cardiovascular events than LDL-C, HDL-C, or plasma TAG levels alone in both men and women with type 2 diabetes. The utility of non-HDL-C in predicting cardiovascular events in nondiabetic subjects is supported by other studies (Frost et al., 1996; Tyroler, 1990), TC:HDL ratio may be a useful maker of cardiovascular risk. When either TC:HDL ratio or non-HDL-C measurement triggers the initiation of drug therapy, clinical judgment must determine which lipid regulating agent is indicated as primary intervention. Statins primarily target LDL-C, whilst fibric acid derivatives and nicotinic acid target VLDL and TAG-rich lipoprotein particles. Maximal event rate reduction results from alterations in both LDL and HDL-C concentrations; in other words, increasing HDL-C augments the effects of LDL-C reduction on CVD (Figure 11.6).

Therefore, optimal risk reduction and lipid regulating therapy may require either monotherapy or combination therapy resulting in the development of a targeted strategy using a variety of agents. The precedent for such an approach has been set in the evolution of pharmacotherapy in hypertension.

11.3.4 Inflammation, coronary heart disease and lipid-lowering therapy

Over the past decade, the role of inflammation in atherosclerotic vascular disease has become increasingly apparent (Libby, 2002). A variety of population-based studies have demonstrated that baseline C-reactive protein (CRP) levels as a marker of inflammation predict future cardiovascular events (Ridker et al., 2001b). Indeed, in a recent study, CRP level was a stronger independent predictor of cardiovascular events than LDL-C levels (Ridker et al., 2002). Such observations suggest that reliance on LDL-C to predict cardiovascular risk may result in suboptimal targeting of lipid-lowering therapy for primary prevention and that measurements of inflammation may be more important (Ridker et al., 2001a).

Based on the ongoing discussion, it is clear that optimal global risk reduction should involve the treatment of both lipids and inflammation. Statin therapy may contribute to atherosclerotic plaque stability by both reducing plaque size and modifying the physicochemical properties. Changes in plaque size occur over extended time and are often minimal. The acute coronary syndrome (ACS) is related to thrombosis superimposed on an unstable atherosclerotic lesion. The clinical benefits of early statin therapy on cardiovascular outcome in ACS (Schwartz et al., 2001) may be related to the anti-inflammatory as well as the lipid lowering effects of statins, in particular the inhibition of matrix metalloproteinases, tissue factor expression and macrophage activation. Indeed, growing evidence indicates that inflammation as measured by CRP predicts an unfavourable outcome in ACS, independent of any other factors (Libby et al., 2002). Thus, CRP estimation in patients at risk of cardiovascular disease may enable more effective targeting of interventions, such as early intensive statin therapy.

Other lipid lowering therapies also exert potentially important anti-inflammatory properties, independent of lipid lowering effects. However, the clinical application of the potential anti-inflammatory properties of both fibrates and TZDs on atherosclerotic vascular disease remains unclear. Nevertheless, TZD therapy may attenuate atherosclerotic disease progression and improve plaque stability effects (Martens et al., 2002; Takagi et al., 2002; Jackson et al., 1999; Claudel et al., 2001), which may be partly related to anti-inflammatory mechanisms. Further randomized clinical studies, however, are required to further evaluate clinical relevance of such observations.

11.3.5 Lipid-lowering therapy in clinical practice

11.3.5.1 Statins

Based on the concept purported by treatment guidelines that stipulate ever lower targets for LDL-C, combined with the growing evidence supporting the notion that lower is better, practicing clinicians face the problem of how best to achieve optimal cholesterol reduction.

Several studies have demonstrated that statins may differ in their lipid-modifying properties. The largest such study, the STELLAR (Jones et al., 2003) study, compared rosuvastatin, atorvastatin, simvastatin, and pravastatin across licensed doses for reducing LDL-C and other lipid parameters in patients with hypercholesterolaemia. This trial found that after 6 weeks of treatment rosuvastatin resulted in significantly greater LDL-C reductions, dose-for-dose, compared with other statins (Figure 11.7).

In addition, the STELLAR study showed that rosuvastatin raised HDL-C better than other statins. Importantly, this effect was maintained across the dose range (Figure 11.8). This study also illustrates the so-called "rule of 6" in relation to statin therapy, in that a doubling in the statin dose only results in additional 6% reduction in

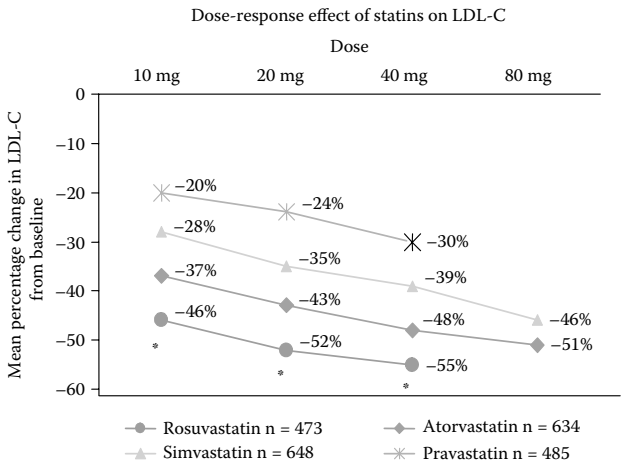


FIGURE 11.7 Dose effects of statins on LDL-C reduction. (From Jones, P.H., et al., 2003.)

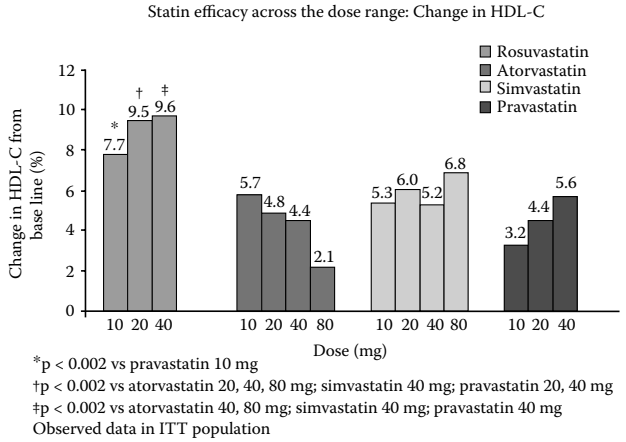


FIGURE 11.8 Dose effects of statins on HDL-C. (From Jones, P.H., et al., 2003.)

LDL-C and that the majority of the LDL-C reduction occurs with the starting dose.

The differing effects on LDL-C reduction of the individual statins relates to the different pharmacological properties of the individual agents (Table 11.9). An important pharmacological property of the statin class relates to their mechanism of elimination and metabolism as this may give rise to potential drug interactions with other compounds that share the same metabolic pathway or that inhibit or increase the activity of these metabolic pathways, thereby affecting statin bioavailability. Such drug interactions exist between statins and numerous compounds including ammioderone, the oral contraceptive pill, diltiazem, erythromycin, grapefruit juice and the azole antifungals, all of which result in increased statin bioavailability and, thus, potential for side effects.

11.3.5.2 Statin safety

Statin treatment represents a life-long therapy, with cumulative benefit year on year. Thus, with any such long-term treatment to what is an asymptomatic therapeutic target, drug safety and tolerability is crucially important.

Overall, the statin class of drugs has a good safety profile and is well tolerated. Indeed, in a recent meta-analysis of 14 statin trials, the therapy was demonstrated to have a similar safety profile to that of a placebo. However, the withdrawal of cerivastatin in 2003 as a result of high rates of muscle toxicity (Staffa et al., 2002) led to questions regarding the safety of statins. The overall adverse event rate associated with statin therapy is low, with the most common side effects reported being headaches, GI disturbance and myalgia (Evans and Rees, 2002). Most currently available statins in the U.K. have a similar safety record (Brewer, 2003). However, as with most drugs, statins exhibit a dose-related adverse events (AEs) profile. One of the side effects sometimes associated with statin therapy relates to muscle toxicity and myopathy (Evans and Rees, 2002). It is important to appreciate that there is a distinct classification of muscle AE, ranging from mild myopathy to frank rhabdomyolysis. Furthermore, rhabdomyolysis and myositis have a strict clinical definition (Table 11.10).

The total reported incidence of statin-associated myotoxicity ranges from between 1 to 7% (Colhoun et al., 2004) and is a function of dose rather than LDL-C reduction. This dose-related effect has been demonstrated across the statin class (Evans and Rees, 2002) and was further supported in another trial with simvastatin (James et al., 2004).

Myalgia is the most common side effect with statins, while rhabdomyolysis and myositis, the most serious of muscle AEs, account for less than 0.1% of all statin-related AEs, with comparable reporting rates across currently available statins (Evans and Rees, 2002). However, cerivastatin post-marketing data revealed the incidence of myotoxicity was greater than 10 times that of other statins (Staffa et al., 2002) (Table 11.11).

TABLE 11.9 Comparative pharmacology of individual statins

	Potency on enzyme IC_{50} (nM)	Cell selectivity log ratio	Hepatic metabolism by 3A4*	Elimination half life (h)	Max % effect on LDL-C
Cerivastatin	10.0	–0.14	Yes	2–3	42
Simvastatin	11.2	0.54	Yes	1–2	47
Fluvastatin	27.6	–0.04	Yes	1–2	35
Atorvastatin	8.2	2.2	Yes	14	61
Rosuvastatin	5.4	3.3	No	20	65
Pravastatin	44.1	3.3	No	1–2	37

* A particular cytochrome P450 pathway.

TABLE 11.10 Definition of muscle side effects associated with statin therapy

Myalgia
Diffuse muscle discomfort
Proximal muscles most often involved
Creatine kinase (CK) levels may be normal or slightly elevated
Completely reversible
Most common feature of statin myotoxicity
Dose related

Myopathy
Muscle pain with elevated CK levels ($>10 \times$ upper limit of normal)
Predominantly affects upper limb proximal muscles
Characteristic muscle fibre necrosis and EMG appearances
Dose related

Myositis
Occurs with or without elevated CK levels
Characterised by muscle weakness
Biopsy — variation in muscle fibre size with associated inflammatory cell infiltrate
Dose related phenomenon

Rhabdomyolysis
Characterised by muscle destruction — release of myoglobin, muscle pain, swelling and CK levels
Myoglobin may cause acute acute renal failure and by inhibiting NO metabolism may cause vasoconstriction and tissue ischaemia
Inherited muscle enzyme defects present in ~25% of cases
Can be fatal

TABLE 11.11 Frequency of serious muscle side effects with statin therapy

Statin	No. of fatal rhabdomyolysis cases	No. of prescriptions (10^6)	No. of cases/10^6 prescriptions
Cerivastatin	31	9.8	3.16
Lovastatin	19	99	0.19
Simvastatin	14	116	0.12
Atorvastatin	6	140	0.04
Pravastatin	3	81	0.04
Fluvastatin	0	37	—
TOTAL	**73**	**484**	**3.55**

In summary, while rhabdomyolysis may be serious and life-threatening, it is very rare in relation to currently available statin therapy. When it does occur, it is most commonly in the context of predisposing risk factors, such as trauma, major surgery, hypothyroidism, liver failure, renal failure, and concomitant drug therapy, such as amiodarone or fibrate therapy (Staffa et al., 2002). The precise mechanisms underlying statin associated myopathy are unclear.

11.3.5.3 Lipid lowering therapy: beyond statins

Statins are not the only lipid-lowering therapy used in clinical practice and Table 11.12 illustrates the indications, contraindications, and cautions for the major classes of lipid-lowering drugs in current use. Table 11.13 illustrates the effects of the various lipid-lowering agents on lipid parameters.

In clinical practice, there can be considerable variation in response to statin therapy. This may be related to a variety of factors including poor compliance, with up to 30% of patients discontinuing statins (Pazzucconi et al., 1995). Concomitant drug therapy is another potential cause of variability in response. One important factor influencing stain response is genetic variability in cholesterol absorption. Subgroup analysis that divided subjects into quartiles according to serum cholestanol:cholesterol ratio (Miettinen et al., 1998), (cholestanol being an index of intestinal cholesterol absorption) showed the reduction in cholesterol in response to simvastatin was significantly lower in those subjects in the highest cholestanol:cholesterol quartile, i.e., high cholesterol absorbers and low synthesizers. The baseline cholestanol:cholesterol ratio was also an important determinant of CHD risk reduction (Naoumova et al., 1996; Hallikainen et al., 2000). Subjects with the highest baseline cholestanol:cholesterol ratio (high absorbers–low synthesizers of cholesterol) demonstrated significantly less reduction in CHD events than those in the lowest quartile (low absorbers–high synthesizers) (Table 11.14).

The most likely explanation for these observations is that down-regulation of HMG-CoA reductase resulting from increased intestinal cholesterol absorption was responsible for the suboptimal response to simvastatin. The fact that this phenomenon was seen in the context of a controlled trial suggests that it may be genetically determined rather than due to dietary or compliance factors.

Therefore, one potential approach to improving the LDL-C response to statin therapy is to inhibit intestinal cholesterol absorption resulting in up-regulation of HMG-Co A reductase expression. Several studies have

TABLE 11.12 Overview of indications, cautions, and contraindications of currently used lipid lowering agents

Class of Drugs	Compelling indications	Possible indications	Caution	Contraindications
Statins	Atherosclerotic vascular disease Type 1 diabetes/Type 2 diabetes aged 40 years or more Type 1/Type 2 diabetes 18–39 years with specific indications: Nephropathy, retinopathy, poor glycaemic control (Hba1c >9%), hypertension, premature family history of CVD, familial hypercholesterolaemia, features of metabolic syndrome	10 year CVD risk > 20% TC: HDL ratio > 6 LDL-C > 5	Nonalcoholic steatohepatosis Untreated hypothyroidism Excess alcohol Certain drugs metabolised through cytochrome P450 3A4 pathway, including grapefruit juice in large quantities	Gemfibrozil Significant liver disease
Fibrates	Type III hyperlipoproteinaemia Severe hypertriglycerideaemia	Type 2 diabetes – low HDL-C, elevated triglycerides Moderate to severe hypertriglyceridaemia; when LDL-C appropriately treated	Chronic renal failure Risk of myopathy	Gemfibrozil with a statin
Anion exchange resins	None to Poor tolerability	Inadequate LDL-C control with statins/ ezetimibe (e.g., FH) Cholestasis with itching	GI upset Exacerbation of hypertriglyceridaemia Reduction of fat soluble vitamins Reduced absorption of other drugs	None
Nicotinic acid	Severe hypertriglyceridaemia Type V hypertriglyceridaemia	Mixed dyslipidaemia, often in combination with other lipid lowering drugs	Impaired renal function Liver disease Diabetes mellitus Gout Peptic ulcer Flushing/Diarrhea as side effects	Worsening of glucose tolerance GI upset/flushing
Cholesterol absorption inhibitors	Familial sitosterolaemia	In combination with a statin when LDL-C not appropriately controlled Statin intolerance	Liver impairment Fibrates	None
Fish oils: Omega-3-acid ethyl esters	Severe hypertriglycerideaemia CHD prevention	Hypertriglyceridaemia	Anticoagulants Haemorrhagic disorders Aspirin-sensitive asthma	None

now demonstrated enhanced LDL-lowering effects of statins when cholesterol absorption inhibitors are given concomitantly. The effects of combination therapy appear additive resulting in decrements in LDL ranging from 10 to 20% compared with statin therapy alone which, in many cases, is greater than that achieved by doubling the dose of statin.

11.3.5.4 Cholesterol absorption inhibitors

The first synthetic cholesterol absorption inhibitor, ezetimibe, has been approved for use both in the U.S. and Europe either as monotherapy or in combination with statins. Dose effects for LDL-C lowering (Kreisberg, 1998) and increasing HDL-C (Ezzet et al., 2001) with no change in plasma TAG levels have been shown. The degree of LDL-C reduction was directly related to dose. Ezetimibe has also been shown to reduce chylomicron cholesterol content by up to 69% (Heek et al., 2001), potentially reducing the atherogenic potential of chylomicron remnants.

In a multicentre study known as the Add-On study, Ezetmibe, when combined with continuing statin therapy, produced an additional 20% reduction in LDL-C (Gagne et al., 2002a, b). Most of this response was seen within 2 weeks of commencing combination therapy, with the additive reduction being consistent across all statins.

TABLE 11.13 Effects of lipid modifying drugs on lipid profiles

Therapy	TC	LDL	HDL	TAG	Patient tolerability
Bile acid sequestrants	Down 20%	Down 15–30%	Up 3–5%	Neutral or up	Poor
Nicotinic acid	Down 25%	Down 25%	Up 15–30%	Down 20–50%	Poor to reasonable
Fibrates	Down 15%	Down 5–15%	Up 20%	Down 20–50%	Good
Probucol	Down 25%	Down 10–15%	Down 20–30%	Neutral	Reasonable
Statins*	Down 15–30%	Down 24–50%	Up 6–12%	Down 10–29%	Good
Eżetimibe	–	Down 18%	Up 1%	Down 8%	Good

TC-total cholesterol, LDL-low density lipoprotein, HDL-high density lipoprotein, TAG-triacylglycerol

* Daily dose of 40 mg of each drug. This side does not include rosuvastatin.

Source: Adapted from Yeshurun D, Gotto AM. *Southern Med J* 1995; 88(4):379–391; Knopp RH. *N. Engl J Med* 1999; 341:498–511, Ezetimibe Prescribing Information.

TABLE 11.14 Distribution of coronary heart disease events in the 4S study based on cholestanol:cholesterol ratio (C:CR) at baseline, prior to simvastatin treatment

	C:CR (mmol/l)	C:CR (mmol/l)	C:CR (mmol/l)	C:CR (mmol/l)
Group	<107	107–126	127–148	>148
Placebo (n = 434)	37	39	36	32
Simvastatin (n = 434)	22	24	31	38
Relative risk of CHD	0.62*	0.66	0.75	1.17*

* denotes $p<0.01$ for CHD risk reduction for subjects in the lowest quartile plasma cholestanol/cholesterol ratio vs. those in the highest quartile.

11.3.5.5 Fibrates

Fibrates currently represent the best therapeutic option for TAG reduction and raising HDL-C. A variety of endpoint studies have assessed the benefits of fibrate therapy on cardiovascular disease. In the VA-HIT study, a 22% reduction in coronary events over 5 years was seen in men with coronary disease and baseline levels of LDL-C < 3.6 mmol/l, plasma TAG < 3 mmol/l, and HDL-C < 1 mmol/l. This risk reduction was associated with no change in LDL-C and an average reduction in TAG of 31% and increase in HDL-C of 6% (Robins et al., 2001). In the BIP study (2000) hypertriglyceridaemic subjects with low HDL-C levels experienced a 39.5% reduction in the probability of a cardiac event following bezafibrate therapy. The DAIS Study (Diabetes Atherosclerosis Intervention Study, 2002), demonstrated the potential benefits of modifying TAG and HDL-C metabolism with micronized fenofibrate.

Patients with type 2 diabetes mellitus are at increased risk of cardiovascular disease, partly owing to dyslipidaemia, which can be amenable to fibrate therapy. The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) (2006) study was designed to assess the effect of fenofibrate on cardiovascular disease events in such patients; 5.9% of patients on placebo and 5.2% of those on fenofibrate had a coronary event. This finding corresponds to a significant 24% reduction in nonfatal myocardial infarction and a nonsignificant increase in coronary heart disease mortality. Total cardiovascular disease events were significantly reduced from 13.9 to 12.5%. This finding included a 21% reduction in coronary revascularisation. Total mortality was 6.6% in the placebo group and 7.3% in the fenofibrate group. Thus, fenofibrate did not appear to significantly reduce the risk of the primary outcome of coronary events. It did reduce total cardiovascular events, mainly due to fewer nonfatal myocardial infarctions and revascularisations. The higher rate of starting statin therapy in patients, who were allocated a placebo, might well have masked a potentially larger treatment benefit.

Consequently, the results of studies evaluating the cardiovascular benefits of fibrate therapy in the setting of concomitant stable background statin therapy are eagerly awaited.

11.3.5.6 Nicotinic Acid

Of the currently available lipid-lowering drugs, nicotinic acid is the most effective at raising HDL-C levels, while also reducing plasma triacylglycerol levels and LDL-C. Nicotinic acid is also the only lipid-modifying agent that

has been shown to reduce the levels of lipoprotein (a) (Lp(a)), a genetic variant of LDL-C, which is a recognised marker of increased cardiovascular risk (Durrington and Sneiderman, 2000). Consequently, nicotinic acid is increasingly used in the management of dyslipidaemia and cardiovascular risk.

Clinical data support the use of nicotinic acid for secondary prevention. In the Coronary Drug Project (1975), nicotinic acid reduced CHD events by 26% and cerebrovascular events by 24% compared to a placebo. Additionally, the Stockholm ischaemic heart study demonstrated a significant reduction in total mortality (26%) and CHD mortality (36%) associated with combination nicotinic acid and clofibrate therapy (Carlson and Rosenhamer, 1988).

Despite these demonstrable outcome benefits, problems with tolerability are well recognised with nicotinic acid, in particular, hepatotoxicity, cutaneous flushing, and deteriorating glycaemic control associated with worsening insulin resistance (Sachter, 2003). Many of these are related to the metabolism of nicotinic acid, which is either via conjugation with glycine to form nicoturinic acid, which is hepatotoxic, or via a series of oxidation reactions forming either nicotinimide or pyridimines, which may induce flushing. The development of a sustained release preparation has circumvented many of these problems and also resulted in a sustained and predictable effect on HDL-C (Sachter, 2003). Along with a 28% increase in HDL-C after 96 weeks' treatment with 2 g/day of nicotinic acid produced a 40% reduction in TAG, 20% in LDL-C, and 40% in Lp(a). Furthermore, patients treated with modified release nicotinic acid have been shown to have beneficial effects on lipoprotein morphology, with significant increases in larger, more cardioprotective HDL-C subclasses and reduced concentrations of atherogenic small dense LDL particles (Sachter, 2003). The tolerability profile of the slow release preparation may be further increased by advising patients to take the medication late at night, following a low fat snack, avoid chewing the capsules, and take with a prior dose of aspirin.

11.3.5.7 Omega-3 fatty acids, fish oils, DHA, and EPA

Omega-3 fatty acids (fish oils) at a dose of 2 to 4 g/day and omega 3-marine TAG at a dose of 5 to 10 g/day reduce plasma TAG by 15 to 20% and increase HDL-C by 5 to 8% (De Filipis and Sperling, 2006). Omega 3 fatty acids are also licensed at the lower dose of 1 to 2 g/day for CHD prevention and can be used in combination with other lipid lowering therapies. Marine omega-3 fatty acids, that is eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, prevent fatal myocardial infarction and sudden cardiac death by their antiarrhythmic effects and presumably also by their effect on infarct size, the latter mediated by plaque stabilization, improvements in endothelial function, and other mechanisms. In contrast, a cardioprotective effect of alpha-linolenic acid, a plant-derived omega-3 fatty acid, remains to be clearly demonstrated in adequate intervention trials.

Other forms of applications, like parenteral use or other indications, such as in the psychiatric field, are currently being actively investigated (von Schacky, 2004). Omega-3 fatty acids may also influence cardiovascular risk through qualitative effects on lipid profiles. In a small study of patients with familial-combined hyperlipidaemia (Callabresi et al., 2004), Omacor (an omega-3 polyunsaturated fatty acid (omega-3 FA) concentrate providing 1.88 g of EPA and 1.48 g of DHA per day) over 8 weeks, caused a selective increase of the more buoyant HDL(2) subfraction; plasma HDL(2) cholesterol and total mass increased by 40% and 26%, respectively. Both HDL(2) and HDL(3) were enriched in cholesteryl esters and depleted of TAG after Omacor.

11.3.5.8 Acyl-coenzyme A-cholesterol acyltransferase inhibitors

Several ACAT inhibitors have been shown to reduce lipid levels and have anti-atherogenic effects in animals, reducing plaque size and progression. Many of these developed agents, however, are poorly absorbed and to date only Avasimibe has progressed to human studies, having reached phase 2/3 trials. In the largest published study to date, no significant changes in TC, LDL-C, HDL-C, or apo-B were seen (Insull et al., 2001b). In contrast, VLDL and TAG were reduced across the Avasimibe dosage range. This was an 8-week placebo controlled study and, across the four doses used, Avasimibe was well tolerated with no clinical or biochemical abnormalities noted.

Although in animal studies, Avasimibe has been shown to reduce LDL-C and apo-B concentrations, in humans Avasimibe appears to be primarily a TAG-lowering agent, with apparent optimal effects at the lowest dose of 50 mg. The long-term potential clinical benefits of Avasmibe on lipid levels and human atherosclerosis require further study.

11.3.5.9 Microsomal triacylglycerol transfer protein inhibitors

Microsomal triacylglycerol transfer protein (MTP) is an important factor in the assembly of VLDL, which is a precursor of LDL. Defects in the MTP gene cause abeta lipoproteinaemia (ABL), in which VLDL and chylomicron production is impaired (Kane and Havel, 1995), resulting in low plasma levels of TAG and LDL-C (95). Thus, MTP inhibition may have therapeutic potential for reducing atherogenic lipoproteins in humans.

In animal studies reductions in TC and plasma TAG of 89 and 81%, respectively, have been achieved with MTP inhibitor therapy (Robl et al., 2001). Human trials have yet to be reported, with initial evidence not being encouraging as the most advanced of these agents, BMS 201038, is found to cause fat infiltration and liver enzyme elevation (Brousseau and Schaefer, 2002).

11.3.5.10 Cholesteryl ester transfer protein inhibition

The most advanced CETP inhibitor in development, JTT-705, in animal studies has been shown to increase HDL-C levels, decrease non-HDL-C and reduce aortic atherosclerosis by up to 70% (de Groot et al., 2002).

In a human study, subjects were treated over a 4 week period with a placebo or different doses of JTT-705. Among the treatment groups, there was a dose-dependent increase in HDL-C. Reductions in LDL-C were also seen. JTT-705 in this study appeared to be well tolerated and exhibited a safety profile similar to placebo. These data thus support the notion that CETP inhibition may represent a promising new target for raising HDL; further clinical studies, however, are required to evaluate both safety and the potential impact on CHD risk.

11.3.5.11 Bile acid transport inhibitors

These agents include both bile acid binding resins, such as cholestyramine, and specific inhibitors of bile acid transport known as Na^+/bile acid transporter inhibitors (IBAT). Cholestyramine proved effective for cholesterol and cardiovascular risk reduction in early studies (Brown, 1990), but is poorly tolerated. A new bile acid sequestrant, colesvelam (WelChol®), has recently been launched, but it is too early to make any judgments on tolerability and acceptance in clinical practice. IBAT inhibitors result in more complete blockade of bile acid reuptake and, thus, have greater potential efficacy than bile acid absorbing resins, which work by competitive inhibition. IBAT inhibitors are undergoing clinical evaluation and have the potential to produce significant LDL-C reductions.

11.3.5.12 HDL-associated enzymes and HDL-derived proteins

HDL is associated with 20 to 30 proteins, many of which have been shown to influence atherogenesis. One of the best characterised is platelet-activating factor hydrolase (PAF-H is a phospholipase A2), which is anti-atherogenic when associated with HDL, but pro-atherogenic when associated with LDL through its ability to oxidise phosphatidylcholine to lyso-phosphatidylcholine (Mertens and Holvoet, 2001). Phospholipase A2 (PLA2) is associated primarily with LDL, released by activated macrophages (foam cells). It is one of the few specific markers associated with small dense LDL, and levels are also associated with the risk of coronary heart disease in statin trials (Packard et al., 2000). PAF-H/PLA2, therefore, is a good candidate for inhibition, and a few compounds have entered early clinical trials after showing benefits in animal models of atherosclerosis (Blackie et al., 2003).

Point mutations in apolipoprotein–A1, the principal protein component of HDL, are associated with a wide variety of clinical phenotypes including amyloidosis, neuropathy, and both increased and decreased rates of atherosclerosis (Srivastava and Srivastava, 2000; Newton and Krause, 2002). Mutations have been shown to reduce HDL levels but, paradoxically, protect against atherosclerosis in man and animal models (Chiesa and Sirtori, 2003; Klon et al., 2000). Infusion of purified apoA-1 protein shows no benefit, as it is degraded in the kidneys. However, recently, it has been proved possible to synthesise pre-HDL discs containing apoA1-*Milano* and phospholipids, which are functional and are not instantly cleared. These are protective against atherosclerosis in animal models and in phase 3 clinical trials in humans (Nissen et al., 2003).

11.3.5.13 Lipoprotein (a) formation inhibitors

Lipoprotein (a) is a lipoprotein associated with excess cardiovascular risk and known to be present in atherosclerotic plaques. Therapy for elevated lipoprotein (a) is limited. Estrogen-containing hormone replacement therapy (HRT) reduces Lp (a) up to 30%, and the elevated Lp (a) subgroup benefited from HRT in the HERS study, while in males, anabolic steroids or androgens can reduce Lp (a) (Shlipak et al., 2000; Berglund et al., 1996). The only other therapies to reduce Lp (a) are physical removal through apheresis and reduction in synthesis by nicotinic acid. A number of specific inhibitors of Lp (a) have been synthesised, but none has reached clinical trials in humans as yet. The most promising approach seems to be the use of peptides that interfere with Lp (a) assembly. The peptide apolipoprotein B 4330–4397, for instance, binds to apolipoprotein (a) and inhibits assembly of Lp (a) in apo (a) transgenic mice (Sharp et al., 2003).

11.3.5.14 Oxidant signal disruptors

Oxidised cholesterol is one of the main promoters of atherosclerosis, but directly through its activation of macrophages and indirectly through its actions on the oxidised LDL receptors on other cells. The antioxidant probucol has been shown to reduce progression of atherosclerosis both in animals and, to a limited extent, in humans. However, its mechanism of action has remained obscure. A derivative of probucol, AGI-1067, interferes with lipid peroxide signalling in a similar manner to pyrrolidine dithiocarbamate, which disrupts activation of intercellular adhesion molecules. In animal models, AGI-1067 reduces coronary artery disease progression (Sundell et al., 2003) and in a phase 2 trial in 305 patients, it inhibited vessel restenosis in a manner similar to some previous studies with probucol (Tardif et al., 2003) and formed the rational for a phase 3 clinical trial programme.

11.3.5.15 Inhibitors of cholesterol particle synthesis

Assembly of apolipoprotein-B containing lipoproteins is dependent on a cholesterol sensor (the sterol regulatory element-activating protein) in the endoplasmic reticulum, which regulates apoB gene expression through cleavage and liberation of sterol regulatory element-binding protein 2 (SREBP-2). This complex pathway offers many opportunities for inhibition of particle assembly or for

increasing degradation and, thus, may provide a therapeutic potential.

11.3.6 Summary

There is convincing clinical trial and epidemiological evidence linking dyslipidaemia to cardiovascular disease morbidity and mortality. It is increasingly clear that perturbations in lipid metabolism form a final common pathway linking many recognized cardiovascular risk factors to the pathogenesis of atherosclerosis. Thus, while cardiovascular risk reduction is a multifactorial approach involving both lifestyle changes and pharmacotherapy, the management of dyslipidaemia is of central importance. There is a wealth of evidence supporting the outcome benefits of intensive LDL-C reduction, a concept that is endorsed by the most recent treatment guidelines. The management of dyslipidaemia extends beyond LDL-C reduction, with growing trial and epidemiological evidence linking low HDL-C and elevated plasma TAG to cardiovascular risk. Thus, while statins remain the mainstay of lipid-lowering pharmacotherapy, there is an increasing vogue for combination therapies tailored to address specific dyslipidaemic profiles. Based on such a concept there is intense interest in the development of novel lipid modifying therapies.

11.3.7 Diabetic dyslipidaemia

11.3.7.1 Introduction

The prevalence of type 2 diabetes continues to rise, with an estimated increase in prevalence in the U.K. of approximately 20% between 2000 and 2036 (Bagust et al., 2002). The rising prevalence of type 2 diabetes is related to a variety of factors including an ageing population, excessive dietary energy intake, a sedentary lifestyle and an increase in obesity. Diabetes leads to a 2- to 4-fold increase in the risk of vascular disease with the pattern of atherosclerosis in patients with type 2 diabetes often being more diffuse and severe than in nondiabetic subjects, resulting in higher morbidity and mortality (Naryan et al., 2003). Overall, up to 80% of patients with type 2 diabetes will die from cardiovascular complications, while the relative increase in cardiovascular risk associated with diabetes is seemingly greater in women than men, which abrogates the normal premenopausal gender-related difference in CVD risk (Howard et al., 2002). Currently, guidelines recommend that people with type 2 diabetes should be considered as coronary heart disease risk equivalents and that dyslipidaemia should be a key therapeutic target in these subjects, irrespective of baseline LDL cholesterol levels or age (Grundy et al., 2004). The typical dyslipidaemia of type 2 diabetes is characterised by moderate elevations in plasma TAGs, low HDL cholesterol levels, and a predominance of small, dense, more atherogenic LDL-cholesterol particles without substantial increase in total or LDL cholesterol (Durrington, 1999). This so-called atherogenic lipoprotein phenotype appears independent of metabolic control, while in patients with type 1 diabetes, dyslipidaemic profile tends to be in association with poor glycaemic status. Over recent years there have been tremendous developments in our understanding of the role of lipids and lipoproteins in the process of atherogenesis, particularly in type 2 diabetes and this is accompanied by the development of a variety of effective therapeutic agents.

There is no evidence for any threshold level below which reductions in LDL cholesterol is not associated with lower risk, for example, the MRFIT study demonstrated that the risk of CHD rises with increasing cholesterol levels in diabetic and nondiabetic subjects, while at any serum cholesterol level the absolute CHD risk is 3 to 5 times higher in diabetic men than those without diabetes (Stamler et al., 1993). Furthermore, the U.K. PDS (Prospective Diabetes Study) provided further evidence of a direct and continuous association between LDL cholesterol levels and CVD risk in patients with type 2 diabetes (Turner et al., 1998). In addition, LDL cholesterol levels may also be an important predictor of diabetic nephropathy, with a direct association between LDL cholesterol levels and microalbuminuria (Jenkins et al., 2003).

In early clinical trials drugs with limited efficacy, poor tolerability or a combination of both were used, resulting in little lipid differences between treatment and placebo groups, and since many of these trials were underpowered, the results were inconclusive. In general, however, there were significant reductions in nonfatal myocardial infarction, but little effects otherwise on fatal heart disease or overall mortality. The discovery of more potent agents, in particular HMG-CoA (hydroxymethylglutaryl-CoA) coreductase inhibitors, have resulted in conclusive clinical trials being performed. These have had the statistical power to address the issues of whether treating dyslipidaemia in patients with type 2 diabetes could significantly impact on the morbidity and mortality of atherosclerotic cardiovascular disease.

11.3.7.2 Pathogenesis of diabetic dyslipidaemia

Dyslipidaemia is present when type 2 diabetes is diagnosed and persists despite intensive glycaemic control. It is characterised by moderately raised plasma TAG, low HDL cholesterol, the accumulation of cholesterol-rich remnant lipoprotein particles and a shift in the density distribution of LDL towards small, dense, more atherogenic particles (Durrington, 1999). Furthermore, there are also qualitative alterations in HDL profile with a shift towards smaller, denser particles, with altered biological properties including reduced antioxidant and antiinflammatory potential, diminished reversed cholesterol-ester transport capacity, reduced potential to attenuate endothelial dysfunction and, thus, an overall reduction in cardio-protective potential. This dyslipidaemic profile may be modified by genetic factors, diet and

life style, obesity, other primary and secondary dyslipidaemias, concurrent medications, nephropathy and, to a certain extent, poor glycaemic control.

Dyslipidaemia is an integral feature of insulin resistance and is present at the stage of impaired glucose tolerance (Isooma et al., 2001). Indeed, insulin resistance is central to the pathogenesis of diabetic dyslipidaemia as a consequence of inappropriate and unsuppressed hepatic and adipocyte free fatty acid release along with defective activity of lipolytic enzymes, in particular lipoprotein lipase and hepatic lipase resulting in the generation of excess triglyceride containing lipoproteins (Durrington, 1999).

The importance of insulin resistance in the etiology of diabetic dyslipidaemia is exemplified by data demonstrating that subjects demonstrating the characteristic clinical phenotype of insulin resistance, termed the metabolic syndrome, are most likely to demonstrate the typical dyslipidaemia of type 2 diabetes (Issoma et al., 2001).

The metabolic syndrome represents a cluster of cardiovascular risk factors, with obesity and insulin resistance as core etiological features (see Figure 11.7). It is identified on the basis of a clinical phenotype (see Figure 11.10 and Figure 11.11) and is associated with a 2-fold increase in both incident type 2 diabetes and cardiovascular events (Eckel et al., 2005; Hanley et al., 2005). Consequently, the most recent treatment guidelines advocate intensive cardiovascular risk factor modification in such patients with target TC levels <4 mmol/l and LDL-C <2 mmol/l (Joint British Societies Guidelines, 2005).

The qualitative lipoprotein abnormalities associated with diabetic dyslipidaemia contributes significantly to the enhanced cardiovascular risk characteristic of type 2 diabetes. Hypertriglyceridaemia is associated with increases in both large and small VLDL subfractions with the increase in large VLDL being relatively greater as total triglyceride concentrations increase. In addition, plasma TAGs increase after a fatty meal more markedly than in subjects without diabetes with peak levels observed for up

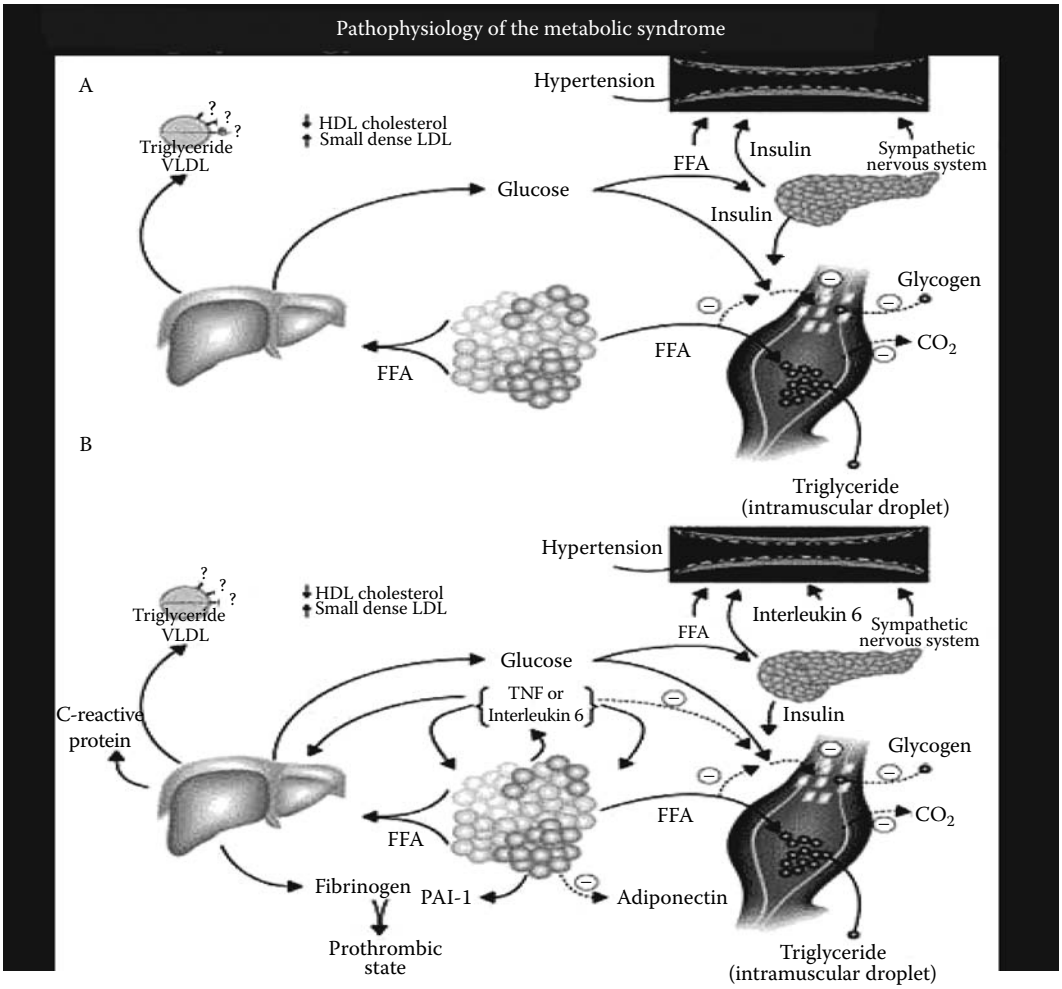


FIGURE 11.9 Pathophysiology of the metabolic syndrome. (See Ferroni et al., 2004, for more details.)

The metabolic syndrome: A clinical classification

Metabolic syndrome

Waist > 102 cm

Triacylglycerols > 1.69 mmol/l

HDL < 1.04 mmol/l

Fasting glucose > = 6.1 mmol/l

SBP > = 130 or DBP > = 85 or anti-hypertension

Metabolic syndrome when 3 or more criteria met
47 million Americans (2001)
1 in 4 men in UK

FIGURE 11.10 NCEP ATP3 Metabolic syndrome criteria. Abbreviations: NCEP ATP3, National Cholesterol Education Panel Adult Treatment Panel 3; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Metabolic syndrome – IDF definition

Central obesity
Waist circumference - > 94 cm (male)
> 80 cm (female)

Ethnic variation in measure of central obesity
+2 of:
Fasting TAG > 1.7 mmol/l
HDL-C < 1.04 mmol/l (Male)
< 1.29 mmol/l (female)
FPG > 5.5 mmol/l
BP > 130/85 mm/hg or anti-hypertensive treatment

FIGURE 11.11 Metabolic syndrome definition. Abbreviations: IDF, International Diabetes Federation; FPG, fasting plasma glucose; BP, blood pressure.

to 6 hours after a meal. Postprandial hypertriglyceridaemia includes particles of an intestinal origin (chylomicrons) and particles of hepatic origin (VLDL) (Evans et al., 1999). There is also reduced removal of TAG-rich remnant particles by the liver and the excess generation of such TAG-rich lipoproteins enhances atherogenesis due to an increased potential to penetrate the arterial intima. This results in increased accumulation of cholesterol esters within foam cells, the potentiation of both enhanced oxidative stress and endothelial dysfunction. These processes consequently promote an ongoing local pro-inflammatory diathesis within the vicinity of the arterial wall, ultimately resulting in the generation of an unstable atherosclerotic plaque.

Reduced HDL-cholesterol concentrations are a characteristic feature of diabetic dyslipidaemia and there is considerable epidemiological support for the association between low HDL-cholesterol concentrations and an increased risk of cardiovascular disease; this association becoming particularly relevant at lower LDL-cholesterol concentrations. In diabetes, there is a shift in HDL phenotype and density distribution from larger, buoyant, to smaller, denser particles. Indeed, lower HDL particle size is a recognised association of hyperinsulinaemia and other components of the insulin resistance clinical phenotype. Moreover, this HDL phenotype is biologically dysfunctional with reduced plasma residence time, decreased transport of antioxidant enzymes, glycation of its major protein apoprotein A1 and reduced reversed cholesterol transport capacity (Pascot et al., 2001).

There are significant qualitative changes within the LDL phenotype contributing to an increased atherogenicity. The LDL subfraction distribution is shifted towards small, denser particles. This is often referred to as Pattern B phenotype, as assessed by gradient gel electrophoresis (Evans et al., 1999). Small dense LDL particles have less phospholipid than larger, more buoyant particles and there is an increased accessibility of apolipoprotein B, including segments which bind the glycosaminaglycans. This leads to increased retention in the arterial wall and, thus, increased potential for accumulation within macrophages resulting in foam cell formation. In addition, small, dense LDL is more susceptible to oxidation and it is well recognised so that oxidised LDL is central to many of the processes involved in atherogenesis including the aetiology of endothelial dysfunction, potentiation of vascular inflammation and the deposition of unstable atherosclerotic plaques that may be prone to rupture and, thus, thrombus formation (Evans et al., 1999).

Smaller, denser LDL particles also demonstrate an increased susceptibility to glycation; this will result in reduced affinity for the LDL receptor and reduced clearance from the plasma, further potentiating the process of macrophage LDL uptake and foam cell formation. Thus, in the context of small, dense LDL, pattern B phenotype, it is important to recognised that for any given LDL-cholesterol concentration there is a significant increase in particle number and, therefore, atherogenic potential (Sniderman et al., 2001).

11.3.7.3 Dyslipidaemia and type 1 diabetes

While the majority of the increasing prevalence of diabetes is in the form of type 2 diabetes, a condition which is classically recognised to be associated with an increased risk of cardiovascular morbidity and mortality, it is important to remember that atherosclerotic disease also represents the leading cause of both morbidity and mortality in patients with type 1 diabetes (Laing et al., 1999). There are a variety of well-described factors that are associated with an increased prevalence of vascular disease in patients with type 1 diabetes. These include the degree of glycaemia, insulin dosage, duration of diabetes, and the presence of microvascular complications, in particular nephropathy and retinopathy (Weis et al., 2001).

Dyslipidaemia in type 1 diabetes is also an important etiological factor in cardiovascular risk. The dyslipidaemia

in type 1 diabetes is different from that seen in type 2 diabetes and epidemiological data has suggested that the prevalence of dyslipidaemia, namely reduced HDL-cholesterol levels, hypertriglyceridaemia and elevated serum LDL-cholesterol levels are lower in patients with type 1 diabetes, compared to the nondiabetic population. Indeed, HDL-cholesterol concentrations are often higher in subjects with type 1 diabetes than nondiabetic individuals and those with type 2 diabetes (Weis et al., 2001). There, however, are significant qualitative abnormalities in lipoprotein metabolism which arise in type 1 diabetes. This particularly involves HDL metabolism (Wadwa et al., 2005) and it is increasingly recognised that HDL-cholesterol particles, from patients with type 1 diabetes, partly as a function of glycation, have a diminished cholesterol-ester transport capacity, reduced antioxidant potential and reduced anti-inflammatory properties resulting in cardioprotective potential.

There is a relative shortage of specific outcome data assessing cardiovascular benefits of lipid-modifying therapy in patients with type 1 diabetes. However, the results of the cholesterol trialists collaborative meta-analysis of statin studies included over 1500 patients with type 1 diabetes and, in keeping with other cardiovascular risk factors, a 1 mmol/l reduction in LDL-cholesterol demonstrated a similar relative risk reduction in terms of cardiovascular morbidity and mortality (Baienet et al., 2005). Consequently, the most recent treatment guidelines (Joint British Societies Guidelines, 2005) advocate a target total cholesterol level of <4 mmol/l and LDL-cholesterol levels below 2 mmol/l in the majority of patients with type 1 diabetes as the primary therapeutic target for dyslipidaemia management. Further studies are awaited assessing the impact of specific therapies targeting HDL metabolism on cardiovascular risk in patients with type 1 diabetes.

11.3.7.4 Diabetic dyslipidaemia: therapeutic considerations

The observed dramatic decline in CHD mortality in the general population has not been accompanied by comparable reduction in patients with type 2 diabetes (Centers for Disease Control, 2003). In the majority of type 2 diabetes patients, LDL-cholesterol lowering remains the primary goal in the treatment of dyslipidaemia. Data from prospective epidemiological studies (Assaman and Schulte, 1992) and from intervention studies, such as HHS, VAHIT, and BIP, indicate that a significant proportion of high-risk patients may benefit not only from LDL-cholesterol reduction, but also from modification of HDL-cholesterol and TAG subfractions; overweight patients and those with highest insulin resistance seemingly deriving the greatest benefit (Manninen et al., 1992; Robins et al., 2001; BIP Study Group, 2000; Tuomilheto and Leiter, 2005). Based on such findings, lipid targets are being established for patients with type 2 diabetes. Current National Cholesterol Education Program (NCEP), joint European and American Diabetes Association recommend the target LDL-cholesterol level of below 2.6 mmol/l. Furthermore, the most recently NCEP guidelines suggest that an LDL-cholesterol goal of < 1.8 mmol/l may be a clinical option in very high risk patients.

The European Diabetes Policy Group guidelines recommend an LDL-cholesterol target of <3 mmol/l while the ADA (American Diabetes Association) and European Diabetes Policy group guidelines also suggest recommended targets for HDL-cholesterol and plasma triglyceride of >1.2 mmol/l and <2.2 mmol/l, respectively. Currently, however, treatment goals for both HDL-cholesterol and plasma TAG are not specified in either the NCEP or Joint European guidelines; the Joint European Guidelines suggest that HDL-cholesterol levels of below 1 mmol/l in men and 1.2 mmol/l in women and triglyceride levels of 1.7 mmol/l should be considered as markers of increased cardiovascular risk (Joint British Societies Guidelines, 2005; Haffner, 2003). The recent Joint British Societies Guidelines advocate target LDL-cholesterol levels in high risk, secondary prevention, and type 2 diabetes patients of <2 mmol/l with a minimum HDL-cholesterol level in patients with type 2 diabetes of 1 mmol/l or above. But, although total and LDL-cholesterol remain current targets of therapy, non-HDL-cholesterol may be a better biological target. This acknowledges that cholesterol within lipoprotein fractions other than HDL contributed to atherogenesis in type 2 diabetes. In the future, apoprotein B reduction may become the prime target as there is one apoprotein B molecule per lipoprotein particle, and apoprotein B concentrations greatly reflect the atherogenic lipoprotein burden. In the setting of low HDL-cholesterol and elevated plasma TAG levels, fibrates or Niaspan may be the therapeutic agent of choice. As of yet, there is little clinical end point data assessing the outcome benefits of such therapies, although angiographic studies, such as HATS (Brown et al., 2001) are suggestive and the FIELD (2005) study provided further data in support of the outcome benefits of fibrate therapy in type 2 diabetes. Many questions remain unanswered due to study design, the low risk patient population studied, and the high penetration of statins into both placebo and fenofibrate groups.

A number of large clinical trials have established statins as effective agents in both primary and secondary CHD prevention with a clear association between cholesterol reduction and outcome benefits (Figure 11.5). In many of these studies there were significant subgroups of patients with type 2 diabetes. In the heart protection study type 2 diabetes was an independent predictor of benefit from statin therapy with a 1 mmol/l reduction in LDL-cholesterol resulting in a 22% reduction in the risk of a first vascular event, independent of baseline LDL-cholesterol levels (Collins et al., 2003). Indeed, meta-analysis of the diabetes subgroups from statin trials has demonstrated that cholesterol reduction may reduce the risk of primary and secondary cardiovascular events by 22% and 24%,

respectively (Vijan and Hayward, 2004). Since patients with known CHD are at greater absolute risk, cholesterol-lowering therapy achieves substantially higher absolute risk reduction in secondary prevention studies than in primary prevention studies.

The CARDS study further illustrated the benefits of cholesterol reduction in patients with type 2 diabetes (Colhoun et al., 2004). In this study of over 2800 patients with type 2 diabetes and at least one other CHD risk factor, an LDL cholesterol reduction of 40% and TAG reduction of 19% were associated with a 37% reduction in major coronary events and a 48% reduction in stroke. Approximately 20% of patients with type 1 diabetes will develop diabetic nephropathy after 20 to 25 years of diabetes duration (Anderson et al., 1983). Of these, more than 40% develop cardiovascular disease by the age of 40 (Tuomileto et al., 1998). LDL-cholesterol has been shown to be closely associated with microalbuminuria, a marker for nephropathy and a strong independent risk factor for the development of coronary heart disease. Abnormalities of lipid metabolism may exacerbate diabetic nephropathy through a variety of mechanisms including perturbations of the coagulation fibrinolytic system, changes in membrane permeability, endothelial dysfunction and enhanced atherosclerosis. There is also evidence that intensive cholesterol lowering therapy may retard the progression of microvascular diseases including nephropathy (Baghdsarian et al., 2004). It is interesting to speculate that dyslipidaemia may be a predictor of nephropathy risk and that lipid-lowering therapy may retard the development of this complication. There, however, is persisting debate regarding optimum treatment lipid levels in patients with type 1 diabetes and the optimum timing of institution for lipid-lowering therapy in such subjects.

A recent meta-analysis of lipid lowering trials in diabetes has concluded that the number needed to treat to prevent one CHD event was 13.8/4.9 years of secondary prevention and 34.5/4.3 years for primary prevention (Vijan and Hayward, 2004). Thus, compared with commonly adopted medical interventions, cholesterol reduction in type 2 diabetes appears to be cost-effective even in the absence of overt cardiovascular disease. Based on such evidence, statins should be prescribed for the vast majority of individuals with type 2 diabetes, irrespective of baseline LDL-cholesterol levels, the target LDL-cholesterol of below 2.5 mmol/l being appropriate for patients with diabetes in the absence of cardiovascular disease, while for those with established cardiovascular disease an even lower LDL-cholesterol target of below 1.8 mmol/l may be considered. Possible exceptions to this approach may include younger patients (under 40 years) for whom there is little current clinical trial evidence, and those with recently diagnosed type 2 diabetes who have no additional CHD risk factors or diabetic complications.

There is much interest in determining which statins demonstrate the greatest LDL-cholesterol lowering efficacy and, thus, the greatest potential clinical benefit. In diabetic populations, significant reductions in cardiovascular risk are usually associated with statin-induced LDL-cholesterol reduction of 30 to 40%; the particular statin chosen and the dosage at which it is prescribed, therefore, should be associated with an LDL-cholesterol reduction of at least this magnitude. The 54-week ASSET trial included a six-week comparison of the efficacy of Atorvastatin 10 mg/daily and Simvastatin 10 mg/daily in both diabetic and nondiabetic subjects (Insull et al., 2001a). In the diabetes subgroup, atorvastatin-treated patients showed a significantly greater reduction in LDL-cholesterol reduction (37.1%) compared to those treated with simvastatin (29.7%). Rosuvastatin is currently the most efficacious of available statins and recent studies have shown that rosuvastatin is highly effective in reducing LDL-cholesterol in patients with type 2 diabetes (Tuomileto et al., 2004).

11.3.7.5 Combination therapy

The HDL and plasma TAG abnormalities characteristic of diabetic dyslipidaemia may be improved by fibrate or niacin therapy. Fibrate therapy should be considered in individuals with severe hypertriglyceridaemia (>4.5 mmol/l); if patients also have an elevated LDL cholesterol, a fibrate-statin combination may be used. However, it is important to remember that fibrate-statin combination therapy may increase the risk of myopathy and, when considering the use of this therapeutic approach, an assessment of the risk factors for myopathy is essential. Combining a statin with niacin may also improve the overall dyslipidaemic profile and may, thus, offer patients more comprehensive lipid control and ultimately greater cardiovascular risk reduction. Studies evaluating statin-fibrate and statin–niacin therapy in patients with dyslipidaemia have demonstrated substantial benefits. For example, the HATS trial which combined simvastatin and niacin therapy lowered LDL-cholesterol by 42% and raised HDL-cholesterol by 25% in patients with coronary artery disease and low HDL-cholesterol. These lipid changes associated with cessation of atherosclerosis progression and a 60% reduction in major clinical events, supporting the notion that CHD risk reduction may be optimised through both LDL-C reductions combined with raising HDL-C (Figure 11.6). The concept of combination therapy is well established in the management of other cardiovascular risk factors in diabetes, such as hyperglycaemia and hypertension and the potential benefits of a combination approach for the management of dyslipidaemia in diabetes is increasingly apparent. The ongoing ACCORD trial is a large-scale primary prevention study involving 10,000 patients with type 2 diabetes, which would evaluate the effects of both intensive glycaemic control along with combination lipid modifying therapy including statin +/– fibrate or antihypertensive therapy. A developing area of therapeutic interest focuses on the role of PPAR alpha and gamma agonists in the management of type 2 diabetes. These

agents have potentially significant effects on diabetic dyslipidaemia, in particular a reduction of plasma TAG levels, increase in HDL-cholesterol levels, and reduction in numbers of small, dense atherogenic LDL particles. Thus, combining statins with such agents may also prove beneficial in the treatment of the multiple features of diabetic dyslipidaemia and may ultimately translate into cardiovascular outcome benefits.

11.3.7.6 Summary

Diabetes is a condition characterised by increased cardiovascular risk, in which dyslipidaemia is an integral factor. Understanding of the mechanisms linking dyslipidaemia to cardiovascular disease has increased over the past decade, with the importance of treating dyslipidaemia in people with diabetes becoming increasingly apparent. This is reflected by the development of ever more stringent treatment guidelines. Although TC and LDL-C are currently the main therapeutic targets, optimal cholesterol risk reduction in people with diabetes involves addressing the entire dyslipidaemic profile.

References

Amarenco, P. et al. (2004). Statins in stroke prevention and carotid atherosclerosis. *Stroke*, **35**, 2902–9.

Anderson, A.R. et al. (1983). Diabetic nephropathy in type 1 diabetes: an epidemiological study. *Diabetologia,* **25**, 496–501.

Asami, Y. et al. (1999). Inhibitory effect of TS-962 on the formation of early atherosclerotic lesions in high fat fed hyperlipidaemic hamsters. *Atherosclerosis* **146**, 237–42.

Assmann, G. (2001). Pro and con: high-density lipoprotein, triglycerides and lipid subfractions are the future of lipid management. *Am. J. Cardiol.,* **87**, 2B–7B.

Assmann, G. and Noffer, J.R. (2003). Atheroprotective effects of high density lipoprotein cholesterol. *Ann. Rev. Med.,* **54**, 324–41.

Assmann, G. and Schulte, H. (1992). Role of triglycerides in coronary artery disease: lessons from the prospective muster cardiovascular study. *Am. J. Cardiol.,* **70**, 10H–13H.

Assmann, G. et al. (1998). The Munster Heart study (PROCAM): results of follow up at 8 years. *Eur. Heart J.,* **19** (Suppl A), A2–A11.

Baghdsarian, S.B. et al. (2004). Association of dyslipidaemia and effects of statins on non-macrovascular disease. *Clin. Ther.,* **26**, 337–51.

Bagust, A. et al. (2002). The projected health care burden of type 2 diabetes in the U.K. from 2000 to 2060. *Diabet. Med.,* **19** Suppl 4:1–5.

Baienet, C. et al. (2005). Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet*, **366**, 1267–78.

Bays, H.E. et al. (2001). Effectiveness and tolerability of ezetimibe in patients with primary hypercholesterolemia: pooled analysis of two phase II studies. *Clin. Ther.*, **23**, 1209–30.

Berglund, L. et al. (1996). Hormonal regulation of serum lipoprotein(a) levels: effects of parenteral administration of estrogen or testosterone in males. *J. Clin. Endocrinol. Metab.*, **81**, 2633–7.

BIP Study Group, The (2000). Secondary prevention by raising HDL cholesterol and reducing triglycerides in patients with coronary heart disease. The bezafibrate infarct prevention study. *Circulation*, **102**, 21–7.

Blackie, J.A. et al. (2003). The identification of clinical candidate SB-480848: a potent inhibitor of lipoprotein-associated phospholipase A (2). *Bioorg. Med. Chem. Lett.*, **13**, 1067–70.

Bolibar, I. et al. (2000). The ECAT Angina Pectoris Study Group. European Concerted Action on Thrombosis and Disabilities. Short-term prognostic value of lipid measurements in patients with angina pectoris. *Thromb. Haemost.*, **84**, 955–60.

Brady, A. and Betteridge, J. (2003). Prevalence and risks of undertreatment with statins. *Brit. J. Cardiol.* **10**, 218–19.

Brewer, H.B. (2003). Benefit-risk assessment of rosuvastatin 10 to 40 milligrams. *Am. J. Cardiol.*, **92**, 23K-29K.

Brijmohun, R.S. et al. (2005). Efficacy and safety of high density lipoprotein cholesterol increasing compounds. A meta-analysis of randomized controlled trials. *J. Amer. Coll. Cardiol.*, **45**, 185–97.

Brousseau, M.E. and Schaefer, E.J. (2002). New targets for medical treatment of lipid disorders. *Curr. Atheroscler. Reports*, **4**, 343–9.

Brown, B.G. et al. (2001). Simvastatin and niacin and antioxidants or the combination for the prevention of coronary disease. *N. Eng. J. Med.*, **345**, 1583–92.

Brown, W.V. (1990). Review of clinical trials: proving the lipid hypothesis. *Eur. Heart J.* II (Suppl H), 15–20.

Brown, W.V. (2001). Novel approaches to lipid lowering: what is on the horizon? *Am. J. Cardiol.* **87**, 23B – 27B.

Callabresi, L. et al. (2004). An omega-3 polyunsaturated fatty acid concentrate increases plasma high-density lipoprotein-2 cholesterol and paraoxonase levels in patients with familial combined hyperlipidemia. *Metabolism*. **53**, 153–8.

Carlson, L.A. and Rosenhamer, G. (1988). Reduction in mortality in the Stockholm ischaemic heart disease secondary prevention study with clofibrate and nicotinic acid. *Acta Med. Scand.*, **223**, 405–18.

Centers for Disease Control and Prevention (CDC). (2003). Self reported heart disease and stroke among adults with and without diabetes: United States, 1991–2001. *MMWR Morb. Mortal. Wkly. Rep.,* **52**, 1056–70.

Chen, Z. et al. (1991). Serum cholesterol concentration and coronary heart disease in a population with low cholesterol levels. *Brit. Med. J.,* **303**, 276–282.

Chiesa, G. and Sirtori, C.R. (2003). Apolipoprotein A-I Milano: current perspectives. *Curr. Opin. Lipidol.*, **14**, 159–63.

Claudel, T. et al. (2001). Reduction of atherosclerosis in apolipoprotein E knockout mice by activation of the retinoid X receptor. *Proc. Natl. Acad. Sci. USA,* **98**, 2610–5.

Colhoun, H.M. et al. (2002). Relationship of phospholipid transfer protein activity to HDL and apolipoprotein B containing lipoproteins in subjects with and without type 1 diabetes. *Diabetes*, 51, 3300–05.

Colhoun, H.M. et al. (2004). Primary prevention of cardiovascular disease with atorvastatin in type 2 diabetes in the Collaborative Atorvastatin Diabetes Study (CARDS): multicentre randomised placebo-controlled trial. *Lancet*, **364**, 641–2.

Collins, R. et al. (2003) MRC/ BHF heart protection study (2003) of cholesterol lowering with simvastatin in 5693 people with diabetes: a randomised placebo controlled trial. *Lancet*, **361**, 2005–16.

Coronary Drug Project Group (1975). Clofibrate and niacin in coronary heart disease. *J. Am. Med. Ass*n., **231**, 360–81.

Crouse, J.R. et al. (1998). HMG-CoA reductase inhibitor therapy and stroke risk reduction: an analysis of clinical trials. *Atherosclerosis*. **138**,11–24.

DAIS Study Group, The (2001). Effect of fenofibrate on progression of coronary-artery disease in type 2 diabetes: the Diabetes Atherosclerosis Intervention Study, a randomised study. *Lancet*, **357**, 905–10.

Davis, H.R. Jr. et al. (2001). Ezetimibe, a potent cholesterol absorption inhibitor, inhibits the development of atherosclerosis in ApoE knockout mice**.** *Arterioscler. Thromb. Vasc. Biol.,* **21**, 2032–8.

de Filipis, A.P. and Sperling, L.S. (2006). Understanding omega-3's. *Am. Heart J.*, **151**, 564–70.

de Groot, G.J. et al. (2002). Efficacy and safety of a novel cholesteryl ester transfer protein inhibitor, JTT-705 in humans: a randomised phase II dose response study. *Circulation,* **105**, 2159–2165.

de Lemos, J.A. et al. (2004). Early intensive vs. a delayed conservative simvastatin strategy in patients with acute coronary syndromes —phase Z of the A to Z trial. *J. Am. Med. Assn.,* **292**, 1307–1316.

Delerive, P. et al. (1999). Peroxisome proliferator-activated receptor alpha negatively regulates the vascular inflammatory gene response by negative cross-talk with transcription factors NF-kappaB and AP-1. *J. Biol. Chem*., **274**, 32048–54.

Delerive, P. et al. (2000). Induction of IkappaBalpha expression as a mechanism contributing to the anti-inflammatory activities of peroxisome proliferator-activated receptor-alpha activators. *J. Biol. Chem*., **275**, 36703–7.

Dujovne, C.A. et al. (2002). Ezetimibe Study Group — Efficacy and safety of a potent new selective cholesterol absorption inhibitor, ezetimibe, in patients with primary hypercholesterolemia**.** *Am. J. Cardiol*., **90**, 1092–7.

Durrington, P.N. (1999). Diabetic dyslipidaemia. *Balliers Best Pract. Res. Clin. Endocrinol. Metab.*, **13**, 265–78.

Durrington, P. and Sneiderman, A. (2000). *Hyperlipidaemia*. Oxford Health Press, Oxford, U.K.

Durrington, P.N. et al. (2001). Paraoxonase and atherosclerosis. *Arterioscler. Thromb. Vasc. Biol*., **21**, 473–80.

Duval, C. et al. (2002). The role of PPARs in atherosclerosis**.** *Trends Mol. Med.,* **8**, 422–30.

Eastern Stroke and Coronary Heart Disease Collaborative Research Group (1998). Blood pressure, cholesterol and stroke in eastern Asia. *Lancet*, **138**, 11–24.

Eckel, R.H. et al. (2005). The metabolic syndrome. *Lancet*, **365**, 1415–28.

Evans, L.M. et al. (1999). Diabetic dyslipidaemia and coronary heart disease: a new perspective. *Curr. Opin. Lipidol.,* **10**, 387–391.

Evans, L.M. et al. (2000). Ciprofibrate therapy improves endothelial function and reduces post-prandial lipaemia and oxidative stress in type 2 diabetes. *Circulation*, **101**, 1773–9.

Evans, M. and Rees, A. (2002). Effects of HMG-CoA reductase inhibitors on skeletal muscle: are all statins the same? *Drug Safety*, **25**, 649–663.

Evans, M. et al. (2004). Medical lipid-regulating therapy: current evidence, ongoing trials and future developments. *Drugs,* **64**, 1181–96.

Expert Panel on Detection Evaluation and Treatment of High Blood Cholesterol in Adults (2001). Executive summary of the third report of the National Cholesterol Education Programme (NCEP) Expert Panel on Detection Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *J. Amer. Med. Assoc.,* **285**, 2486–2497.

Ezzet, F. et al. (2001). The plasma concentration and LDL-C relationship in patients receiving ezetimibe. *J. Clin. Pharmacol.*, 41, 943–9.

Ferroni, P. et al. (2004). Inflammation, insulin resistance, and obesity. *Curr. Atheroscler. Rep.,* **6**, 424–31.

Field Investigators, The (2005). Effects of long term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes (The FIELD study): randomized controlled trial. *Lancet*, **366**, 1849–61.

Frost, P.H. et al. (1996) Serum lipids and incidence of coronary heart disease. Findings from the Systolic Hypertension in the Elderly Program (SHEP). *Circulation*, **94**, 2381–8.

Gaede, P. et al. (2003). Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes. *N. Engl. J. Med*., **348**, 383–93.

Gagne, C. et al. (2002a). Ezetimibe Study Group. Efficacy and safety of ezetimibe added to ongoing statin therapy for treatment of patients with primary hypercholesterolemia. *Am. J. Cardiol.,* **90**, 1084–91.

Gagne, C. et al. (2002b). The Ezetimibe Study Group. Efficacy and safety of ezetimibe coadministered with atorvastatin or simvastatin in patients with homozygous familial hypercholesterolemia. *Circulation*, **105**, 2469–75.

Gotto, A.M., Jr. et al. (2000). Relation between baseline and on-treatment lipid parameters and first acute major coronary events in the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS). *Circulation,* **101**, 477–84.

Gould, A.L. et al. (1998). Cholesterol reduction yields clinical benefit: impact of statin trials. *Circulation*. **97**, 946–52.

Grundy, S.M. and Cleeman, J.I. (2004). Bairey Merz CN for the co-ordinating committee of the national cholesterol education program adult treatment panel III guidelines. *Circulation*, **110**, 227–39.

Haffner, S.M. (2003). American Diabetes Association. Management of diabetic dyslipidaemia in adults with diabetes. *Diab. Care*, **26** (Suppl 1): s83–86.

Hallikainen, M.A. et al. (2000). Plant stanol esters affect serum cholesterol levels of hypercholesterolaemic men and women in a dose dependent manner. *J. Nutrit*., **130**, 767–776.

Hanley, A.J.G. et al. (2005). Prediction of type 2 diabetes mellitus with alternative definitions of the metabolic syndrome. *Circulation*, 3713–21.

Heek, M. et al. (2001). The cholesterol absorption inhibitor, ezetimibe, decreases diet-induced hypercholesterolemia in monkeys. *Eur. J. Pharmacol*., **415**, 79–84.

Howard, B.V. et al. (2002). Prevention conference VI: Diabetes and cardiovascular disease. *Circulation*, **105**, 132–7.

Insull, W. et al. (2001a) Comparison of the efficacy and safety of atovastatin and simvastatin at 6 weeks. *Am. J. Cardiol*., **87**, 554–9.

Insull, W., Jr. et al. (2001b). Efficacy and short-term safety of a new ACAT inhibitor, avasimibe, on lipids, lipoproteins, and apolipoproteins, in patients with combined hyperlipidemia. *Atherosclerosis*, **157**, 137–44.

Isooma, B. et al. (2001). The metabolic syndrome influences the risk of chronic complications in patients with type 2 diabetes. *Diabetologia*, **44**, 1148–54.

Jackson, S.M. et al. (1999). Peroxisome proliferator-activated receptor activators target human endothelial cells to inhibit leukocyte-endothelial cell interaction. *Arterioscler. Thromb. Vasc. Biol*., **19**, 2094–104.

JBS 2 (2005). Joint British Societies' guidelines on prevention of cardiovascular disease in clinical practice. *Heart*, **91**, 1–52.

Jenkins, A.J. et al. (2003). Lipoproteins in the DCCT/EDIC cohort: associations with diabetic nephropathy. *Kidney Int.,* **64**, 817–28.

Jones, P.H. et al. (2003). Comparison of the efficacy and safety of rosuvastatin versus atorvastatin, simvastatin and pravastatin across doses (STELLAR Trial). *Am. J. Cardiol.,* **92**, 152–160.

Joyce, C.W. et al. (2002). The ATP binding cassette transporter A1 (ABCA1) modulates the development of aortic atherosclerosis in C57BL/6 and apo E knockout mice. *Proc. Natl. Acad. Sci*., **99**, 407–12.

Kane, J.P. and Havel, R.J. (1995). Disorders of biogenesis and secretion of lipoproteins containing B apolipoproteins. In *The Metabolic and Molecular Basis of Inhertited Disease.* Eds. Servier, C.R., Beaudet, A.R., Sly, W.S., Valle, D., McGraw-Hill, New York, pp. 417–433.

Kannel, W.B. and Larson, M. (1993). Long-term epidemiologic prediction of coronary disease. The Framingham experience. *Cardiology,* **82**, 137–52.

Klon, A.E. et al. (2000). Molecular belt models for the apolipoprotein A-I Paris and Milano mutations. *Biophys. J*., **79**, 1679–85.

Koba, S. et al. (2002). Significance of small dense low-density lipoproteins and other risk factors in patients with various types of coronary heart disease. *Am. Heart J*., **144**, 1026–35.

Koizumi, J. et al. (1985). Deficiency of serum cholesteryl-ester transfer activity in patients with familial hyperalphalipoproteinaemia**.** *Atherosclerosis*, **58**, 175–86.

Kreisberg, R.A. (1998). Diabetic dyslipidemia. *Am. J. Cardiol*., **82**, 67U-73U.

La Rosa, J.C. et al. (2005). Treating to new targets (TNT) Investigators. Intensive lipid lowering with atorvastatin in patients with stable coronary disease. *N. Engl. J. Med*., **352**, 1425–35.

Laing, S.P. et al. (1999). The British Diabetic association cohort study II. Cause specific mortality in patients with insulin dependent diabetes. *Diabet. Med*., 16, 466–71.

Law, M.R. et al. (2003). Quantifying effect of statins on low density lipoprotein cholesterol, ischaemic heart disease and stroke: Systematic review and meta-analysis. *Brit. Med. J.,* **326**, 1423–29.

Libby, P. (2002). Inflammation in atherosclerosis**.** *Nature*, **420**, 868–74.

Libby, P. et al. (2000). Inflammation and atherosclerosis. *Circulation*, **105**, 1135-43.

Manninen, V. et al. (1992). Joint effects of serum triglyceride and LDL and HDL cholesterol concentrations on coronary heart disease risk in the Helsinki heart study. Implications for treatment. *Circulation*, **85**, 37–45.

Martens, F.M. et al. (2002). Metabolic and additional vascular effects of thiazolidinediones. *Drugs*, **62**, 1463–80.

Mbewu, A.D. et al. (1993). The immediate effect of stroptokinase on serum lipoprotein (a) concentration and the effect of myocardial infarction on serum lipoprotein (a), apolipoprotein A1 and B lipids and C-reactive protein. *Atherosclerosis*, **103**, 65–71.

Mertens, A. and Holvoet, P. (2001). Oxidized LDL and HDL: antagonists in atherothrombosis. *FASEB J*., **15**, 2073–84.

Miettinen, T.A. et al. (1998). Baseline serum cholestanol as predictor of recurrent coronary events in subgroup of Scandinavian simvastatin survival study. Finnish 4S Investigators. *Brit. Med. J.,* **316**, 1127–30.

MRC/BHF Heart Protection Study (2002). Heart protection study of cholesterol lowering with simvastatin in 20,536 high risk individuals: a radomised placebo controlled trial. Heart Protection Study Collaberative group. *Lancet*, **360**, 7–22.

Naoumova, R.P. et al. (1996). Plasma mevalonic acid, an index of cholesterol synthesis in vivo, and responsiveness to HMG-CoA reductase inhibitors in familial hypercholesterolaemia. *Atherosclerosis*, **119**, 203–13.

Naryan, K.M. et al. (2003). Lifetime risk for diabetes mellitus in the United States. *J. Am. Med. Assoc*., **290**, 1884–90.

Newton, R.S. and Krause, B.R. (2002). HDL therapy for the acute treatment of atherosclerosis. *Atheroscler. Suppl*., **3**, 31–8.

Nicholls, S.J. et al. (2006). Relationship between atheroma regression and change in lumen size after infusion of apolipoprotein A-I Milano. *J. Am. Coll. Cardiol.*, **47**, 992–997.

Nissen, S.E. et al. (2003). Effect of recombinant ApoA-I Milano on coronary atherosclerosis in patients with acute coronary syndromes: a randomized controlled trial. *J. Am. Med. Assoc*., **290**, 2292–300.

Nissen, S.E. et al. (2006). Effect of very high-intensity statin therapy on regression of coronary atherosclerosis: the ASTEROID trial. *J. Am. Med. Assoc*., **295**, 1556–65.

Norata, G.D. and Catapano, A.L. (2004). Lipid lowering activity of drugs affecting cholesterol absorption. *Nutr. Metab. Cardiovasc. Dis.,* **14**, 42–51.

O'Keefe, J.H., Jr., et al. (2004). Optimal low density lipoprotein is 50-70mg/dl — Lower is better and physiologically normal. *J. Am. Coll. Cardiol*., **43**, 2142–46.

Okamato, H. et al. (2000). A cholesteryl ester transfer protein inhibitor attenuates atherosclerosis in rabbits. *Nature*, **406**, 203–7.

Ordovas, J.M. et al. (2000). Association of cholesteryl ester transfer protein-TaqIB polymorphism with variations in lipoprotein subclasses and coronary heart disease risk: the Framingham study. *Arterioscler. Thromb. Vasc. Biol*., **20**, 1323–9.

Packard, C.J. et al. (2000). Lipoprotein-associated phospholipase A2 as an independent predictor of coronary heart disease.

West of Scotland Coronary Prevention Study Group. *N. Eng. J. Med.*, **343**, 1148–55.

Pascot, A. et al. (2001). Reduced HDL particle size as an additional feature of atherogenic dyslipidaemia of abdominal obesity. *J. Lipid Res.*, **52**, 2007–14.

Patrick, J.E. et al. (2002). Disposition of the selective cholesterol absorption inhibitor ezetimibe in healthy male subjects. *Drug Metab. Dispos.*, **30**, 430–37.

Pazzucconi, F. et al. (1995). Therapy with HMG-CoA reductase inhibitors: characteristics of the long-term permanence of hypocholesterolemic activity. *Atherosclerosis*, 11, 7189–98.

Piscon, T. et al. (2005). Non-high-density lipoprotein cholesterol and apolipoprotein B in the prediction of coronary heart disease in men. *Circulation,* **112**, 3375–83.

Repa, J.J. et al. (2000). Regulation of absorption of and ABC1-mediated efflux of cholesterol by RXR heterodimers. *Science*, **289**, 1524–29.

Ridker, P.M. et al. (1999). Long-term effects of pravastatin on plasma concentration of C-reactive protein. The Cholesterol and Recurrent Events (CARE) Investigators. *Circulation*, **100**, 230–35.

Ridker, P.M. et al. (2001). Air Force/Texas Coronary Atherosclerosis Prevention Study Investigators. Measurement of C-reactive protein for the targeting of statin therapy in the primary prevention of acute coronary events. *N. Engl. J. Med.*, **344**, 1959–65.

Ridker, P.M. et al. (2001). Novel risk factors for systemic atherosclerosis: a comparison of C-reactive protein, fibrinogen, homocysteine, lipoprotein(a), and standard cholesterol screening as predictors of peripheral arterial disease. *J. Am. Med. Assoc*. **285**, 2481–85.

Ridker, P.M. et al. (2002). Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N. Engl. J. Med.*, **347**, 1557–65.

Robins, S.J. et al. (2001). VA-HIT study group. Veterans' Affairs HDL intervention trial. Relation of Gemfibrozil treatment and lipid levels with major coronary events. *J. Am. Med. Assoc*., **285**, 1585–91.

Robl, J.A. et al. (2001). A novel series of highly potent benzimidazole-based microsomal triglyceride transfer protein inhibitors. *J. Med. Chem*., **44**, 851–56.

Ross, R. (1993). The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature*, **362**, 801–809.

Sachter, M. (2003). Modified release nicotinic acid for dyslipidaemia: novel formulation improves tolerability and efficacy. *Br. J. Cardiol.*, **10**, 462–68.

Sacks, F.M. et al. (2000). Effect of pravastatin on coronary disease events in subgroups defined by coronary risk factors: the Prospective Pravastatin Pooling Project**.** *Circulation,* **102**, 1893–900.

Scandanavian Simvastatin Survival Study (1994). Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease. *Lancet*, **344**, 1383–89.

Schwartz, G.G. et al. (2001). The effects of atorvastatin on early recurrent ischaemic events in acute coronary syndromes. The MIRACL study. *J. Am. Med. Assoc.*, **285**, 1711–18.

Sever, P.S. et al. (2003). Prevention of coronary and stroke events with atorvastatin in people with average or lower than average cholesterol concentrations, The Anglo-Scandanavian outcomes trial — lipid lowering arm (ASCOT-LLA). *Lancet*, **361**, 1149–58.

Sharp, R.J. et al. (2003). A synthetic peptide that inhibits lipoprotein (a) assembly. *Arterioscler. Thromb. Vasc. Biol*., **23**, 502–07.

Sheperd, J. et al. (2002). Pravastatin in elderly individuals at risk of vascular disease (PROSPER): A randomised controlled trial. *Lancet*, **360**, 7–22.

Shlipak, M.G. et al. (2000). Estrogen and progestin, lipoprotein (a), and the risk of recurrent coronary heart disease events after menopause. *J. Am. Med. Assoc*., **283**, 1845–52.

Sniderman, A.D. et al. (2001). Hypertriglyceridaemic hyperapoB. The unappreciated dyslipoproteinaemia in type 2 diabetes mellitus. *Ann. Intern. Med.*, **135**, 447–59.

Srivastava, R.A. and Srivastava, N. (2000). High density lipoprotein, apolipoprotein A-I, and coronary artery disease. *Mol. Cell. Biochem*., **209**, 131–44.

Staffa, J.A. et al. (2002). Cerivastatin and reports of fatal rhabdomyolysis. *N. Eng. J. Med*., **346**, 539–540.

Stamler, J. et al. (1993). Diabetes, other risk factors, and 12-year cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. *Diabetes Care,* **16**, 434–44.

Stamler, J. et al. (2000). Relationship between baseline serum cholesterol levels in 3 large cohorts of younger men to long-term coronary, cardiovascular and all cause mortality and longevity. *J. Am. Med. Assoc.,* **284**, 311–18.

Suckling, K.E. and Stange, E.F. (1985). Role of acyl-CoA: cholesterol acyltransferase in cellular cholesterol metabolism. *J. Lipid Res*., **26**, 647–71.

Sundell, C.L. et al. (2003). AGI-1067: a multifunctional phenolic antioxidant, lipid modulator, anti-inflammatory and anti-atherosclerotic agent. *J. Pharmacol. Exp. Ther.,* **305**, 116–23.

Takagi, T. et al. (2002). Impact of troglitazone on coronary stent implantation using small stents in patients with type 2 diabetes mellitus**.** *Am. J. Cardiol.,* **89**, 318–22.

Tall, A.R. (1993). Plasma cholesteryl ester transfer protein. *J. Lipid Res*., **34**, 1255–74.

Tardif, J.C. et al. (2003). Effects of AGI-1067 and probucol after percutaneous coronary interventions. *Circulation*, **107**, 552–58.

Tuomilheto, J. and Leiter, L.A. (2005). Defining the role of statins in diabetes. *Br. J. Diab. Vasc. Dis.*, **5**, 55–62.

Tuomileto, J. et al. (1998). Incidence of cardiovascular disease in subjects with and without diabetic nephropathy in Finland. *Diabetologia*, **41**, 784–90.

Tuomileto, J. et al. (2004). A review of the efficacy of rosuvastatin in patients with type 2 diabetes. *Int. J. Clin. Pract. Suppl.,* **143**, 30–40.

Turner, R.C. et al. (1998). Risk factors for coronary artery disease in non-insulin dependent diabetes mellitus: United Kingdom Prospective Diabetes Study (UKPDS: 23) *Br. Med. J.,* **316**, 823–28.

Tyroler, H.A. (1990). Serum lipoproteins as risk factors: recent epidemiologic studies in individuals with and without prevalent cardiovascular disease. *Eur. Heart J.*, **11** Suppl. H, 21–25.

Verschuuren, W.M. et al. (1995). Serum total cholesterol and long-term coronary heart disease mortality in different cultures: twenty-five year follow up of the seven countries study. *J. Am. Med. Assoc*., **274**, 131–136.

Vijan, S. and Hayward, R.A. (2004). American college of physicians. Pharmacologic lipid lowering therapy in type 2 diabetes: Background paper for the American College of Physicians. *Ann. Intern. Med.*, 140, 650–58.

Vogel, R.A. (1997). Coronary risk factors, endothelial function and atherosclerosis: a review. *Clin. Cardiol.,* **54**, 1–8.

von Schacky, C. (2004). Omega-3 fatty acids and cardiovascular disease. *Curr. Opin. Clin. Nutr. Metab. Care*, **47**, 131–36.

Vosper, H. et al. (2002). Peroxisome proliferator-activated receptor agonists, hyperlipidaemia, and atherosclerosis. *Pharmacol. Ther. Pent.,* **95**, 47–62.

Wadwa, R.P. et al. (2005). Awarness and treatment of young adults with type 1 diabetes. *Diab. Care*, **28**, 1051–56.

Wang, N. et al. (2002). Constitutive activation of peroxisome proliferator-activated receptor-gamma suppresses pro-inflammatory adhesion molecules in human vascular endothelial cells. *J. Biol. Chem.*, **277**, 34176–81.

Weis, U. et al. (2001). Long term predictors of coronary artery disease and mortality in type 1 diabetes. *Q. J. Med.*, **94**, 623–30.

Weitz-Schmidt, G. (2002). Statins as anti-inflammatory agents. *Trends Pharmacol. Sci.*, **23**, 482–86.

Wiviott, S.D. et al. (2005). Can low-density lipoprotein be too low? The safety and efficacy of achieving very low low-density lipoprotein with intensive statin therapy: a PROVE IT-TIMI 22 substudy. *J. Am. Coll. Cardiol.,* **46**,1411–16.

Wood, D. et al. (1998). Prevention of coronary heart disease in clinical practice: recommendations of the second joint task force of European and other societies on coronary prevention. *Eur. Heart J.,* **19**, 1434–503.

Yusuf, S. et al. (on behalf of the INTERHART investigators) (2004). Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet*, **364**, 937–952.

Zhang, X. et al. (2003). Cholesterol, coronary heart disease and stroke in the Asia Pacific Region. *Int. J. Epidemiol.*, **32**, 563–72.

11.4 Skin lipids and medical implications

The surface cells representing the epidermis play an obvious role in providing a protective permeability barrier between animals and their environment. The mammalian epidermis consists of an undifferentiated germinative layer surmounted by progressively flattened cells that eventually lose recognisable organelles as they transform into corneocytes. Studies on the different layers of the epidermis have shown that, while the basal cells have a lipid composition that is typical of other epithelial cells (see Lampe et al., 1983b), transformation of the stratum granulosum into the stratum corneum is accompanied by a number of dramatic lipid changes. These include depletion of phospholipids with relative retention of neutral lipids, generation and retention of large amounts of sphingolipids, and an enrichment of sphingolipids composed of longer-chain, more saturated fatty acids than are present in lipids in the subjacent viable epidermis (Lampe et al., 1983b; Table 11.15). Possible functions for these changes include both the maintenance of the normal barrier against water loss and the control of stratum corneum desquamation. Elias and Menon (1991) provide a full discussion of the role of lamellar bodies with their complement of lipids, as well as lipid-metabolising enzymes in the generation of the unique composition of the stratum corneum.

A detailed discussion of the biochemistry and functions of stratum corneum lipids is given by Schurer and Elias (1991). Although it is accepted generally that the stratum compactum layer, the lowest region of the stratum corneum, possesses formidable barrier properties (Bowser and White, 1985), most, if not all, of the cells of the stratum corneum also participate. The ability of the intercellular lipids (in the almost complete absence of phosphoglycerides) to do this is dependent on the amphipathic properties of ceramides, nonesterified fatty acids and cholesterol and on the stabilisation of lamellar bilayers (in an aqueous environment) by van der Waals' interactions and hydrogen bonds (Rehfeld et al., 1988, 1990).

A comprehensive discussion of the lipid phase behaviour of the stratum corneum and consequential properties is given by Bouwstra et al. (2003). The presence of ceramide 1 (Figure 11.12) appears to be crucial. A model for the organisation of the 13 nm lamellar phase (known as "the sandwich model") has been proposed in which crystalline and liquid domains co-exist.

When (human) skin lipids are analysed, it should be appreciated that there are two contributing sources. The epidermis itself (discussed above) contributes only a proportion, the remainder originating from the secretion of the sebaceous glands (see below). An approximate composition of lipids from these two sources is shown in Table 11.16. Depending on the site of collection, the contribution of sebum lipids to the total skin lipids will, of course, depend on the number of sebaceous glands. For example, skin lipids from the scalp (where there are 900 pilosebaceous structures/cm^2) have the same overall composition as those from pilosebaceous glands (see Table 11.16), whereas skin lipids from the palms or soles (where sebum is virtually absent) have a composition resembling that of the stratum corneum (Schurer and Elias, 1991). In addition, human stratum corneum lipids display striking regional variations, which could reflect differences in stratum corneum thickness, turnover, desquamation, and/or permeability (Elias et al., 1981; Lampe, et al., 1983a). In addition to endogenous lipids, external sources, such as soaps, cosmetics, or atmospheric pollutants, may also be significant (Burton, 1979).

Sphingolipids are major components of the stratum corneum and are of special interest because it is this class that provides the major polar lipid from which the extracellular membrane structues of the stratum corneum are constructed. Structures of ceramides and glucosylceramides isolated from human epidermis are shown in Figure 11.12. One of the most interesting sphingolipids is one that is

TABLE 11.15 Changes in overall lipid composition during epidermal differentiation and cornification (% total lipids)

	Tissue		
Lipid	**Basal**	**Granular**	**Cornified**
Phospholipids	44.5 ± 3.4	25.3 ± 2.6	6.6 ± 2.2
Cholesterol sulfate	2.6 ± 3.4	5.5 ± 1.3	2.0 ± 0.3
Neutrals	45.4 ± 4.5	56.5 ± 2.8	66.9 ± 4.8
Sterols	11.2 ± 1.7	11.5 ± 1.1	18.9 ± 1.5
Fatty acids	7.0 ± 2.1	9.2 ± 1.5	26.0 ± 5.0
Triacylglycerols	12.4 ± 2.9	24.7 ± 4.0	Variable
Sterol/wax esters	5.3 ± 1.3	4.7 ± 0.7	7.3 ± 1.2
Squalene	4.9 ± 1.1	4.6 ± 1.0	6.5 ± 2.7
n-Alkanes	3.9 ± 0.3	3.8 ± 0.8	8.2 ± 3.5
Sphingolipids	7.5 ± 1.0	12.7 ± 2.7	24.4 ± 3.8
Glucosylceramides	3.5 ± 0.3	5.8 ± 0.2	Trace
Ceramides	3.8 ± 0.2	6.8 ± 0.2	24.4 ± 3.8

Source: Lampe, M.A. et al. (1983b) and Elias, P.M. and Menon, G.K. (1991).

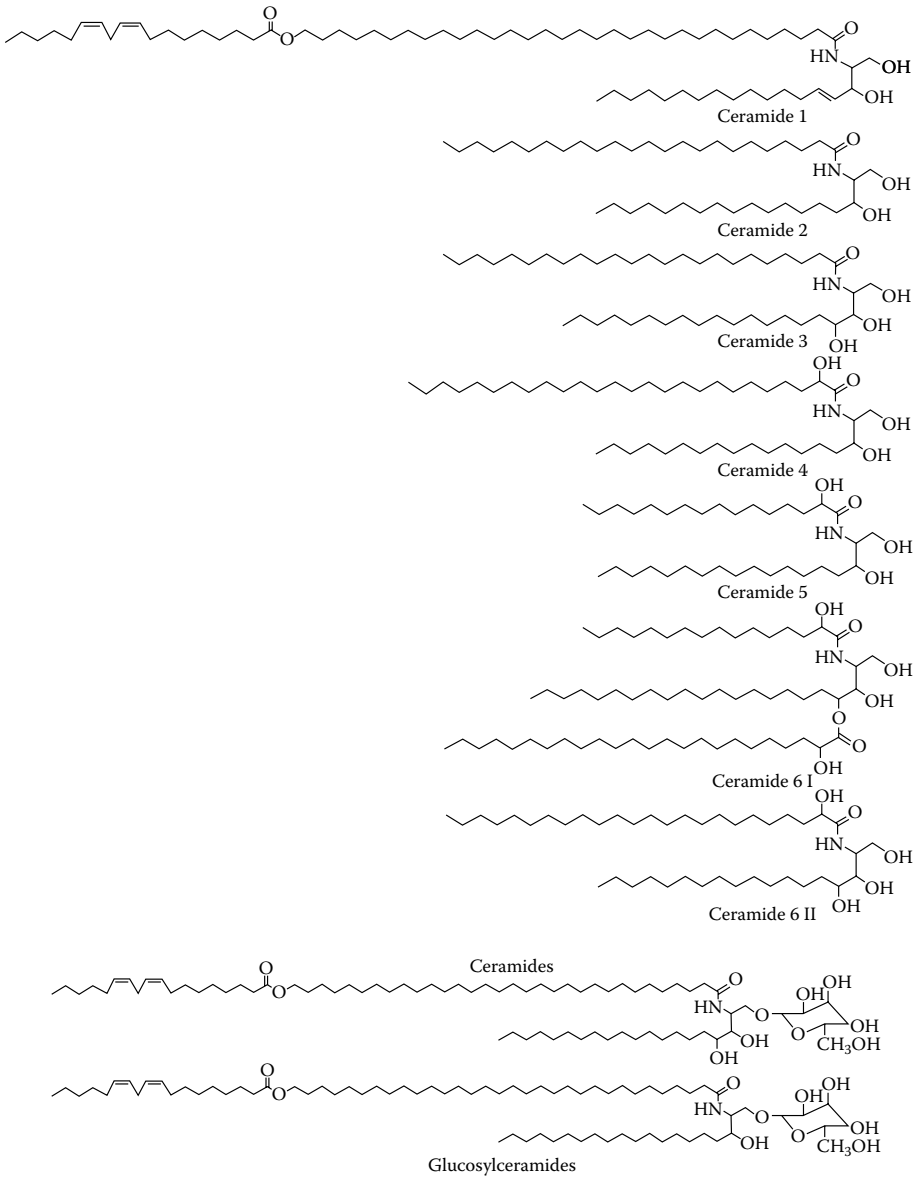


FIGURE 11.12 Ceramides and glucosylceramides isolated from human epidermis. (See Schurer and Elias (1991) for details.)

apparently unique to epidermis, and consists of a glycosylated or nonglycosylated sphingosine base with an amide-linked, long-chain, nonhydroxy, or ω-hydroxy acid with an additional ω-esterified nonhydroxy fatty acid (usually linoleate). Because the major ω-esterified acid is linoleic, this may indicate a special role for this component in barrier function (Hansen, 1986). Indeed, the proportion of linoleate esterified in acylsphingolipids varies from 35% in mice to 50% in humans to 77% in pig (see Schurer and Elias, 1991). Acylated fatty acids containing significant quantities of linoleate are also found (see Hansen, 1986; Schurer and Elias, 1991). The regulation and function of epidermal lipid synthesis have been reviewed by Feingold (1991). Some discussion is also made there about factors, such as diet, diabetes, and the topical application of detergents and other agents that can effect epidermal lipid synthesis and normal function. In addition, the role of lipid modulators of epidermal proliferation and differentiation has been discussed by Holleran (1991). Particular attention was drawn to platelet-activating factor, phospholipase-generated signalling mechanisms (see Section 10.2.2 and Section 10.6) and the role of sphingolipids, especially gangliosides, in mediating the action of epidermal growth factor. The metabolism and function of skin lipids have also been reviewed by Ziboh and Chapkin (1988).

Because of the importance of the epidermis as a permeability barrier, much research has been made to understand its properties, particularly since such knowledge is needed in order to understand pathological problems (such as found in essential fatty acid deficiency) as well as in finding more efficient ways for the topical delivery of pharmaceutical agents in creams and other vehicles. A useful review is that of Potts et al. (1991). In the past 2 decades, various chemicals (e.g., dimethylsulfoxide, n-decylmethylsulfoxide, 1-dodoecylazacycloeptan-2-one (Azone), pyrrolidones, oleic acid) have been investigated for their skin permeability-enhancing action and, hence, their usefulness in the topical administration of therapeutic agents (see Hou et al., 1991).

One of the approaches is the application of drugs in formulations containing vesicles. The efficiency of transport across the skin depends on such factors as the effect of vesicles on drug permeation rate, the permeation pathway and pertubations of the skin ultrastructure (Bouwstra et al., 2003). In model systems, the effect of different formulations can be easily studied and molecular mechanisms proposed (see Heard et al., 2005).

Normal desquamation and correct maintenance of the stratum corneum are orderly and continuous processes. However, disorders of desquamation are seen: *hyperproliferation* results in excessive numbers of incompletely differentiated cells in the outer layer, and *retention hyperkeratoses* result in increased thickness of the stratum corneum due to a failure of desquamation, which may be caused as a side-effect of drugs. Particular problems are known to occur with hypocholesterolaemic agents (Table 11.17). Metabolic defects associated with disorders of cornification include ichthyosis (vulgaris, recessive X-linked, or harlequin), Refsum's disease, neutral lipid storage disease, multiple sulphatase deficiency, and CHILD syndrome (see Williams, 1991).

A most obvious connection of skin lipids with medical problems is with acne vulgaris. It is often stated that the severity of acne correlates with the amount of seborrhoea (Cunliffe and Shuster, 1969). Moreover, nonesterified fatty acids are thought to play a role in its pathogenesis, since they are both irritant and comedogenic (Stillman et al., 1975). However, others have doubted the importance of unesterified fatty acids in the pathogenesis of acne (e.g., Voss, 1974). Alternatively, it has been suggested that the dilutional effect of increased sebaceous gland activity on sebum linoleate may cause acne because local essential fatty acid deficiency is induced (Downing et al., 1986).

Sebum is produced by cells of the sebaceous glands as they move towards the latter's centre and then disintegrate. The lipid cell contents are discharged into the sebaceous duct and then to the pilosebaceous follicle. From there they are excreted to the skin surface. It takes 2 to 3 weeks for a sebaceous cell to travel from the sebum to reach the skin surface. Rates of discharge of sebum are, of course, dependent on changes in sebaceous gland size and mitotic activity but, normally, the rates remain fairly constant for an individual. Methods for studying sebum secretion rates are discussed by Burton (1979). The most reliable of these techniques is a simple gravimetric method in which sebum is collected onto an absorbent paper in contact with forehead skin (see Burton, 1979). Normal values for the sebum secretion rate for subjects of different ages or sex are available in Pochi and Strauss (1974) and Burton et al. (1975), though, because of differences in

TABLE 11.16 The main lipid classes of the stratum corneum and sebum

Sebum lipids		Stratum corneum lipids	
Triacylglycerols	41%	Ceramides	50%
Wax monoesters	25%	Fatty acids	25%
Fatty acids	16%	Cholesterol	19%
Squalene	12%	Cholesterol sulfate	4%
Sterol esters	3%	Phospholipids	1%
Free sterol	1%	Glucosyl ceramides	1%
Unidentified	2%		

TABLE 11.17 Systemic hypocholesterolaemic agents that can cause hyperkeratotic skin conditions

Drug	Therapeutic use	Dermatological side effects	Effect(s) on cholesterol metabolism
Nicotinic acid	Hypocholesterolaemic agent	Generalized dry skin	Decreases hepatic VLDL synthesis; inhibits HMG-CoA reductase; increases cholesterol oxidation and excretion
Triparanol (Mer-29)	Hypocholesterolaemic agent	Ichthyosis; hair loss and depigmentation	Inhibits Δ^{24} (side chain) reduction
WY-3457 (butyrophenone)	Antipsychotic agent	Ichthyosis; hair loss and depigmentation	Inhibits post-squalene step(s)?
Azacosterol (20,25-diazacholesterol)	Hypocholesterolaemic agent	Hyperkeratosis of palms and soles	Inhibits Δ^{24} (side chain) reduction
Gemfibrozil	Hypocholesterolaemic agent	Exacerbation of psoriasis	Decreases hepatic VLDL secretion; increases peripheral VLDL hydrolysis; inhibits HMG-CoA reductase

Source: Williams, M.L. (1991).

experimental technique, normal ranges should be obtained by each investigator. Drug treatment and hormonal or other factors that affect sebum secretion rates have been reviewed (e.g., Shuster and Thody, 1974). The selection of "normal" controls for such testing poses a number of problems (cf. Burton, 1979). The sebum secretion rate is normally expressed in terms of the area of skin studied. It could also be related to the number of functional glands (cf. Burton, 1979).

The chemistry and function of mammalian sebaceous lipids have been reviewed (Stewart and Downing, 1991). Apart from details of human sebum, they discuss other animals, including agriculturally important species (for example, sheep wool lipids). The function of sebum in humans is controversial. It has been suggested to have bactericidal activity and to minimise water loss through the epidermis. However, Kligman (1963) proposed that sebum has none of these effects. Another possible role is to provide a dynamic seal around the hair shaft (Stewart and Downing, 1991).

In acne, a major problem seems to relate to the microorganisms that are capable of hydrolysing the triacylglycerols of sebum and, hence, generate the irritant and comedogenic fatty acids. Three microorganisms have been implicated in acne — *Propionibacterium acne, Staphylococcus* spp. and *Pityrosporum ovale* — with the former being the most important. However, micrococci, sarcina and yeasts are also present, and may predispose the patient to acne (Holland and Cunliffe, 1979). The hormonal control of sebum secretion, which is critical to the appearance of acne in early puberty, is discussed by Stewart and Downing (1991). Oral medications that reduce sebum secretion, such as estrogens, anti-androgens, and 13-*cis*-retinoic acid, are used to alleviate the symptoms of acne.

In addition to acne, abnormal essential fatty acid deficiency has been cited as a cause of atopic dermatitis and psoriasis. In atopic dermatitis it has been suggested that a deficiency of Δ6-desaturase reduces the amount of γ-linolenic acid available for subsequent eicosanoid synthesis (Horrobin, 1989, 1992). Topical application of this acid is reported to be efficacious in atopic dermatitis treatment. Psoriasis, which is a widespread complaint affecting 2% of the population worldwide, has been suggested to be associated with increased phospholipase A_2 activity. This causes increased levels of unesterified arachidonic acid, and, in addition, there is a relative shift towards lipoxygenase rather than cyclooxygenase activity for eicosanoid production (see Section 10.1.8). An imbalance in eicosanoid proportions would then cause changes in cellular metabolism and differentiation (see Kragballe and Voorhees, 1985).

References

Bouwstra, J.A. et al. (2003). Structure of the skin barrier and its modulation by vesicular formulations. *Prog. Lipid Res.* **42**, 1–36.

Bowser, P.A. and White, R.J. (1985). Isolation, barrier properties and lipid analysis of stratum compactum, a discrete region of the stratum corneum. *Br. J. Dermatol.*, **112**, 1–14.

Burton, J.L. (1979). Looking at skin lipids. In *Investigative Techniques in Dermatology*, Ed. R. Marks, Blackwell, Oxford, U.K., pp. 1–20.

Burton, J.L. et al. (1975). The sebum excretion rate in rosacea. *Br. J. Dermatol.*, **92**, 541–543.

Cunliffe, W.J. and Shuster, S. (1969). The rate of sebum excretion in man. *Br. J. Dermatol.*, **81**, 697–704.

Downing, D.T., Stewart, M.E., Wertz, P.W. and Strauss, J.S. (1986). Essential fatty acids and acne. *J. Am. Acad. Dermatol.*, **14**, 221–225.

Elias, P.M. and Menon, G.K. (1991). Structural and lipid biochemical correlates of the epidermal permeability barrier. *Adv. Lipid Res.*, **24**, 1–26.

Elias, P.M., Cooper, E.R., Korc, A. and Brown, B.E. (1981) Percutaneous transport in relation to stratum corneum structure and lipid composition. *J. Invest. Dermatol.*, **76**, 297–301.

Feingold, K.R. (1991). The regulation and role of epidermal lipid synthesis. *Adv. Lipid Res.*, **24**, 57–82.

Hamanaka, S., Asagami, C., Suzuki, M., Inagaki, F. and Suzuki, A. (1989). Structure determination of glucosyl beta 1-N-(omega-O-linoleoyl)-acylsphingosines of human epidermis. *J. Biochem. (Tokyo)*, **105**, 684–690.

Hansen, H.S. (1986). The essential nature of linoleic acid in mammals. *Trends Biochem. Sci.*, **11**, 263-265.

Heard, C.M. et al. (2005). Preferential π-π complexion between tamoxifen and borage oil γ-linolenic acid: transcutaneous delivery and NMR spectral modulation. *J. Pharmaceut.,* **302**, 47–55.

Holland, K.T. and Cunliffe, W.J. (1979). Methods for microbiological investigation of acne. In *Investigative Techniques in Dermatology*, Ed. R. Marks, Blackwell, Oxford, U.K., pp. 21–35.

Holleran, W.M. (1991). Lipid modulators of epidermal proliferation and differentiation. *Adv. Lipid Res.*, **24**, 119–39.

Horrobin, D.F. (1989). Essential fatty acids in clinical dermatology. *J. Am. Acad. Dermatol.*, **20**, 1045–1053.

Horrobin, D.F. (1992). Nutritional and medical importance of gamma-linolenic acid. *Prog. Lipid Res.*, **31**, 163–194.

Hou, S.Y.E. et al. (1991). X-ray diffraction and electron paramagnetic resonance spectroscopy of mammalian stratum corneum lipid domains. *Adv. Biol. Skin*, **24**, 141–171.

Kligman, A.M. (1963). The uses of sebum. *Adv. Biol. Skin,* **4**, 110–124.

Kragballe, K. and Voorhees, J.J. (1985). Arachidonic acid and leukotrienes in clinical dermatology. *Curr. Probl. Dermatol.*, **13**, 1–10.

Lampe, M.A. et al. (1983a). Human stratum corneum lipids: characterization and regional variations. *J. Lipid Res.*, **24**, 120–130.

Lampe, M.A. et al. (1983b). Human epidermal lipids: characterization and modulations during differentiation. *J. Lipid Res.*, **24**, 131–140.

Melnik, B.C. (1989). Epidermal lipids and the biochemistry of keratinisation. In *The Ichthyoses*, Ed. H. Traupe, Springer, Berlin, pp. 15–42.

Pochi, P.E. and Strauss, J.S. (1974). Endocrinologic control of the development and activity of the human sebaceous gland. *J. Invest. Dermatol.*, **62**, 191–201.

Potts, R.O. et al. (1991). Strategies to enhance permeability via stratum corneum lipid pathways. *Adv. Lipid Res.*, **24**, 173–210.

Rawlings, A.V. (1995). Skin waxes: their composition, properties, structures and biological significance. In *Waxes: Chemistry, Molecular Biology and Functions,* Ed. R.J. Hamilton, Oily Press, Dundee, U.K., pp. 223–256.

Rehfeld, S.J. et al. (1988). Calorimetric and electron spin resonance examination of lipid phase transitions in human stratum corneum: molecular basis for normal cohesion and abnormal desquamation in recessive X-linked ichthyosis. *J. Invest. Dermatol.*, **91**, 499–505.

Rehfeld, S.J. et al. (1990). Localization of lipid microdomains and thermal phenomena in murine stratum corneum and isolated membrane complexes: an electron spin resonance study. *J. Invest. Dermatol.*, **95**, 217–223.

Schurer, N.Y. and Elias, P.M. (1991). The biochemistry and function of stratum corneum lipids. *Adv. Lipid Res.*, **24**, 27–56.

Shuster, S. and Thody, A.J. (1974). The control and measurement of sebum secretion. *J. Invest. Dermatol.*, **62**, 172–190.

Stewart, M.E. and Downing, D.T. (1991). Chemistry and function of mammalian sebaceous lipids. *Adv. Lipid Res.*, **24**, 263–301.

Stillman, M.A. et al. (1975). Relative irritancy of free fatty acids of different chain length. *Cont. Derma.*, **1**, 65–69.

Voss, J.G. (1974). Acne vulgaris and free fatty acids. A review and criticism. *Arch. Dermatol.*, **109**, 894–898.

Williams, M.L. (1991). Lipids in normal and pathological desquamation. *Adv. Lipid Res.*, **24**, 211–262.

Ziboh, V.A. and Chapkin, R.S. (1988). Metabolism and function of skin lipids. *Prog. Lipid Res.*, **27**, 81–105.

11.5 Sphingolipidoses

There are over a dozen inherited disorders of metabolism that are characterised by the accumulation of different types of sphingolipids in various organs and tissues. Many of these diseases are fatal in the first few years of life, although, when the central nervous system is unaffected, there are possibilities for treatment (see below). The sphingolipidoses are caused by deficiency of a single lysosomal enzyme, which leads to the accumulation of the enzyme's substrate in affected tissues. The general topic of lysosomal storage diseases has been comprehensively reviewed (Beaudet, 1994).

The structure of sphingolipids was discussed in Section 1.2.4 and their metabolism in Section 10.5.

The specific metabolic defect in each of the lipid storage diseases has been shown in all cases (Table 11.18), with the exceptions of mucolipidosis IV and multiple sulphatase deficiency and the possible exception of lactosylceramidosis. The enzymic defects have been reviewed many times (e.g., Neufeld et al., 1975; Desnick et al., 1976; Neufeld, 1991), and they are also summarised in Table 11.18. Recently, there have been advances in our knowledge concerning the molecular basis of the disorders. It has been proposed that, in these diseases, there is a genetic mutation that leads to a change in the primary structure of the catabolic enzyme. Thus, in patients with adult or juvenile Gaucher's disease, residual glucocerebrosidase activity was found in the tissues. However, the glucocerebrosidase in a pathological spleen was found to have a K_m that was an order of magnitude larger than that in control samples (Brady, 1978). Inactive or less-efficient enzymes have been found in metachromatic leukodystrophy and in G_{M1} gangliosides. In the latter disease, the enzyme from the affected liver had a K_m for G_{M1} that was five times that of controls (Norden and O'Brien, 1975).

Much of the progress in our knowledge of the genetics of the sphingolipidoses has come from cell hybridisation experiments. Cultured skin fibroblasts have been used to study the isoenzyme abnormalities in Tay-Sachs disease and Sandhoff-Jatzkewitz disease. In the former, only hexosaminidase isoenzyme A is lacking (Okada and O'Brien, 1969), while in the latter, both hexosaminidase A and hexosaminidase B are deficient (Sandhoff et al., 1968). The deficiency of hexosaminidase A in these two

TABLE 11.18 Summary of enzyme deficiencies and accumulating lipids in the sphingolipidoses

Disease	Signs and symptoms	Major lipid accumulation	Enzyme defect
Ceramide lactoside lipidosis	Slowly progressing brain damage	Ceramide lactoside	Neutral β–galactosidase
Fabry's disease	Reddish purple skin rash, kidney failure, pain in lower extremities	Gal-Gal-Glu-ceramide	α-Galactosidase
Farber's disease	Hoarseness, dermatitis, skeletal deformation, mental retardation	Ceramide	Ceramidase
Gaucher's disease	Spleen and liver enlargement, erosion of long bones and pelvis, mental retardation only in infantile form	Glucocerebroside	Glucocerebrosidase
Generalized gangliosidosis (GM_1 gangliosidosis)	Mental retardation, liver enlargement, skeletal deformities, about 50% with red spot in retina	Ganglioside GM_1	β-Galactosidase
Krabbe's disease (globoid leukodystrophy)	Mental retardation, almost total absence of myelin, globoid bodies in white matter of brain	Galactocerebroside	Galactocerebroside β-galactosidase
Metachromatic leukodystrophy (two forms)	Mental retardation, psychological disturbances in adult form, nerves stain yellow brown with cresyl violet dye	Sulfatide	
Niemann-Pick disease	Liver and spleen enlargement, mental retardation, about 30% with red spot in retina	Sphingomyelin	Sphingomyelinase
Tay-Sachs disease	Mental retardation, red spot in retina, blindness, muscular weakness	Ganglioside GM_2	Hexosaminidase A
Tay-Sachs variant	Same as Tay-Sachs disease, but progressing more rapidly	Globoside (and ganglioside GM_2)	Hexosaminidase A and B

Source: Brady, R.O. (1978). *Annu. Rev. Biochem.*, **47**, 687–713. With permission. Modified with reference to Neufeld (1991).

disorders is due to different mutations, and activity can be restored by complementation techniques (e.g., Rattazzi et al., 1976). The studies also provided support for the idea that hexosaminidase A is composed of two nonidentical subunits {(αβ)n} and hexosaminidase B is {(ββ)n} (cf. Brady, 1978). Hybridisation studies have now identified the chromosomes that carry the genetic code for the enzymes. The cistron for the α subunits is on chromosome 5. Also, chromosome 1 seems to carry the cistron for α-L-fucosidase (Brady, 1978).

Pathways for the synthesis, transport and processing of lysosomal enzymes and aspects relevant to the sphingolipidoses have been summarised (Neufeld, 1991). Moreover, we also have information of cDNAs and a number of genes encoding such proteins. Sequence homologies have been noted in several cases and there are families of, for example, α-galctosidases. Molecular analysis of mutations has revealed extensive heterogeneity. Though one or two mutations, often clustered in particular ethnic groups, account for a significant fraction of patients or carriers, a very large number of rare mutations account for the remainder. Thus, patients are very likely to be compound heterozygotes (Neufeld, 1991).

Detailed analysis of genetic mutations has been made in a number of sphingolipidoses. These include GM2 gangliosidosis, Gaucher's disease, metachromatic leukodystrophy and Fabry's disease (see Neufeld, 1991, for details).

Models have been used to study some sphingolipidoses, an example being a mouse model for Gaucher's disease (Sun et al., 2005). These have led to increased knowledge of molecular mechanisms involved, such as the use of activator proteins (saposin C in the case of the defective glucosidase in Gaucher). Furthermore, increased understanding of the detailed characteristics of sphingolipidoses has allowed recognition of alterations in cellular characteristics outside the lysosomal compartment. For example, changed lipid rafts and, consequently, insulin receptor activation are manifestations of Niemann-Pick type C disease in hepatocytes (Vainio et al., 2005).

Because of the difficulty of treating a number of the sphingolipidoses, clinical biochemists have been very interested in the development of simple diagnostic tests that can also be used for heterozygous individuals. Fortunately, the enzyme deficiency is the same in all tissues. Thus, easily available materials can be used for the assay of the enzymes concerned in each disease. Such material includes leuko-

cytes, cultured skin fibroblasts, serum and hair follicles. Urine and tears have also been used. In most cases the enzymes remain stable when frozen, so that samples may be shipped to diagnostic centres where automated assays have been developed for many of the determinations (e.g., Peters et al., 1975; cf. Brady, 1978). For a general discussion of diagnostic methods, refer to Beaudet (1994).

For heterozygous carriers of sphingolipidoses, enzyme assays with leukocytes and, especially, cultured skin fibroblasts have proved the most useful (Table 11.19). However, in a large screening programme for Tay-Sachs disease, a number of mistakes occurred. These problems can be alleviated by the very careful use of the test and maintenance of quality controls (Kaback et al., 1977).

With the exception of Fabry's disease, all of the sphingolipidoses are transmitted as autosomal recessive disorders. In short, both parents must be carriers in order to produce an affected child. When both parents are heterozygous, then there is a 1 in 4 chance of an affected child, 1 in 4 of a normal child, and 1 in 2 for carriers. In Fabry's disease, only the female needs to be a carrier to have an affected son; 50% of the sons will be homozygous (affected) and 50% of the daughters will be carriers. When monitoring pregnancies at risk, samples are obtained by transabdominal aminiocentesis at around week 14 of gestation. Viable fetus skin cells are obtained from a 20 ml sample of amniotic fluid and cultured for the enzymic test. Such methods have been shown to be reliable for most of the sphingolipidoses (Milunsky, 1975).

Although the above techniques have proved very useful, occasional problems arise. For example, in the case of Tay-Sachs disease assayed by the use of 4-methylumbelliferyl-β-D-*N*-acetylglucosaminide, certain so-called normal individuals have lowered enzyme activities. Thus, in this case, it is important to use radiolabelled G_{M2} substrate in order to prevent the incorrect diagnosis of individuals with unusual hexosaminidase mutations (Tallman et al., 1974)..

Some clinicians are critical of the use of amniotic materials as described above and prefer to use cells that are more definitely fetal in origin. Therefore, more rapid assay procedures have been developed by increasing the sensitivity of the enzyme assays and reducing the number of tissue culture passages (Brady, 1978). Alternatives include the isolation of fetal cells from maternal blood, fetal blood sampling, fetal tissue biopsy and somatic cell hybridization. The latter involves the formation of hybrids between deficient parenchymal cells and cultured amniotic fluid cells.

Another possible diagnostic method is based on the fact that alkaline phosphatase activity is increased when the unmetabolizable substrate is added to the medium that bathes cells of an affected individual (Hösli, 1976). This technique has been used for hereditable diseases, including the sphingolipidoses, and may be used to help identify unknown metabolic defects.

Since the discovery that the sphingolipidoses are enzyme deficiency diseases, it has been suggested that a method of treatment would be the administration of exogenous enzymes. Initial trials with cells in culture were encouraging, but early trials in which either purified enzymes or plasma and leukocytes as their source were given to patients with lysosomal storage diseases were disappointing (Neufeld and Muenzer, 1989). Three problems were significant. First, many sphingolipidoses affect brain tissue and the blood–brain barrier is an important obstacle, although its permeability can be altered under certain conditions. For example, α-mannosidase can enter the brain if the barrier is altered by injecting hyperosmotic solutions of mannitol into the external carotid artery. The blood–brain barrier can also be altered by intermittent periods of hyperbaric oxygen (Brady, 1978). Both methods

TABLE 11.19 Detection of heterozygous carriers of lipid storage diseases

Disorder	Substrate	Enzyme source	Activity (% of normal)	
Caramide lactoside lipidosis	[^{3}H]Ceramide lactoside	Cultured skin fibroblasts	52	
Fabry's disease	*p*-Nitrophenyl-α-D-galactopyranoside	Leukocytes	31	
		Cultured skin fibroblasts	57	
Gaucher's disease	[^{14}C]Glucocerebroside	Circulating leukocytes	59	
		Cultured fibroblasts	47	
Generalized gangliosidosis	*p*-Nitrophenyl-β-D-galactopyranoside	Leukocytes	48	
Krabbe's disease	[^{3}H]Galactocerebroside	Serum	26	
Metachromatic leukodystrophy	*p*-Nitrocatechol sulphate	Leukocytes	49	
		Cultured skin fibroblasts	19	
Niemann-Pick disease	[^{14}C]Sphingomyelin	Leukocytes	55	
		Cultured skin fibroblasts	64	
Tay-Sachs disease	4-Methylumbelliferyl-β-D-*N*-acetylglucosaminide	Serum	HexA	HexB
O variant			56	61
B variant			55	84

Source: Brady, R.O. (1972). In *Current Trends in the Biochemistry of Lipids*, Eds. J. Ganguly and R.M.S. Smellie, Academic Press, New York, pp. 113–127. With permission.

have inherent dangers. Secondly, it is necessary to have large amounts of enzyme available for therapy. In early trials, there was probably not enough administered in the first place (Neufeld, 1991) and, moreover, because of continuous protein turnover in cells, repeated doses are needed. Thirdly, a signal is needed for receptor-mediated endocytosis and, also, a method needs to be found to target the administered liposomes containing the exogenous enzyme to the appropriate tissue(s).

A study of enzyme replacement for Gaucher's disease has shown more success (Barton et al., 1990). The enzyme was given in substantial amounts for a 2-year period, and the enzyme was modified to expose terminal mannose residues, which aided its binding to mannose receptors present on the surface of reticuloendothelial cells (Stahl et al., 1984).

Because of the failure of early trials with enzyme replacement, other alternatives have been tried, including bone-marrow transplantation. Some limited benefits have been reported in a few cases (see Neufeld, 1991). However, the newer and potentially very beneficial method of gene replacement by transfection of haematopoietic progenitor cells has been tested with considerable success *in vitro.* Thus, for example, retroviral vectors containing human glucocerebrosidase cDNA have been used to express the enzyme in cultured fibroblasts from Gaucher patients (Sorge et al., 1987). Insertion of the human β-glucuronidase gene into the gene line of a completely deficient mouse model resulted in expression of activity in all tissues of the transgenic animal and a completely normal phenotype (Kyle et al., 1990). See also Beaudet (1994) for a general discussion of treatments.

These experimental results offer real hope to patients suffering from the sphingolipidoses. Moreover, molecular biology can also help in the use of recombinant DNA technology to produce large quantities of lysosomal enzymes for replacement therapy. In the meantime, genetic counselling remains a way of providing some relief for those families in which these lysosomal storage diseases are known to occur.

References

Barton, N.W. et al. (1990). Therapeutic response to intravenous infusions of glucocerebrosidase in a patient with Gaucher disease. *Proc. Natl Acad. Sci. USA*, **87**, 1913–1916.

Beaudet, A.L. (1994). Lysosomal storage diseases. In *Principles of Internal Medicine,* 13th edition, Eds. Isselbacker, K.J. et al., McGraw-Hill, New York, pp. 2090–2099.

Brady, R.O. (1972). Disorders of lipid metabolism. In *Current Trends in the Biochemistry of Lipids*, Eds. J. Ganguly and R.M.S. Smellie, Academic Press, New York, pp. 113–127.

Brady, R.O. (1978). Sphingolipidoses. *Annu. Rev. Biochem.*, **47**, 687–713.

Desnick, R.J. et al. (1976). Toward enzyme therapy for lysosomal storage diseases. *Physiol. Rev.*, **56**, 57–99.

Hosli, P. (1976). Simple ultra-microtechniques for genetic complementation analysis and early prenatal diagnosis of sphingolipidoses. *Adv. Exp. Med. Biol.*, **68**, 1–13.

Kaback, M.M. et al. (1977). *Tay-Sachs Disease: Screening and Prevention*, Alan R. Liss, New York.

Kyle, J.W., Birkenmeier, E.H., Gwynn, B., Vogler, C., Hoppe, P.C., Hoffmann, J.W. and Sly, W.S. (1990). Correction of murine mucopolysaccharidosis VII by a human beta-glucuronidase transgene. *Proc. Natl Acad. Sci. USA*, **87**, 3914–3918.

Milunsky, A. (1975). *The Prevention of Genetic Disease and Mental Retardation*, Saunders, Philadelphia.

Neufeld, E.F. (1991). Lysosomal storage diseases. *Annu. Rev. Biochem.*, **60**, 257–280.

Neufeld, E.F. and Muenzer, J. (1989). The mucopolysaccaridoses. In *The Metabolic Basis of Inherited Disease*, 6th ed. (Eds. C.R. Scriver, A.L. Beaudet, W.S. Sly and D. Valle), McGraw-Hill, New York, pp. 1565–1587.

Neufeld, E.F. et al. (1975). Inherited disorders of lysosomal metabolism. *Annu. Rev. Biochem.*, **44**, 357–376.

Norden, A.G. and O'Brien, J.S. (1975). An electrophoretic variant of beta-galactosidase with altered catalytic properties in a patient with GM1 gangliosidosis. *Proc. Natl. Acad. Sci. USA*, **72**, 240–244.

Okada, S. and O'Brien, J.S. (1969). Tay-Sachs disease: generalized absence of a beta-D-N-acetylhexosaminidase component. *Science*, **165**, 698–700.

Peters, S.P. et al. (1975). A microassay for Gaucher's disease. *Clin. Chim. Acta*, **60**, 391–396.

Rattazzi, M.C., Brown, J.A., Davidson, R.G. and Shows, T.B. (1976). Studies on complementation of beta hexosaminidase deficiency in human GM2 gangliosidosis. *Am. J. Hum. Genet.*, **28**, 143–154.

Sandhoff, K. et al. (1968). Deficient hexosaminidase activity in an exceptional case of Tay-Sachs disease with additional storage of kidney globoside in visceral organs. *Life Sci.*, **7**, 283–288.

Sorge, J. et al. (1987). Complete correction of the enzymatic defect of type I Gaucher disease fibroblasts by retroviral-mediated gene transfer. *Proc. Natl. Acad. Sci. USA*, **84**, 906–909.

Stahl, P.D. et al. (1984). Mannose-specific oligosaccharide recognition by mononuclear phagocytes. *Biol. Cell.*, **51**, 215–218.

Sun, Y. et al. (2005). Gaucher disease mouse models: point mutations at the acid β-glucosidase locus combined with low-level prosaposin expression lead to disease variants. *J. Lipid Res.* **46**, 2102–2113.

Tallman, J.F. et al. (1974). Ganglioside catabolism in hexosaminidase A-deficient adults. *Nature*, **252**, 254–255.

Vainio, S. et al. (2005). Defective insulin receptor activation and altered lipid rafts in Niemann-Pick type C disease hepatocytes. *Biochem. J.,* **391**, 465–472.

11.6 Other disorders of lipid metabolism

In addition to sphingolipidoses, there are a number of other rare disorders where lipids accumulate in tissues — apparently because of enzymic deficiencies.

Phytanic oxidase deficiency is also known as Refsum's disease after the original discoverer. In this disease, there is an accumulation of phytanic acid (3,7,11,15-tetramethylhexadecanoic acid), which may account for 10 to 30% of the total plasma fatty acids of patients. Phytanic acid originates from dietary phytols (such as those contained in the side

chain of chlorophylls) and treatment consists of a low-phytol diet. The familial disease is characterized by ataxic neuropathy, anosmia, retinitis pigmentosa, dry skin, skeletal deformities and ichthyosis and is most common in children and young adults of Scandinavian descent. Parents may have high plasma levels of phytanic acid without clinical symptoms. The most important defect is probably a block in the α-oxidation system (Section 10.1.6) so that phytanic acid is no longer metabolised. Diagnosis is usually made by chromatographic analysis of plasma (Brown and Goldstein, 1994).

Apart from Refsum's disease, there are a number of other complaints where there are problems with the degradation of phytanic and/or pristanic acids (Verhoeven and Jakobs, 2001). There are also several diseases where peroxisome biogenesis is defective and which show Zellweger Syndrome as the most severe clinical phenotype. These diseases give rise to several problems with lipid metabolism including accumulation of phytanic and pristanic acids (Verhoeven and Jakobs, 2001).

Genetic disorders of mitochondrial fatty acid β-oxidation are important causes of mobidity and mortality. At present there are over 20 known inherited diseases of the pathway. They can be catergorised as those which involve disorders of (1) plasma membrane functions, (2) mitochondrial fatty acid transport, (3) long-chain fatty acid β-oxidation, (4) medium-chain fatty acid β-oxidation, (5) short-chain fatty acid β-oxidation and (6) other fatty acid oxidation problems, including secondary effects from respiratory disorders. Good reviews on the subject are those by Wanders et al. (1999) and Rinaldo et al. (2002).

Other rare disorders of lipid metabolism include Wolman's disease (triacylglycerol and cholesterol accumulation), hepatic cholesterol ester storage disease, ceroid storage disease, histiocytosis X, lipid proteinosis, lipid dermatoarthritis and Farber's disease (see Table 11.18; involves storage of acid mucopolysaccharides also). Many chronic infections are accompanied by exudates and other proliferative processes, which may be associated with collections of foam cells (xanthomas) and sometimes crystals of cholesterol.

Abetalipoproteinemia, Tangier disease, lecithin:cholesterol acyltransferase (LCAT) deficiency and sitosterolemia are other rare (autosomal recessive) disorders of lipid metabolism (Brown and Goldstein, 1994).

Although covered in Section 11.2 (in relation to cardiovascular disease), the hyperlipoproteinemias have a common occurrence and should be mentioned here. The categories of plasma lipoprotein elevation are summarised in Table 11.20. Other diseases that are due to single-gene mutations and which result in elevation of plasma lipid levels are shown in Table 11.21. These are discussed more fully in Brown and Goldstein (1994).

A number of adipose tissue disorders have been reported. These include conditions of lipodystrophy (where adipose tissue is abnormal, but not necessarily absent) and lipoatrophy. Lipoatrophy may be partial or total. The lipodystrophies are characterised by generalised or partial loss of body fat and various metabolic abnormalities. They are classified as follows (see Foster, 1994):

1. Generalised:
   Congenital (familial or sporadic)
   Acquired (sporadic)

TABLE 11.20 Hyperlipoproteinemias

Class	Characteristic elevation	
	Lipid	**Lipoprotein**
Type 1	TAG	Chylomicrons
Type 2a	Cholesterol	LDL
Type 2b	Cholesterol, TAG	LDL, VLDL
Type 3	TAG, cholesterol	Chylomicron remnants, IDL
Type 4	TAG	VLDL
Type 5	TAG, cholesterol	VLDL, chylomicrons

Note: TAG, triacylglycerols; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein. Note: chylomicron remnants are enriched in cholesteryl esters and apoproteins B48 and E.

TABLE 11.21 Primary hyperlipoproteinemias caused by single gene mutations

Disorder	Cause	Plasma lipoprotein increased
Familial lipoprotein lipase (LPL) deficiency	LPL deficiency	Chylomicrons (Type 1)
Familial apoprotein CII deficiency	Apoprotein CII deficiency	Chylomicrons (Type 1 or 5)
Familial Type 3 hyperlipoproteinemia	Abnormal apoprotein E in VLDL	Chylomicron remnants, IDL (Type 3)
Familial hypercholesterolemia	Deficiency of LDL receptor	LDL (Type 2a, sometimes Type 2b)
Familial hypertriglyceridemia	Unknown	VLDL, rarely chylomicrons (Type 4, rarely Type5)
Familial-combined hyperlipidemia	Unknown	LDL, VLDL (Type 2a, 2b or 4, rarely 5)

Note: For abbreviations see Tables 11.6 and 11.20. For more details refer to Brown and Goldstein (1994).

2. Partial:
   Common (sporadic)
   Dominant (familial)
   Limb and trunk
   With Rieger anomaly
3. Localised:
   Inflammatory
   Noninflammatory

These are discussed in detail by Foster (1994).

Lipids are an important muscle energy source during rest and prolonged, moderately intense exercise. Oxidation of stored fat involves carnitine as a transport vehicle (see Section 10.1.6.2), and carnitine deficiency (myopathic or systemic) and/or carnitine palmitoyltransferase deficiency have been described (Mendell and Griggs, 1991).

Production of inflammatory mediators by the host in response to bacterial endotoxin is believed generally to be responsible for the pathogenesis of septic shock. Among the many mediators produced by the major effector cells, the macrophages, are arachidonic acid metabolites (Geisel et al. 1991). Changes in polyunsaturated fatty acid metabolism have been suggested to predispose patients to endotoxic shock (Stark et al., 1990).

References

Brown, M.S. and Goldstein, J.L. (1983). The hyperlipoproteinemias and other disorders of lipid metabolism. In *Principles of Internal Medicine*, 10th ed., Eds. R.G. Petersdorf et al., McGraw-Hill, New York, pp. 547–559.

Brown, M.S. and Goldstein, J.L. (1994). The hyperlipoproteinemias and other disorders of lipid metabolism. In *Principles of Internal Medicine*, 13th ed., Eds. R.G. Petersdorf et al., McGraw-Hill, New York, pp. 2058–2069.

Foster, D.W. (1991). The lipodsytrophies and other rare disorders of adipose tissue. In *Principles of Internal Medicine*, 12th ed., Eds. J.D. Wilson et al., McGraw-Hill, New York, pp. 1883–1887.

Foster, D.W. (1994). The lipodystrophies and other rare disorders of adipose tissue. In *Principles of Internal Medicine*, 13th ed., Eds. E. Braunwald et al., McGraw-Hill, New York, pp. 2132–2136.

Frederickson, D.S. (1970). Disorders of lipid metabolism and xanthomatosis. In *Principles of Internal Medicine*, 6th ed., (Eds. M.M. Wintrobe et al., McGraw-Hill, New York, pp. 629–639.

Geisel, J. et al. (1991). Endotoxin-induced arachidonic acid metabolism requires de novo protein synthesis and protein kinase C activation. *Biochim. Biophys. Acta*, **1085**, 15–20.

Mendell, J.R. and Griggs, R.C. (1991). Muscular dystrophy. In *Principles of Internal Medicine*, 12th ed., Eds J.D. Wilson et al., McGraw-Hill, New York, pp. 2112–2118.

Rinaldo, P. et al. (2002). Fatty acid oxidation disorders. *Annu Rev. Physiol.* **64**, 477–502.

Stark, J.M. et al. (1990). The effect of endotoxin on membrane fatty acid composition in BCG-sensitized mice. *Experientia*, **46**, 472–474.

Verhoeven, N.M. and Jakobs, C. (2001). Human metabolism of phytanic acid and pristamic acid. *Prog. Lipid Res.* **40**, 453–466.

Wanders, R.J. et al. (1999). Disorders of mitochondrial fatty acyl-CoA β-oxidation. *J. Inher. Metab.Dis.* **22**, 442–487.

11.7 Pulmonary surfactant (lung surfactant)

Pulmonary surfactant is a complex lipoprotein material (King, 1974; Harwood, 1987; van Golde et al., 1988), which is present at the air/water interface in the pulmonary alveoli. It lowers the surface tension to about 10 nN/m, reduces the contractile force of the surface, the work of lung expansion and the tendency of alveoli to collapse during expiration (Clements, 1977; Goerke, 1975). A deficiency of surfactant in immature infants causes neonatal respiratory distress syndrome (RDS), a major cause of neonatal death (Farrell and Avery, 1975). Abnormal surfactant may also contribute to the pathogenesis of adult RDS (Lachmann and Danzmann, 1984). In addition, pulmonary surfactant may be involved in several defence mechanisms in the lung (Wright, 1997).

11.7.1 Composition

The definition of pulmonary surfactant and a discussion of the properties of various candidate fractions can be found in Clements (1970) and Goerke (1975). This discussion is necessary because the composition and properties of biological materials are, obviously, influenced by the methods used for their isolation. Isolation of surfactant requires careful endobranchial infusion and withdrawal of isotonic solutions, such as Ringers, to yield a cloudy white fluid with the surface-active material in suspension. Pulmonary surfactant has then been purified from the lavage fluid by a number of different procedures using differential and density-gradient centrifugation in either salt (King, 1974; Harwood et al. 1975) or sucrose (Stein et al., 1969; Clements and King, 1976) solutions. The material is isolated at a density of 1.08 to 1.09 g/ml in salt density gradients. Pulmonary surfactant is a lipid-rich lipoprotein containing little carbohydrate and a large quantity of diplamitoylphosphatidylcholine (see below and Table 11.22).

As shown in Table 11.22, about 80% of isolated pulmonary surfactant is lipid, with phospholipids predominating. Most recent studies suggest that cholesterol comprises the largest amount of nonpolar lipids. Phosphatidylcholine is the main phospholipid (usually 70 to 80%) and disaturated species are prominent (mainly dipalmitoyl-PC). This is the main surface-active component (Harwood et al., 1975). Plasmalogen derivatives are minor (Batenburg and Haagsman, 1998). Smaller amounts of unsaturated species seem essential to maintain fluid properties of the monolayer at body temperature and to help with spreading (Goerke,

TABLE 11.22 Composition of isolated lung surfactant

	Rat	Rabbit	Ox	Sheep
	(% w/w)			
Total protein	10	8	18	14
Total lipid	88	90	79	86
	(% w/w of total lipid)			
Triacylglycerol	5	4	15	7
Unesterified fatty acid	3	2	3	10
Cholesterol (total)	3	3	1	3
Phosphatidylethanolamine	4	1	3	2
Phosphatidylcholine	73	83	71	58
Phosphatidylglycerol	5	3	2	4
Phosphatidylinositol	5	2	3	4
Sphingomyelin	2	2	1	3
Lysophosphatidylcholine	tr.	tr.	tr.	11

Source: Harwood et al. (1975).

1974). In many animal species, including humans, phosphatidylglycerol is a significant component (up to 10%). This is unusual and the reason is not understood because some animals (e.g., rhesus monkeys, cats) survive with only small amounts. In humans, the PG/PI ratio in surfactant can be used to estimate maturity (Egberts and Noort, 1986). More details of surfactant lipids will be found in Harwood et al. (2002).

As well as lipids, surfactant contains a number of specific proteins: SP-A, SP-B, SP-C, and SP-D (Hawgood, 1989; Possmayer, 1990; Weaver and Whitsett, 1991). The most abundant protein is SP-A, which has a monomeric mass of 28 to 36 kDa, depending on glycosylation. Its sequence is highly conserved between species and is similar to the collagen family of proteins. In its functional form, it is an oligomeric complex of 18 monomers (in 6 trimers).

SP-B and SP-C are both very small hydrophobic proteins. SP-B (about 8 kDa) is a hydrophobic basic protein with nearly 10% of its residues as cysteine. SP-C is about 4 kDa and, in species that contain two cysteines near its N-terminus, is also doubly palmitoylated there. SP-D, like SP-A, is a C-type lectin and is not thought to be involved in defence mechanisms (Wright, 1997). It does not appear to interact directly with surfactant lipids (Batenburg and Haagsman, 1998). The structures, processing, regulation of expression and functions of those proteins have been well reviewed (Weaver and Whitsett, 1991; Batenburg and Haagsman, 1998).

11.7.2 Metabolism

The pulmonary surfactant lipids are formed in the alveolar type II epithelial cells (Van Golde et al., 1988; Wright and Clements, 1987), which, in adults, comprise about 15% of the total lung cells (Crapo et al., 1982). A schematic diagram of surfactant metabolism is shown in Figure 11.13. Surfactant material is stored in lamellar bodies, where it can be visualised as closely packed bilayers. These contents are secreted into the alveolar humour where they can form a lattice-like structure known as tubular myelin. The surface film at the air/water interface in the alveoli is replenished from the tubular myelin and, furthermore, the molecular composition of the phospholipid monolayer changes during the respiratory cycle (Batenburg and Haagsman, 1998). Used surfactant and material not taken up by or squeezed out of the surface layer is removed from the alveoli by several routes of which endocytic uptake into type II cells is the most important. Some of the lipids and proteins are recycled intact and some are broken down to contribute to resynthesis of surfactant (Figure 11.13). Of the total disaturated phospholipid in rat lungs (6.3 mg/pair) about 1.1 mg were calculated to be in the Type II cells (0.86 mg in lamellar bodies) and up to 1.2 mg in the alveoli.

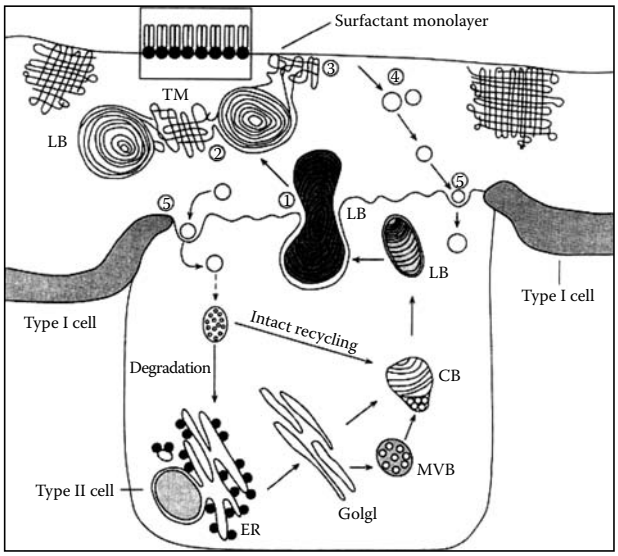


FIGURE 11.13 Schematic diagram of surfactant metabolism. N: nucleus; ER: endoplasmic reticulum; CB: composite body; LB: lamellar body; MVB: multivescular body; TM: tubular myelin; 1: secretion of LB; 2: conversion of LB into TM; 3: generation of monolayer from TM material; 4: formation of small aggregate material from monolayer; 5: reuptake of surfactant material. (From Batenberg, J.J. and Haagsman, H.P. (1998). *Prog. Lipid Res.*, **37**, 233–276. With permission.)

TABLE 11.23 Effects of glucocorticoids and estrogen on various parameters of surfactant production in the fetal rabbit lung 26 to 27 days gestation

	Effect (% increase over control)		
	Glucocorticoids		17β-estrogen to doe
	Cortisol to fetus	*Betamethasone to doe*	
Phosphatidylcholine content of lung lavage	130	70	320
L/S ratio of lavage	80	78	190
Rate of choline incorporation into PC of lung slices	90	64,125	–
Enzyme activities:			
Cholinephosphate cytidylyltransferase	22	50	72
Phosphatidate phosphatase	–	50,94	–
Lysolecithin acyltransferase	33	–	26

Source: Rooney, S.A. (1979). *Trends Biochem. Sci.*, **4**, 189–191. With permission.

Of the amount in the alveoli, over half is needed to form the surface monolayer (Wright and Clements, 1987). This means that there are not great reserves of material to support the vital respiratory function of lung surfactant (Batenburg and Haagsman, 1998) and it is not surprising that adult RDS can occur following lung damage.

As mentioned above, Type II alveolar cells contain characteristic lamellar inclusion bodies. Disaturated phosphatidylcholine appears to be synthesised in the endoplasmic reticulum and then stored in the lamellar bodies, where it may also be combined with other surfactant by exocytosis (Goerke, 1975; Rooney, 1979) (see Figure 11.13). Lung synexin may be involved in membrane fusion between the plasma membrane and lamellar bodies during exocytosis (Chander and Wu, 1991). Phosphatidylcholine is synthesised *de novo* in the lung mainly by the CDP-base pathway, and this results in 1-saturated, 2-unsaturated molecular species. These phosphatidylcholines are converted to disaturated species ("remodelling") by deacylation to 1-saturated, 2-lysophosphatidylcholine and then either transacylated with lysophosphatidylcholine or reacylated with palmitoyl-CoA. The latter appears to be more important (Rooney, 1979; Post and van Golde, 1988).

The beginning of pulmonary surfactant synthesis occurs towards the end (last 10%) of gestation and there is a further increase (tenfold or so) immediately after birth. During these periods the relative amount of sphingomyelin in lung lavage decreases from 38% of the total phospholipids to 7% at full term and 2% after birth. The ratio of phosphatidylcholine/sphingomyelin in lavage fluid changes, therefore, from less than 1 to 10 at full term to 30 to 40 after birth. An analysis of the phosphatidylcholine (lecithin)/sphingomyelin (L/S) ratio can be used clinically to predict suitable times for elective delivery (Rooney, 1979).

Although changes in a number of enzymes involved in phosphatidylcholine synthesis have been noted during lung development (Rooney, 1979), it seems most likely that the activity of cytidylytransferase is particularly important (Vance and Choy, 1979). Other enzymes that show changes around birth and may, in consequence, influence the rate of surfactant synthesis include choline kinase, choline phosphotransferase, phosphatidate phosphatase, phospholipase A_2 and lysolecithin acyltransferase (Rooney, 1979). The production of surfactant has been shown to be stimulated by glucocorticoids, oestrogen, thyroxine and thyrotropin-releasing hormone. It is influenced by neurohormonal agents and stress factors, such as pregnancy and cyclic AMP (Rooney, 1979). Of these effects, those caused by glucocorticoids are the most studied and these hormones increase the activities of a number of enzymes involved in surfactant lipid synthesis (Table 11.23) and accelerate maturation of fetal lungs. Hormonal regulation of lung development and surfactant synthesis and secretion are reviewed by Post and van Golde (1988) and by van Golde et al. (1988).

For general reviews of the biochemistry of lung surfactant lipids, refer to Robertson et al. (1984), Harwood (1987), Post and van Golde (1988), van Golde et al. (1988), Batenburg and Haagsman (1988), and Harwood et al. (2002).

11.7.3 Respiratory distress syndrome (hyaline membrane disease)

Respiratory distress syndrome is a developmental disorder that is caused by immaturity of the lungs, which do not produce the required quantity of pulmonary surfactant. The lack of the normal complement of surfactant results in morphological alterations (hyaline membranes, atelectasis) and physiological alterations (decreased compliance, shunting of blood, hypoxaemia) and is a major cause of death in premature infants in the U.K. Because surfactant is lacking, the baby has difficulty in expanding his or her lungs and they tend to collapse in expiration, leading to a great increase in the work of breathing, respiratory failure and death unless ventilatory support is given. However, it must be stressed that lung surfactant deficiency may not be the only contributing factor to the disease symptoms (Morley, 1985), thus making treatment more problematic. Indeed, for adult respiratory distress (which is more common and where the prognosis is worse), surfactant deficiency is usually a complication of lung injury rather than a primary etiological factor (Lachmann and Danzmann, 1984).

The maturity of fetal lungs can be assessed by obtaining amniotic fluid by transabdominal amniocentesis. It is advisable to perform two types of tests on this fluid, including either a determination of the L/S ratio (see above) or phosphatidylcholine concentrations as well as a measurement of surface characteristics, such as the Clement's foam test (Diedrich et al., 1981). In Table 11.24, various test methods are shown with cautionary comments. A lung maturity test may be positive before week 34 of gestation in cases of gestosis, hypertensive illnesses, or previous premature rupture of the membranes. If the mother is diabetic, then lung maturation is delayed. The predictive accuracy of different tests for neonatal respiratory distress syndrome has been discussed (Harwood and Richards, 1985). Normal values for amniotic fluid phosphatidylcholine increase from about 2.5 mg/100 ml at 34 weeks to 8 mg/100 ml at 39 weeks gestation.

There have been considerable efforts to treat neonatal respiratory distress syndrome. Apart from the use of early continuous positive airway pressure and artificial ventilation (Jonson et al., 1984), pharmacological methods have been used to hasten lung maturity and, hence, increase the production of endogenous surfactant (Avery, 1984). Alternatively, surfactant replacement has been used for treatment (Fujiwara, 1984). The efficacy of the latter therapy is now well established both in animal models and in several randomized clinical trials (van Golde et al., 1988; Robertson and Taeusch, 1995). In fact, it is fair to say that replacement therapy is a common treatment for premature infants in Western countries.

The biosynthesis and secretion of the surfactant are subject to multiple hormonal controls (van Golde et al., 1988; Batenburg and Haagsman, 1988). First, β-adrenergic stimulation is involved in type II cell development, phosphatidylcholine synthesis and surfactant secretion (Walters, 1985). Secondly, glucocorticoids have extensive activity, particulary with regard to lung maturation and the development of lung surfactant formation (see van Golde et al., 1988; Table 11.23). Glucocorticoids have been used in several clinical trials (see, e.g., Avery, 1984). Thyroid hormones may affect lung development and surfactant lipid synthesis and interact with β-adrenergic regulation. Other hormonally related complications are the more severe symptoms in male human neonates and for infants of diabetic mothers (van Golde et al, 1988; Post and van Golde, 1988).

Surfactant replacement via the airways can be used prophylactically at birth or as a rescue operation for patients in respiratory failure. A number of different substitutes have been used — nearly all of which have proven clinically efficacious. Most of the preparations are derived from mammalian sources. Their preparation involves organic extraction of lung tissue or, more usually, lung washings. This process removes SP-A, but leaves the hydrophobic proteins SP-B and SP-C still present. The preparations can then be supplemented by addition of lipids and/or SP-A (see van Golde et al, 1988; Cockshutt et al., 1991). Because surfactant-associated proteins are, in principle, antigenic, efforts have been made to design effective protein-free surfactants. An example is the dipalmitoylphosphatidylcholine-phosphatidylglycerol mixture introduced by Morley et al. (1981). This preparation does not have immediate benefit in patients with manifest respiratory distress syndrome (Greenough et al., 1984), but does reduce infant mortality and improve lung function when given prophylactically at birth (Morley, 1987). Another protein-free surfactant substitute is one based on a mixture of dipalmitoylphosphatidycholine, hexadecanol, and tyloxapol (Tooley and Clements, 1984). However, ex-mammalian isolates are used currently.

Adult respiratory distress is a very important problem, which even now has a 50 to 60% mortality rate (Ingram, 1994). A conservative estimate lists about 150,000 cases per year in the U.S. alone. It is a multifactorial problem, but inactivation and decreased production of pulmonary surfactant are frequent characteristics (Fiser, 1993; Ingram, 1994). Therefore, the possible benefits of replacement therapy have been considered (Fujiwara, 1984; Pattishall and Long, 1992; Seeger et al., 1993). Because of the beneficial effects of surfactant-associated proteins, such as SP-B (Yu and Possmayer, 1992), on surfactant dynamics, their inclusion might be beneficial despite the possibility of allergic responses (Strayer et al., 1986). In any case there is increasingevidence that surfactant replacement therapy can be of benefit (Lachman and Danzmann, 1984; Fuhrman, 1990; Seeger et al., 1993; Griese, 1999).

TABLE 11.24 Some measurements with amniotic fluid that have been used to predict respiratory distress syndrome

Measurement	Comments
PC/Sph ratio	Significant number of false negatives; ratio >2 assures lung maturity; charring methods on TLC needs to be modified for DPPC
Relative % of PI and/or PG	Many babies lack both detectable PG and RDS
Surfactant apoprotein (35 KDa)	Probably will gain increasing popularity; sensitive if linked to ELISA assay
Bubble stability (shake test)	Simple, low cost; number of false negatives high
Fluorescence popularization	High cost instrument, but simple to use and reliable
Enzyme assays	Phosphatidate phoshohydrolase only useful constituent

Note: Sph = Sphingomyelin; PG = phosphatidylglycerol; PI = phosphatidylinositol; PC = phosphatidylcholine; ELISA = enzyme-linked immunosorbent assay; RDS = respiratory distress syndrome.
Source: Harwood, J.L. (1987). *Prog. Lipid Res.*, **26**, 211–256. With permission.

11.7.4 Other aspects of pulmonary surfactant

Sudden infant death syndrome (SIDS) is the most common cause of death in infancy. It affects about one baby in every 500 that die between 1 week and 2 years in the U.K. (Morley et al., 1982). It has been found that surfactant from SIDS babies contained significantly less phospholipid and DPPC and relatively more phosphatidylglycerol than did that of babies dying from other causes (Morley et al., 1982). These results have been confirmed, but it is not yet clear whether surfactant deficiency and abnormality is a primary or secondary phenomenon in SIDS (James et al., 1990).

A number of environmental conditions influence pulmonary surfactant levels in adult lungs. Exposure of animals to silica (Heppleston et al., 1974) or asbestos dusts (Tetley et al., 1977) causes massive increases in pulmonary surfactant and results in pathological conditions similar to human alveolar lipoproteinosis (Sahu et al., 1976). The surfactants from these dust-treated animals also display abnormal surface tension characteristics (McDermott et al., 1977). Asbestos and silica fibres have been shown to be very cytotoxic to cells *in vitro* and these particles are known to be highly damaging *in vivo.* It has been suggested that the increase in pulmonary surfactant is an attempt by animals to neutralize the effect of the inhaled dust. Tests *in vitro* have shown that pulmonary surfactant is strongly bound to the surface of mineral particles and that this surfactant is a very effective material in preventing asbestos-induced lysis of cells (Desai et al., 1975). Alternatively, the increase in surfactant may help in the physical movement of dust particles out of alveoli (Scarpelli, 1968).

The increase in pulmonary surfactant as a result of environmental factors may be generalized lung defence mechanism (Witschi, 1976), but exposure to several compounds, such as paraquat, glass powder, and anaesthetics, causes a decrease in this material. In addition to stabilizing the aleveolar surface, pulmonary surfactant may have a number of vital roles. It has been proposed that it aids in gaseous exchange, helps prevent pulmonary edema, and provides a milieu for movement of macrophages and other cells (Scarpelli, 1968).

The massive increases in surfactant levels following dust exposure may have important industrial implications, and changes are also noticed in many other diseases (Scarepelli, 1968), such as cystic fibrosis, sudden unexpected death in infants, and for adults suffering from multiple trauma, trauma to the lungs, acute pancreatitis and viral infections. In all of these cases, the changes noted in pulmonary surfactant would, at the very least, cause an impairment of lung function. A variety of other conditions, including those caused by chemicals, have been associated with changes in surfactant (see Harwood et al., 2002).

General reviews of surfactant biochemistry in health and disease have been made by Kikkawa and Smith (1983) and by Griese (1999), and the book by Robertson et al. (1984) also contains a wealth of information. Notter (2000) also covers clinical aspects in some detail.

References

Avery, M.E. (1984). Prevention of neonatal RDS by pharmacological methods. In *Pulmonary Surfactant*, Eds. B. Robertson et al., Elsevier, Amsterdam, pp. 449–457.

Batenberg, J.J. and Haagsman, H.P. (1998). The lipids of pulmonary surfactants: dynamics and interactions with proteins. *Prog. Lipid Res.*, **37**, 233–276.

Chander, A. and Wu, R.D. (1991). *In vitro* fusion of lung lamellar bodies and plasma membrane is augmented by lung synexin. *Biochim. Biophys. Acta*, **1086**, 157–166.

Clements, J.A. (1977). Functions of the alveolar lining. *Am. Rev. Respir. Dis.*, **115**, 67–71.

Clements, J.A. and King, R.J. (1976). Composition of surface active material. In *The Biochemical Basis of Pulmonary Function*, Ed. R.G. Crystal, Marcel Dekker, New York, pp. 363–387.

Clements. J.A. (1970). Pulmonary surfactant. *Am. Rev. Respir. Dis.*, **101**, 984–990.

Cockshutt, A.M. et al. (1991). The role of palmitic acid in pulmonary surfactant: enhancement of surface activity and prevention of inhibition by blood proteins. *Biochim. Biophys. Acta*, **1085**, 248–256.

Crapo, J.D. et al. (1982). Cell number and cell characteristics of the normal human lung. *Am. Rev. Respir. Dis.*, **126**, 332–337.

Desai, R. et al. (1975). The prevention of asbestos-induced hemolysis. *Life Sci.*, **16**, 1931–1938.

Diedrich, K. et al. (1981). Diagnostic problems of surfactant defects during birth. *Prog. Respir. Res.*, **15**, 155–167.

Egberts, J. and Noort, W.A. (1986). Gestational age-dependent changes in plasma inositol levels and surfactant composition in the fetal rat. *Pediatr. Res.*, **20**, 24–27.

Farrell, P.M. and Avery, M.E. (1975). Hyaline membrane disease. *Am. Rev. Respir. Dis.*, **111**, 657–688.

Fiser, D.H. (1993). Adult respiratory distress syndrome. *Pediatr. Rev.*, **14**, 163–166.

Fuhrman, B.P. (1990). Surfactant therapy. *Crit. Care Med.* **18**, 682–683.

Fujiwara, T. (1984). Surfactant replacement in neonatal RDS. In *Pulmonary Surfactant*, Eds. B. Robertson et al., Elsevier, Amsterdam, pp. 479–503.

Goerke, J. (1974). Lung surfactant. *Biochim. Biophys. Acta*, **344**, 241–261.

Greenough, A. et al. (1984). Effect of artificial surfactant on lung function in preterm neonates. *Prog. Respir. Res.*, **18**, 263–266.

Griese, M. (1999). Pulmonary surfactant in health and human lung disease: state of the art. *Eur. Resp. J.* **13**, 1455–1476.

Harwood, J.L. (1987). Lung surfactant. *Prog. Lipid Res.*, **26**, 211–256.

Harwood, J.L. and Richards, R.J. (1985). Lung surfactant. *Mol. Aspects Med.*, **8**, 423–514.

Harwood, J.L. et al. (1975). Characterization of pulmonary surfactant from ox, rabbit, rat and sheep. *Biochem. J.*, **151**, 707–714.

Harwood, J.L. et al. (2002). Alveolar surfactant. In *Pulmonary Biology in Health and Disease,* (Ed. E.E. Bittar), Springer, Berlin, pp. 44–63.

Hawgood, S. (1989). Pulmonary surfactant apoproteins: a review of protein and genomic structure. *Am. J. Physiol.*, **257**, L13–L22.

Heppleston, A.G. et al. (1974). Changes in the composition of lung lipids and the "turnover" of dipalmitoyl lecithin in experimental alveolar lipo-proteinosis induced by inhaled quartz. *Br. J. Exp. Pathol.*, **55**, 384–395.

Hills, B.A. (1988). *The Biology of Surfactant*, Cambridge University Press, Cambridge, U.K.

Ingram, R.H. (1994). Adult respiratory distress syndrome. In *Principles of Internal Medicine,* 13th ed., Eds. K.J. Isselbacher et al., McGraw-Hill, New York, pp. 2090–2099.

James, D. et al. (1990). Surfactant abnormality and the sudden infant death syndrome — a primary or secondary phenomenon. *Arch. Dis. Child.,* **65**, 774–778.

Jonson, B. et al. (1984). Early continuous positive airway pressure and artifical ventilation in treatment of neonatal RDS. In *Pulmonary Surfactant*, Eds. B. Robertson et al., Elsevier, Amsterdam, pp. 459–477.

Kikkawa, Y. and Smith, F. (1983). Cellular and biochemical aspects of pulmonary surfactant in health and disease. *Lab. Invest.*, **49**, 122–139.

King, R.J. (1974). The surfactant system of the lung. *Fed. Proc.*, **33**, 2238–2247.

Lachmann, B. and Danzmann, E. (1984). Acute respiratory distress syndrome. In *Pulmonary Surfactant* Eds. B. Robertson et al., Elsevier, Amsterdam, pp. 505–548.

McDermott, M. et al. (1977). The effect of inhaled silica and chrysotile on the elastic properties of rat lungs; physiological, physical and biochemical studies of lung surfactant. In *Inhaled Particles,* vol. 4, Ed. W.H. Walton, Pergamon, Oxford, U.K., pp. 415–427.

Morley, C.J. (1985). Respiratory distress syndrome and its treatment with surfactant. *Biochem. Soc. Trans.*, **13**, 1091–1092.

Morley, C.J. (1987). The Cambridge experience of artificial surfactant. In *Physiology of the Fetal and Neonatal Lung*, Eds. D.V. Walters, L.B. Strang and F. Geubelle, MTP Press, Lancaster, PA, pp. 255–274.

Morley, C.J. et al. (1981). Dry artificial lung surfactant and its effect on very premature babies. *Lancet*, **1**, 64–68.

Morley, C.J. et al. (1982). Surfactant abnormalities in babies dying from sudden infant death syndrome. *Lancet,* 1320–1322.

Notter, R.H. (2000). *Lung Surfactants: Basic Science and Clinical Applications.* Marcel Dekker, New York.

Pattishall, E.N. and Long, W.A. (1992) Surfactant treatment of adult respiratory distress syndrome. In *Update: Pulmonary Diseases and Disorders,* Ed. A.P. Fishman, McGraw-Hill, New York, pp. 225–236.

Possmayer, F. (1990). The role of surfactant-associated proteins. *Am. Rev. Respir. Dis.*, **142**, 749–752.

Post, M. and van Golde, L.M.G. (1988). Metabolic and developmental aspects of the pulmonary surfactant system. *Biochim. Biophys. Acta*, **947**, 249–286.

Robertson, B. and Taeusch, H.W. Eds. (1995). *Surfactant Therapy for Lung Disease*, Marcel Dekker, New York.

Robertson, B. et al. Eds. (1984). *Pulmonary Surfactant*, Elsevier, Amsterdam.

Rooney, S.A. (1979). Biosynthesis of lung surfactant during fetal and early postnatal development. *Trends Biochem. Sci.*, **4**, 189–191.

Sahu, S. et al. (1976) Lipids found in pulmonary lavage of patients with alveolar proteinosis and in rabbit lung lamellar organelles. *Am. Rev. Respir. Dis.*, **114**, 177–185.

Scarpelli, E.H. (1968). *The Surfactant System of the Lung*, Lea and Febiger, Philadelphia.

Seeger, W. et al. (1993). Alveolar surfactant and adult respiratory distress syndrome. Pathogenetic role and therapeutic prospects. *Clin. Investig.*, **71**, 177–190.

Stein, J.M. et al. (1969). Isolation and characterization of lung surfactant. *Biochem. Biophys. Res. Commun.*, **34**, 434–440.

Strayer, D.S. et al. (1986). Surfactant-anti-surfactant immune complexes in infants with respiratory distress syndrome. *Am.J. Pathol.* **122**, 353–362.

Tetley, T.D. et al. (1977). Changes in pulmonary surfactant and phosphatidylcholine metabolism in rats exposed to chrysotile asbestos dust. *Biochem. J.*, **166**, 323–329.

Tooley, W. and Clements, J.A. (1984). Clinical trial of Exosurf protocol. In *Clinical Trials of the Use of Surfactant in the Neonatal Respiratory Diseases Syndrome*, Ed. J.F. Lucey, Ross Labs, Columbus, OH, pp. 136–144.

van Golde, L.M.G. et al. (1988). The pulmonary surfactant system: biochemical aspects and functional significance. *Physiol. Rev.*, **68**, 374–455.

Vance, D.E. and Choy, P.C. (1979). How is phosphatidylcholine biosynthesis regulated? *Trends Biochem. Sci.*, **4**, 145–148.

Walters, D.V. (1985). The role of beta-adrenergic agents in the control of surfactant secretion. *Biochem. Soc. Trans.*, **13**, 1089–1090.

Weaver, T.E. and Whitsett, J.A. (1991). Function and regulation of expression of pulmonary surfactant-associated proteins. *Biochem. J.*, **273**, 249–264.

Witschi, H. (1976). Proliferation of type-2 alveolar cells — review of common responses in toxic lung injury. *Toxicology*, **5**, 267–277.

Wright, J.B. and Clements, J.A. (1987). Metabolism and turnover of lung surfactant. *Am. Rev. Respir. Dis.*, **135**, 426–444.

Wright, J.R. (1997). Immunomodulatory functions of surfactant. *Physiol. Rev.*, **77**, 931–962.

Yu, S-H. and Possmayer, F. (1992). Effect of pulmonary surfactant protein B (SP-B) and calcium on phospholipid absorption and squeeze-out of phosphatidylglycerol from binary phospholipids monolayers containing dipalmitoylphosphatidylcholine. *Biochim. Biophys. Acta* **126**, 26–34.

11.8 Agricultural aspects

11.8.1 Introduction

Lipids and their derivatives have a huge range of uses both for edible and nonedible purposes. Virtually all of these lipid-based products are derived from living organisms that are exploited via agricultural or biotechnological processes. To a great extent, the particular use of a given

biological fat or oil depends on its acyl composition. A major purpose of the end products of agricultural production is to provide human food. Lipids are important components of food, providing dietary energy, fat-soluble vitamins, and essential fatty acids (Gurr, 1983; Vergrosen, 1975; Wiseman, 1984; Murphy, 2007). They also enhance the palatability of food by producing flavour and aroma compounds and by influencing "mouth feel" (Forss, 1972). However, the lipid compositions of the domesticated plant and animal species that are our main sources of such products are relatively limited. For example, most oil crops overwhelmingly produce C16 and C18 acyl lipids, with palmitic, stearic, oleic, linoleic, and linolenic acids as the predominant components. Typical animal lipids, whether from milk or flesh, are also relatively restricted in their acyl compositions, being dominated by C12 to C18 saturates and C16 and C18 mono- and diunsaturates.

There are many additional sources of a wider variety of acyl and nonacyl lipids, but such organisms are often restricted in their availability. A good example is the very long-chain polyunsaturates found in many marine animals. These nutritionally desirable fatty acids are actually made by microalgae and ingested by fish, such as trout and salmon, which then accumulate them as part of their lipid stores. Declining fish stocks are threatening the availability and increasing the price of such marine lipids, which has led to a search for alternative sources. For these reasons, there is an increasing interest in the sourcing of novel lipids from hitherto unexploited organisms, and/or in the manipulation of existing domesticated plants, animals, or microbes to produce additional types of lipid that are especially suited to specific edible or nonedible applications.

11.8.2 Plant lipids

In global terms, plant lipids (see also Section 2.2 and Section 2.3) are the second most important source of edible calories in the human diet (after carbohydrates). About two-thirds of the 107 million tonnes of commercially produced plant oil is obtained from just three crops, namely soybean, palm, and canola (genetically improved rapeseed). Plant sources supply about 80% of the total worldwide demand for traded fats and oils — the nonplant fats and oils are mainly obtained from animal, fish, and dairy products.

Plant lipids are also sources of several essential vitamins and nutrients. For example, plant lipids are the ultimate source of the so-called "essential fatty acids" that are an obligatory component of the diet of all mammals (Section 11.1). Since the dawn of agriculture, certain plant species have been cultivated specifically for their lipid composition. The earliest olive plantations have been dated to more than 9 millennia before the present day and maize may have been domesticated in Mesoamerica as early as 10 millennia ago. In addition to their acyl lipid ingredients, plants are also important dietary sources of a host of other lipophilic compounds, including vitamins A and E, and a range of phytosterols.

Most of our dietary plant lipid is derived from oil crops and is in the form of either "visible" (e.g., oils, margarines, chocolate) or "invisible" (cakes, confectionary, processed foods) fats. Many of these products, especially the spreads, depend on solid fats. Because most vegetable oils are unsaturated and, hence, liquid at room temperature, they cannot be used in many food products without being modified to remove double bonds. This form of chemical manipulation of lipid content was only developed as a commercial process in the early 20th century, following the invention of catalytic hydrogenation (Murphy, 2005). Over the past 100 years, the lipid compositional requirements for many products, therefore, have been provided by bulk-refined, commodity plant oils that are blended and/or chemically modified (e.g., by hydrogenation) for a particular application. More recently, there has been a move towards a greater segmentation of the commodity plant oils market, with far more stress paid to the initial composition of the plant oil itself. Consumers are increasingly willing to pay a premium for identity-preserved oils that can be traced to a specific source, preferably from a single crop and a single country or region. This trend has been coupled with a greater health awareness in the general population, which has fuelled an increasing demand for plant oils that are enriched in monounsaturates, very long-chain ω-3 fatty acids, carotenoids, phytosterols, and tocotrienols. With the increased willingness of shoppers to pay a premium for such nutritionally enhanced oils, it is becoming more economic for growers and processors to breed specific lipid compositions into certain crops and then to segregate such value-added products. In this section, we will look at how plants are being manipulated through various forms of breeding in order to supply this wide range of dietary lipids, with a special focus on fatty acid composition.

11.8.2.1 Edible lipid products

In the past few decades, there has been an increasing segmentation of the plant oils market as food producers seek to highlight oils from particular crops, which may have special attributes that can add value to an end product. For example, high-linoleate sunflower oil is favoured for certain high polyunsaturated margarines, while cold-pressed, unprocessed virgin olive oil is favoured for its organoleptic qualities. In contrast, other plant oils, such as soy and rape, have tended to remain as relatively low-cost, generic commodity products. In the case of rape oil, this is rather odd because it has a very high oleic acid content, which makes it suitable to be branded as high-quality oil that is high in monounsaturates. There are also new varieties of rape seed oil that have less than 4% linolenic acid, which avoids the need for hydrogenation and allows the oil

to be marketed as "low in *trans* fatty acids." Despite these favourable attributes, however, rape oil still tends to be treated as a low-cost commodity, rather than as a higher value segmented product like olive oil.

This brings us to an important point about the reasons for seeking to manipulate plant lipid composition. Over the past 2 decades, many new types of plant oil crops have been developed, and many more are in the pipeline. However, most of these new plant oils have not been taken up by the market, or have not been exploited to their full potential. Part of the reason for this is that many of the modifications of plant lipid composition, especially by genetic engineering, have been technology-driven, rather than being market-led. This means that markets may be unprepared, unaware, or simply unwilling to take on the new products. It will be important in the future for breeders and chemical modifiers of plant lipids to work closely with the retail sector to ensure that viable markets exist or can be readily created to utilise the huge diversity of lipid products that is potentially available from modern biological and chemical technologies. For example, there are at least 500,000 species of higher plants and many of these accumulate storage oils in either their seeds and/or fruits. Unlike membrane lipids, which are extremely constrained in their acyl chain lengths and functionalities, storage lipids appear to be able to contain virtually any type of acyl moiety with chain lengths extending from as short as C8 all the way up to C24, and with many combinations of double or triple bonds, epoxy and hydroxyl groups, etc., as shown in Table 11.25. From the 1960s, the regional research division of the U.S. Department of Agriculture (USDA) at Peoria, IL (New Crops Research Unit at the National Center for Agricultural Utilization Research) has been undertaking a survey of some of the amazing diversity in acyl lipid composition of oils from plants collected from around the world. It has been found that there are many hundreds of plants, which are currently not grown as crops, but which have oil-rich seeds that accumulate novel and potentially useful fatty acids.

In recent years, much of the focus on such plants has entailed the isolation of genes that regulate the formation of the exotic fatty acids and their transfer to mainstream oil crops like soy or rape. However, as we shall see below, this genetic engineering approach has not been without its problems. Quite apart from the technical problems of persuading an existing crop to accumulate novel fatty acids in the right place and in the appropriate quantity, there is the problem of managing their cultivation and processing. After all, a soy seed that contains regular soy oil looks exactly the same as one that has been engineered to contain a nonedible and possibly toxic oil. Plant genetic engineering has also encountered considerable resistance from influential anti-genetic modification (GM) lobby groups, most notably in Europe, who have delayed its commercial application, especially in the food sector.

11.8.2.2 Domesticating new oil crops

An increasingly attractive strategy for the production of new types of plant oil is to domesticate some of the tens of thousands of wild species that already accumulate useful oils, but are not yet grown as crops. The great advantage of domesticating existing plants rather than either

TABLE 11.25 Accumulation of novel fatty acids by some oil-producing plants

Fatty acid[a]	Amount[b]	Plant species	Actual or potential uses
8:0	94%	*Cuphea avigera*	Fuel, food
10:0	95%	*Cuphea koehneana*	Detergents, food
12:0	94%	*Litsea stocksii*	Detergents, food
14:0	92%	*Knema globularia*	Soaps, cosmetics
16:0	92%	*Myrica cerifera*	Food, soaps
18:0	65%	*Garcinia cornea*	Food, confectionery
20:0	33%	*Nephelium lappaceum*	Lubricants
22:0	48%	*Brassica tournefortii*	Lubricants
24:0	19%	*Adenanthera pavonina*	Lubricants
18:1 Δ6 cis	76%	*Coriandrum sativum*	Nylons, detergents
18:1 Δ9 cis	78%	*Olea europaea*	Food, lubricants
22:1 Δ13 cis	58%	*Crambe abyssinica*	Plasticisers, nylons
18:2 Δ9,12 cis	75%	*Helianthus annuus*	Food, coatings
α18:3 Δ9,12,15 cis	60%	*Linum usitatissimum*	Paints, varnishes
γ18:3 Δ6,9,12 cis	25%	*Borago officinalis*	Therapeutic products
18:1–hydroxy	90%	*Ricinus communis*	Plasticisers, cosmetics
18:1–epoxy	60%	*Crepis palestina*	Resins, coatings
18:2 9c12a	70%	*Crepis alpina*	Coatings, lubricants
18:3–oxo	78%	Oiticica(*Licania sigida*)	Paints, inks
18:3–conj	70%	Tung(*Aleurites fordii*)	Enamels, varnishes
20:1/22;1wax	95%	*Simmondsia chinensis*	Cosmetics, lubricants

[a] Fatty acids are denoted by their chain length followed by the number of double bonds or nature of other functionalities.

[b] Percentage of total seed or mesocarp fatty acids.

Source: Murphy, D.J. (2001).

conventional breeding or the development of transgenic crops is that the existing wild plants are already adapted to accumulate their exotic fatty acids in the appropriate cellular compartment, namely in their storage oils. In the native plants, these exotic fatty acids are hardly ever found in cell membranes or in any other location where their presence could be damaging. A further advantage of novel oilseed crops is that the seed oils already contain accessory stabilising agents, such as antioxidants, which prevent the breakdown of some of the more highly reactive fatty acids, such as conjugated polyunsaturates and those containing acetylenic bonds.

The challenge to the domestication of new crops is simple. Although many of the potential new crops may already be excellent sources of useful products, such as novel fatty acids, they are often not suitable for large-scale agriculture. The reason for this is that these plants have not been bred specifically for agronomic performance over centuries or even millennia, as have some of our more familiar crops. They suffer from many of the usual adverse characteristics of wild plants; for example, they tend to flower asynchronously throughout the summer and, therefore, do not produce their seeds at a single time, which makes harvesting very difficult. They often produce seedpods that are prone to shattering before or during harvest, resulting in a loss of many of the seeds. Often, the canopy architecture of the plant is not suitable for existing harvesting machinery. They may be susceptible to a variety of diseases or pests, including fungi and insects. Finally, in the case of oilseeds, although they may contain as much as 90% of a novel fatty acid in their seed oil, the overall oil yield in tonnes per hectare ($T.ha^{-1}$) may be relatively low.

The improvement of these important agronomic characters requires the manipulation of numerous complex traits. Seed companies are often dismayed by the prospect of domesticating new species, citing the example of a major crop, such as wheat, which is still being improved after more than 10,000 years of domestication. Nevertheless, we can now be more optimistic about the prospects for crop domestication by the use of modern breeding techniques. Examples of such relatively new crops include hybrid maize, rapeseed, sunflower, and soybean, which have only been grown as mainstream commercial crops for a century or less. There is also now the prospect of using new advanced biotechnological methods, such as marker-assisted selection (see below) and genomics, to accelerate the development of new oil crops.

11.8.2.3 Oil crop modification

Over the past 50 years, several established oilseed crops have been successfully modified in order to improve their edible qualities, while in other cases new crops have been more or less developed from scratch. Such developments have been facilitated by advances in plant breeding and by improved analytical techniques that have allowed for the rapid and accurate mass screening of plant samples for possible changes in lipid composition.

11.8.2.3.1 Canola — a new oilseed crop

Probably the most impressive example of conventional genetic manipulation of an oilseed crop to enhance its edible performance was the modification of the existing high-erucic form of oilseed rape in the 1960s to produce the current high-oleic form of canola oil. Prior to this time, oilseed rape, and indeed all the other brassica species, produced seed oil that was highly enriched in the very long-chain C22 erucic acid. In Europe and North America, this oil was normally used for nonedible purposes and oilseed rape was a relatively minor crop with a limited market. Following animal studies in the 1950s, it was also claimed that dietary erucic oils were associated with certain cancers and the U.S. authorities removed rapeseed as an approved crop for human consumption. In the 1960s, the Canadians were looking for new crops to grow on their huge prairie farms and one possibility was to breed an edible form of oilseed rape. The challenge was to reduce the amount of erucic acid and instead to increase the amount of one of the more useful fatty acids made by rapeseed, such as oleic acid (18:1). Genetic analysis showed that the pathway for the elongation of oleate to erucate was controlled by only a few genes and, thus, was potentially amenable to manipulation by a classical mutation breeding approach (Downey and Craig, 1964).

Because several genes were involved, it was necessary to cross some of the mutants with intermediate amounts of erucic to produce varieties with very low levels of the fatty acid. Although this took a few years of hard work, by 1964 the project was eventually rewarded with success as the team developed the first zero-erucic acid variety of oilseed rape, which they christened "canola." The remarkable achievement of these breeders has been highlighted by recent molecular genetic data. It was found that canola plants contain single-point mutations in two genes encoding isoforms of the enzyme β-ketoacyl-CoA synthase. This protein is part of the fatty acid elongase complex now known to mediate the formation of erucic acid from oleate (Fourmann et al, 1998). Therefore, our modern canola/rapeseed varieties are the consequence of the alteration of just two nucleotides in a genome that contains over 1.2 billion nucleotides. This demonstrates the power of genetics as applied to plant breeding. Such a result would be the envy of any latter-day biotechnologist and is a useful reminder that genetic engineering is not the only way to achieve the precise genetic manipulation of a crop. For the past 30 years, canola has been a mainstay of Canadian prairie agriculture and a major export earner for the country. Canola-standard oilseed rape has also been adopted enthusiastically as an edible oil crop around the world with an annual value in excess of $6 billion (Oil World, 2005). Since the development of high-oleic canola varieties in the 1960s, rapeseed has been improved further as an edible oil

crop by reductions in its content of oxidation-prone linolenic acid so as to avoid the need for hydrogenation and the accumulation of *trans* fatty acids, as we will now discuss.

11.8.2.3.2 Other modified oilseed crops

The need to reduce levels of *trans* fats in foods has driven breeders to develop several new high oleic varieties of the major oil crops over the past decade. For example, there are now commercial high-oleic varieties of the "big three" oilseed crops: soy, rape, and sunflower, all of which have been produced by conventional breeding. Efforts are also underway to produce high-oleic oil palm varieties, either by screening for existing variation or by transgenic methods, although both strategies may take several decades. A particular attraction of high-oleic plant oils is that they have applications both as premium edible oils and as high-grade feed stocks for the manufacture of oleochemicals in the nonfood sector. An example of the latter market is the use of high-oleic soybean oils as biodegradable lubricating fluids that have relatively long working lives and low susceptibility to oxidation at high temperatures (Cahoon, 2003).

High-oleic soybean varieties with as much as 83% oleate and less than 3% α-linolenate have been developed (Rahman et al., 2001) and are now being marketed by major seed companies. Breeders have also developed other lines of soybean that have high levels of stearic acid (Rahman et al., 2003) and other nutritionally relevant fatty acids. Several high-oleic canola/rape varieties have been developed that typically contain about 70 to 80% oleate, 15% linoleate, and only 3% α-linolenate. Major seed companies such as Cargill, Dow Agrosciences, and Bayer are now developing such varieties for various end-use markets, both edible and nonedible. By 2004, high-oleic rape/canola was already being planted on about 250,000 ha in Canada, which is 5% of the total area of canola cultivation (AgCanada, 2004).

Sunflower oil, once the commercial high polyunsaturate edible oil *par excellence*, is also steadily being rebranded as a high-oleate oil. Traditional sunflower oil consists of about 68% linoleic acid, 20% oleic acid, and 10% saturates, which means that hydrogenation is still necessary for many food uses. During the 1990s in the U.S., there was great interest in newly developed very high-oleic sunflower varieties (with 80% oleic acid, 10% polyunsaturates, and 10% saturates) that had already been developed by breeders. But these varieties were only available to farmers in very limited quantities because of a patent on the hybrid planting seed with oleic levels at or above 80%. The patent holders chose not to license the breeding material to other companies, thus high-oleic sunflower production was very limited and the price of the oil was quite high compared to commercial oils. By 1999, these problems were resolved with the development of a new intermediate oleate hybrid variety called NuSun, which was commercially launched in that year. NuSun oil contains about 65% oleic, 25% polyunsaturates, and 10% saturates; it does not require hydrogenation and performs especially well in larger-scale commercial frying applications. By 2001, over 200,000 tonnes of NuSun oil were being produced and the hoped-for business breakthrough came in the same year with the announcement the Proctor & Gamble would be using NuSun oil exclusively in its popular Pringles range of potato chips (Kleingartner, 2002).

11.8.2.3.3 Advanced breeding for oil crop modification

During the mid to late 20th century, oil crop breeding was driven by advances in analytical technologies and by a vastly improved knowledge of lipid metabolism. Over the past few decades, the task of the oilseed breeder has been facilitated further by new techniques of advanced breeding, such as the use of DNA-based molecular markers, greatly improved tissue culture methods, and more recently the application of genomics and proteomics (Murphy, 2005).

DNA marker-assisted selection — Plant breeding has always relied on the selection of agronomically favourable characters from the diverse gene pool that is present in any crop species, even if many elite commercial cultivars tend to be highly inbred. Often these agronomic characters are visible and easily identified, e.g., height or flower colour or resistance to fungal attack. In other cases, the characters can be much more subtle and sometimes can only be measured by sophisticated analytical techniques, e.g., the amounts of certain secondary products or the fatty acid composition of the seed oil. In all of these cases, it was formerly necessary for the breeder to grow up and analyse each new generation before it was possible to measure the character, or phenotype, and select the appropriate plants. The advent of marker-assisted selection has changed this as breeders can now select a few plants that are likely to express the required characters from amongst tens of thousands of progeny even before the plants have developed to maturity. The basis of the method is DNA-fingerprinting and is, in principle, no different from the methods used with such great effect in modern medical diagnostics or in forensic science (Gill, 1985).

Molecular markers, such as microsatellites, RFLPs (restriction fragment length polymorphisms), and RAPDs (random amplified polymorphic DNA) have now been developed for many oil crops, including trees, such as oil palm. The markers can be used to track the presence of valuable characters in large segregating populations as part of a crop-breeding programme. For example, if a useful trait like disease resistance, improved nutritional quality, or higher yield can be linked with a specific marker, many hundreds or even thousands of young plantlets can be screened for the likely presence of the trait without the necessity of growing all the plants to maturity or doing costly and time-consuming physiological or biochemical assays. The use of molecular markers can decrease the timescale of crop breeding programmes by

several years and can substantially reduce costs. Although largely limited to the major temperate crops at present, the same technology can be applied to assist the breeding of any crop and even to domesticating entirely new crops.

A good example of the potential for marker-assisted selection can be seen with edible tree crops, many of which are major export earners for developing countries. Examples of such crops include oil palm, coconut, coffee, tea, cocoa, and the many fruit trees like bananas and mangoes. In the case of oil palm, the fruits that are the source of the oil are not produced for 5 to 7 years after planting. This means that a breeder must wait for at least 5 to 7 years before being able to determine the oil composition of a particular experimental cross. In contrast, breeders of annual crops have to wait only a few months before a plant like soy or oilseed rape sets seed. However, by using DNA markers, the oil palm breeder can now (in theory) identify whether new plantlets carry the required gene when they are only weeks old. This type of molecular marker technology is reasonably well developed for the major annual oilseed crops like soy or rape (Quiros and Paterson, 2004), but is still very much under development for more complex crops like oil palm (Basri et al., 2004).

Tissue culture and mass-propagation — The use of modern techniques of cell, tissue and organ culture is central to many crop improvement programmes. Tissue culture has been widely used in crop breeding programmes for over 50 years (Phillips, 1993). For example, the use of embryo rescue techniques has enabled the incorporation of characters like disease resistance from wild relatives of crops into elite breeding lines. It is now possible to make wide crosses between hexaploid wheat and barley, rye, or diploid wheat. The hybrids of such crosses are sometimes sterile due to embryo abortion, but can be "rescued" by culturing or transplanting the embryos. Another important technique increasingly used in crop breeding programmes is the production of doubled haploids. The repeated selection of heterozygous materials in a breeding programme can increase uniformity, but many generations are required to reach homozygosity in loci associated with agronomic traits. The artificial production of haploid plants followed by chromosome doubling offers the quickest method for developing homozygous breeding lines from heterozygous parental genotypes in a single generation. Haploid gamete cells from anthers or ovaries can be converted into diploids after colchicine treatment and then regenerated to yield doubled haploid plants. This technique is now used widely for the improvement of many of our most important oil crops, including maize, rapeseed, and soybean (Forster et al., 2000).

Yet another useful application of tissue culture methods is the mass clonal propagation of certain crops, in particular trees. Clonal propagation has not always been commercially successful, however. In the 1980s, a scheme to mass propagate millions of oil palm plantlets from a superior breeding line foundered when many of the maturing trees were discovered to have an abnormality in their floral development (Corley, 2000). This led to a failure of fruit formation and, since the major products of the crop are fruit oils, the trees were effectively useless. The abnormality is now known to be due to a tissue culture effect whereby the expression of homeotic genes regulating meristem identity is disrupted. Although the problem is now in the process of being rectified by further research, commercial confidence in clonal propagation has not recovered and relatively little planting of clonal oil palm has been done over the past 20 years (Corley, 2000).

Genomics — Genomics is the term given to the massively parallel study of the DNA and protein sequences in an organism and the specification of when and where such sequences are expressed. However, genomics is much more than the mere assembly of DNA or protein sequence information or gene expression catalogues. It can also be used as a tool in crop breeding programmes and even for the domestication of new plant species as future crops. Many characters of agricultural importance in crop plants, including some fatty acid traits, appear to be regulated by a large number of genes and, therefore, do not segregate into simple Mendelian ratios, as would be expected if only one or two genes were involved. The application of information from genomics is enabling crop scientists to identify key genes that regulate the accumulation of specific lipids that breeders may wish to either eliminate (e.g., α-linolenate for *trans*-free foods) or up-regulate (e.g., oleate for high monounsaturate oils). This information can then be used to develop DNA-based markers for marker-assisted selection as described above.

11.8.2.3.4 Transgenic oil crop modification

Transgenesis is the addition of exogenous (i.e., externally derived) DNA sequences and their incorporation into the genome of a recipient organism, such as a plant or animal. In the case of plants, the DNA can be added to cells directly by propelling small gold particles coated with DNA into plant tissues. Alternatively, the DNA can be added in a more controlled fashion by means of a bacterial vector, e.g., *Agrobacterium tumefaciens*, that is able to insert a specific region of DNA into the genome of the plant. Even with their various uncertainties, both of these methods of DNA transfer, or transgenesis, are much more efficient than alternative methods of crop genetic manipulation, such as induced mutation or wide crosses. For example, the creation of new varieties via radiation, chemical, or somaclonal mutation normally involves the repeated treatment of tens of thousands of tissue explants or seeds. These extremely drastic procedures result in hundreds of mutated genes, nearly all of which will be undesirable, and perhaps even lethal, to the crop. It then takes many years of backcrossing and selection to obtain a plant that carries a mutation in the desired gene(s), but not in other essential genes. Even then, it is still possible that there may be undetected hidden, or cryptic, muta-

tions that only manifest themselves in later generations as the crop is tested or grown in commercial cultivation. Another significant drawback of mutagenesis is that the breeder can only manipulate genes that already exist in the crop genome. Furthermore, nearly all mutations result in a loss of gene function, so mutagenesis is nearly always about reducing the effect of unwanted genes, rather than increasing the expression of desirable genes.

The concept of "designer oil crops" — During the late 1980s and early 1990s, oilseeds like soy and rape were at the forefront of attempts to produce commercial transgenic (genetically modified) crops. These efforts were pioneered by researchers in small biotech companies, such as Calgene, as well as the large multinationals, such as DuPont, Monsanto, and Zeneca (now Syngenta). Part of the rationale for these efforts was a belief that fatty acid biosynthesis was well understood at the biochemical level and that relatively few genes would be needed to affect substantial changes in oil profiles in a seed. This led to the concept of "designer oil crops" as described in the book of the same name that appeared in 1994 (Murphy, 1994). As pointed out at that time, there are three major challenges that confront those who wish to use transgenesis to modify plant lipid composition. These are to ensure that the new transgene is only expressed in the appropriate place (normally the fruit or seed), to ensure that novel fatty acids are segregated into storage lipids and away from membrane lipids, and to ensure that the transgenic crop varieties and their products are adequately segregated from other identical-looking varieties of the same crop that produce different oils. In the early to mid 1990s, oilseed rape was regularly cited as an archetypical designer oil crop that could be engineered to produce as many as a dozen different oils to supply products ranging from margarines and pharmaceuticals to bioplastics and lubricants (Murphy, 1994: Budzisewski et al., 1996).

Despite the initial optimism of many researchers (including the author), the use of agbiotech to manipulate the fatty acyl composition of oils has turned out to be more complex than was first thought. Indeed, very recent findings suggest that our understanding of even the basic pathway of triacylglycerol oil biosynthesis is far from complete and that there are probably multiple pathways rather than just one (Murphy, 2004). The consequence of these complexities of plant lipid metabolism has been that, although there have been many impressive achievements in isolating oil-related genes and producing transgenic plants with modified oil compositions, it has not yet been possible to achieve the kind of high levels, i.e., 80 to 90% of novel fatty acids that will make possible their widespread commercial exploitation (Murphy, 1999). As of the 2005 season, there were no significant commercial plantings of transgenic oil crops with modified fatty acid profiles. Although there are over 80Mha of transgenic oilseeds, such as soybean, canola, maize, and cotton, these varieties are all modified to carry input-related genes involved in herbicide tolerance or insect resistance traits, rather than seed oil content (James, 2004).

The only commercially grown transgenic crop with modified seed oil is the laurical variety of canola (rapeseed) originally marketed by Calgene in 1995. From an original level of 40% lauric acid, newer laurical varieties have been produced with 40 to 60% lauric by the insertion of several additional transgenes (Voelker et al., 1996). However, this crop remains far from being a commercial success and cannot compete with cheaper tropical lauric oils from coconut and oil palm. Many genes that regulate the formation and accumulation of other exotic, nonedible fatty acids were isolated during the 1990s, including hydroxylases, conjugases, desaturases, and epoxidases, but so far it has not been possible to use any of these genes to effect the production of any commercially useful oils in transgenic plants. Two of the major challenges facing designer oil crops are to prevent the novel fatty acids from leaking into cellular membranes and to segregate the seeds and oils during cultivation and processing, as we will now consider.

Segregation of novel fatty acids from membrane lipids — The cellular membranes of all organisms are crucial to their metabolism and survival. Biological membranes are made up of a lipid bilayer into which is embedded various proteins that mediate such processes as transport, respiration, photosynthesis, and signal transduction. The fatty acid composition of a given membrane is closely regulated and the presence of inappropriate acyl groups often leads to serious disruption of membrane, and, hence, cellular function. In plants that accumulate fatty acids that differ significantly from those of membrane lipids, specific mechanisms have evolved that prevent the "leakage" of unwanted acyl groups into membrane lipid pools. Biochemically speaking, this is not a trivial task, because storage lipids and many membrane lipids are assembled on the same organelle — the endoplasmic reticulum.

As yet, we do not completely understand the mechanisms by which some plants are able to segregate unusual fatty acids away from membranes (Millar et al., 2000). We know that this mechanism is very important because the leakage of a novel fatty acid, namely the saturate, stearic acid, resulted in very poor seed germination rates in a transgenic variety of oilseed rape that had been engineered to have an elevated stearate oil for use in the manufacture of edible spreads (Thompson and Li, 1997). It was found that a small amount of stearate had leaked into cell membranes, resulting in a reduction in membrane fluidity and impairment of function that affected the development of the entire plant. But although, in this case, the transgenic rapeseeds only accumulated about 40% stearate and just 3 to 5% leaked into cell membranes, naturally occurring oilseeds like mangosteen (*Garcinia cornea*) can accumulate over 65% stearate in their seed oil without any detectable leakage into cell membranes.

Two hypotheses have been proposed to explain the sort of lipid segregation that plants like mangosteen seem

capable of, but which seems to be lacking in major oil crops like oilseed rape. The first hypothesis is that there is a compartmentation of membrane and storage lipid synthesis in specific membrane domains of the endoplasmic reticulum. The other hypothesis is that there is a selective accumulation of the novel fatty acids in triacylglycerols after synthesis (Millar, 2000). Research is currently underway to address these issues, but until we understand more about fatty acid segregation, the production of most exotic fatty acids in transgenic crops will remain more of an aspiration than a reality (Thelen and Ohlrogge, 2002).

11.8.2.4 Plant lipid manipulation in the 21st century

The current development of plant lipids for edible production is marked by two apparently contradictory trends. First, there has been a huge consolidation and concentration in the number of the major oil crops, the suppliers of plant material for growing such crops, and the retail sector that delivers food to the domestic consumer. This trend leads to a more generic, commodity-based market. Secondly, however, there is a trend towards segmentation and identity preservation of individual oils that may carry a considerable premium, e.g., extra-virgin olive oils from a specific region or red palm oils from Africa. These divergent tendencies are driven by different forces that favour the cheapest generic oils, especially for use in mass-produced processed food, on one hand, while also favouring the creation of niche products with specialised identity-preserved oil compositions that are often based on health claims. The speed with which such dietary fads can come and go makes it difficult for breeders to produce appropriate varieties, given the decades-long timescale of most breeding programmes. For example, just in the past few decades, we have gone from advice to reduce all dietary saturates, to increase polyunsaturates, to monounsaturates, to ω-3 acids, and so on. The most recent consumer interest seems to be in low-*trans*, high-monounsaturate oils, and in ω-3 acid oils, ideally with some lipophilic antioxidants, such as tocols or carotenoids, thrown in for good measure. Plant oils with each of these profiles are currently being developed by breeders, as we will now see.

11.8.2.4.1 Low trans oils

The market for oils that contain reduced or zero levels of *trans* fatty acids is driven by health concerns that have led to the imposition of labelling requirements to state whether a product contains over a given threshold of these fatty acids. Such labelling requirements were introduced into the U.S. in January 2006 and are likely to be required in the European Union in the near future. Typical threshold levels of *trans* fatty acids that would trigger compulsory labelling are in the region of 0.5 to 1.0%, whereas some existing foods can contain as much as 40% *trans* fatty acids. The solution in most cases will be to develop high-oleic oil crops and, as we have seen above, breeders have been gradually producing such varieties of the major oilseeds over the past decades. There are still challenges for breeders to reduce further or to eliminate altogether α-linolenate from seed oils and to ensure that the high-oleic traits are crossed into their highest performing elite commercial lines.

For most crops, it is possible to produce high-oleic varieties by nontransgenic routes and, given the sentiment about genetically modified crops in Europe, such a route is obviously preferable at the present time. Given the huge potential market for foodstuffs that are free of *trans* fatty acids, it is possible that high-oleic oil crops will become the mainstream commodities over the next decade, rather than existing polyunsaturate-rich varieties. Such a development would certainly be welcome to processors as it would obviate the need to segregate the oil, but it would be less well received by farmers and seed suppliers who would lose their premium for a value-added variety. The types of product, e.g., a spread or liquid oil, that would result from a high monounsaturate plant variety, would also be potentially suitable for the delivery of ω-3 fatty acids, as long as they were kept down to about 10% to avoid oxidation problems. However, if customers required higher levels of ω-fatty acids, e.g., in a neutraceutical format, it would be better to produce a dedicated high ω-3 oil crop. We will now look at the rationale for and recent progress towards this objective.

11.8.2.4.2 Can plants substitute for fish oils?

Oils rich in ω-3 fatty acids include the so-called "fish oils" (or more correctly "marine oils'), which are characterised by relatively high levels of very long-chain polyunsaturated fatty acids (VLCPUFAs), such as eicosapentaenoic acid (20:5 ω-3, EPA) and docosahexaenoic acid (22:6 ω-3, DHA). These compounds are part of the group of ω-3 fatty acids that are essential components of mammalian cell membranes, as well as being precursors of the biologically active eicosanoids and docosanoids (Funk, 2001; Hong et al., 2003). There have been numerous reports concerning the importance of dietary supplementation with these fatty acids for human health and well-being. For example, dietary VLCPUFAs have been shown to confer protection against common chronic diseases, such as cardiovascular disease, metabolic syndrome, and inflammatory disorders, as well as enhancing the performance of the eyes, brain, and nervous system (Crawford et al., 1997; Benatti et al., 2004; Spector, 1999).

Consumption of fish is currently recommended in most Western countries as part of a balanced diet; and much of the nutritional benefit of the fish actually comes from the VLCPUFAs of the fish oils. These fatty acids can be synthesised by the fish themselves, or derived from microorganisms, especially photosynthetic microalgae, that are ingested as part of their diet. As an alternative to fish consumption, therefore, it is possible to purchase VLCPUFA dietary supplements that are derived from cultured

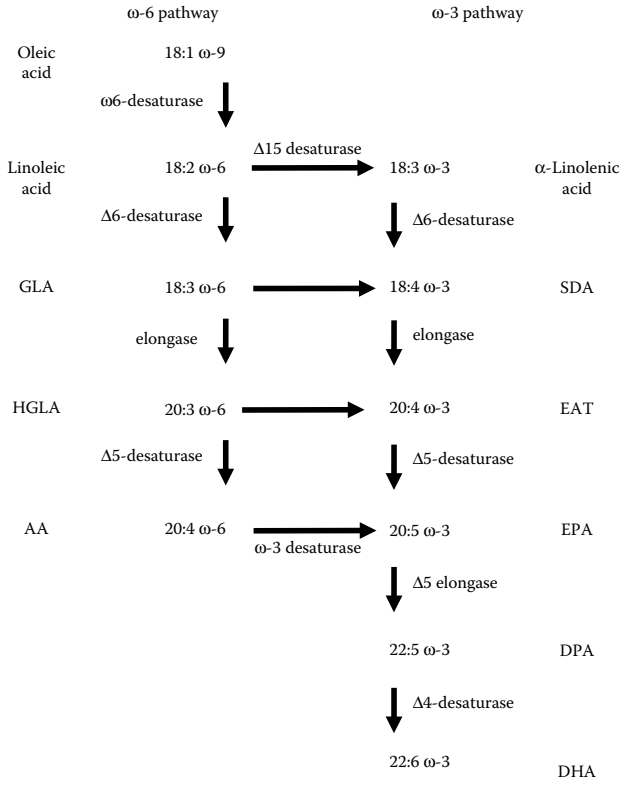


FIGURE 11.14 Biosynthetic pathways for very long-chain polyunsaturated fatty acids. This figure shows the complexity of the various pathways involved in the synthesis of the kinds of the ω-6 and ω-3 enriched "marine oils" that various research groups are attempting to engineer into oil crops. The starting substrates are linoleic acid and α-linolenic acid in the ω-3 and ω-6 pathways, respectively. The Δ6 desaturation of linoleic acid and α-linolenic acid gives rise to γ-linolenic acid (GLA) and stearidonic acid (SDA). γ-Linolenic acid is then elongated progressively to the ω-6 series of dihomo-gamma-linolenic acid (HGLA) and arachidonic acid (AA), while stearidonic acid (SDA) is elongated progressively to the ω-3 series that includes eicosatetraenoic acid (ETA) and eicosapentaenoic acid (EPA). An ω-3 desaturase interconnects the ω-6 and ω-3 pathways for more efficient EPA production. Finally, EPA is elongated by a Δ5 fatty acid elongase and desaturated by a Δ4 desaturase to produce docosahexaenoic acid (DHA). (See also Section 10.1.)

microalgae or fungi. However, low oil yields and high costs of oil extraction have limited the scope for this production method, and ever-dwindling fish stocks are also threatening supplies of the main source of marine oils. This situation has led to renewed interest in the possibility of breeding oilseed crops that are capable of producing significant quantities of VLCPUFAs in their storage oils. Higher plants do not normally accumulate such fatty acids, but they can accumulate C22 and C24 monounsaturates and C18 polyunsaturates in their seed oils, so it seemed possible that C20 and C22 polyunsaturates might also be accumulated providing the plants were able to synthesise these fatty acids.

The most serious of several technical challenges to the engineering of VLCPUFA production in plants is the number of enzymes that are needed for the conversion of a typical plant C18 PUFA, such as linoleate or linolenate, to the C20 and C22 PUFAs with up to six double bonds that are characteristic of fish oils, as shown in Figure 11.14. Other key challenges are similar to those that have confronted previous attempts to engineer transgenic oilseed, namely to ensure seed-specific expression of the transgenes and to channel the novel fatty acids towards oil accumulation and away from membrane lipids. During 2004 and 2005, there were several reports that encourage the view that the economic production of VLCPUFAs in transgenic plants might be possible (Abbadi et al., 2004; Qi et al., 2004; Wu et al., 2005). In one rather heroic experiment, no fewer than nine genes from various fungi, algae, and higher plants were inserted into the oilseed, *Brassica juncea*, with the resultant accumulation of as much as 25% arachidonic acid and 15% eicosapentaenoic acid (Wu et al., 2005).

11.8.2.5 Modifying nonacyl lipids in plants

In the past, the majority of attention by researchers has been paid to modification of the relatively abundant acyl lipids in oil crops. However, there is now increasing awareness of the importance of many nonacyl lipids that may be present in insufficient quantities or may be removed or destroyed during the processing of plants for food. We will consider three examples here. First, there is the case of conventional rice, which is almost entirely deficient in carotenoids, such as β-carotene. This can result in vitamin A deficiency in those (mostly poor) people who are excessively reliant on rice in their diet. Secondly, vitamin E lipids are present in oil palm and can be manipulated to higher levels to serve as antioxidant-rich nutraceuticals. Thirdly, several plant sterols and stanols that have well-characterised hypocholesterolemic functions, and are already produced in some plant tissues, but may be engineered into a wider range of transgenic oil crops in the future (Broun et al., 1999; Corbin et al., 2001).

11.8.2.5.1 Golden rice

Probably one the best-known recent examples of a nutritionally enhanced crop developed by genetic engineering is the transgenic "golden rice," which was produced by a Swiss-based group (Ye et al., 2000). This transgenic rice contains three inserted genes encoding the enzymes responsible for conversion of geranyl geranyl diphosphate to β-carotene. It is claimed that consumption of this rice by at-risk populations may alleviate the vitamin A deficiency, which leads to night blindness, that currently afflicts some 124 million children worldwide. Interestingly, the rights for the commercial exploitation of "golden rice" in developed countries, including the U.S. and Europe, have now been acquired by Syngenta. It is possible that this could lead to the marketing of "vitamin-enhanced" food products

derived from golden rice, e.g., in breakfast cereals, which may be more acceptable to the public than the current generation of food from input trait modified GM crops.

One of the reservations expressed about the original varieties of golden rice was the relatively low content of provitamin A. More recently, this problem has been solved by replacing a daffodil phytoene synthase gene with a similar gene from maize. The addition of this maize transgene to rice led to a 23-fold increase in provitamin-A levels (Paine et al., 2005). It must be emphasised that this improved variety of golden rice still has many years of backcrossing into local varieties and field testing, before it will be known whether it is a viable and useful crop. One of the major challenges is to ensure that the provitamin A in the rice grains is in a form that can withstand processing, storage, and cooking, but is also completely bioavailable following consumption by people.

11.8.2.5.2 The vitamin E group

The vitamin E group of compounds includes four tocopherols, and four tocotrienols, all of which have significant antioxidant properties. These lipophilic vitamins can be found in most nonprocessed seed oils, but are often lacking in foods made from processed oils. There is interest in trying to increase the levels of this group of lipidic vitamins in plant oils using a variety of approaches. For example, transgenic plants that accumulate 10- to 15-fold higher levels of vitamin E compounds have been engineered by adding homogentisic acid geranyl geranyl transferase genes from several cereals to Arabidopsis plants (Cahoon et al., 2003). As described above, unrefined palm oil also contains significant amounts of vitamin E group compounds (Han et al., 2004). In recent years, several varieties of oil palm have been identified that produce oil that is highly enriched in tocols to levels in excess of 1500 ppm, which would be of great interest as potential health food products.

11.8.2.5.3 Phytosterols and stanols

Another category of plant lipid of interest to the food industry is the phytosterols. Margarines enriched in phytosterols extracted from (nontransgenic) wood pulp or vegetable oils have recently been marketed and, despite an appreciable price premium compared to conventional margarines, they have enjoyed modest commercial success. The appeal of the phytosterol-enriched margarines is based on evidence that they may help to reduce blood cholesterol levels and, hence, combat heart disease. Such products could be made more cheaply if more of the phytosterols were synthesised in the same seeds as the oil from which the margarine is derived. Efforts are now underway to up-regulate phytosterol biosynthetic pathways in transgenic plants. The impact on human health of such products could be considerable. Indeed, it has been surmised that the wide-spread availability and consumption of low-cost, phyto-sterol-enriched margarines could eventually lead to a quantifiable reduction in national rates of cardiovascular disease, which is still the most common cause of mortality, especially in low-income groups, in all industrial societies (Plat and Mensink, 2001).

11.8.3 Animal lipids (see also Section 2.4 and Sections 2.6 to 2.8)

Lipids of animal origin are overwhelmingly used for edible purposes. In many regions of the world, animal-derived lipids constitute the bulk of the 20 to 50% of dietary calories that are typically obtained from fats. Although the recommended range for dietary fat intake is 25 to 35% of total calories, the typical Western diet today can contain as much as 40 to 50% fat. Much of this dietary fat is of animal origin and is not always optimally balanced in terms of overall nutrition, especially with regard to its fatty acid profile. Therefore, in addition to efforts to decrease overall fat consumption in the general population, there has been a great deal of interest in altering the chemical nature of these fats. This, in turn, has stimulated a large amount of research into the possibility of manipulating animal lipid compositions by a wide range of techniques that include dietary modification, conventional genetic selection, genomics and marker-assisted selection, and genetic engineering.

Animal lipids can be divided into two major categories, namely structural and storage lipids. As with plants, all animal cells contain membrane lipids and various nonstructural lipidic components, in addition to their pools of storage lipids (Murphy, 2001). The membrane lipids normally consist mainly of phospho- and glyco-based diacylglycerols and cholesterol, although sphingosine-based and other minor lipids are also found in certain parts of the body, particularly in the nervous tissue. The less abundant, nonstructural, nonstorage lipids include certain classes of hormone and other mediators, such as eicosanoids and steroids. The acyl compositions of the bulk membrane lipid are relatively restricted in order to maintain the optimum fluidity of cell membranes. This, in turn, is regulated by the complex mechanism of homeoviscous adaptation (Sinenski, 1974; Hazel, 1995; Scislowski et al., 2004). This means that there is little scope for the deliberate modification of such membrane and nonstructural lipids in animals without the possibility of potentially deleterious physiological side effects.

In contrast to membrane lipids, it appears that the acyl composition of most animal storage lipids can often be modified without adverse consequences (although note the new findings described in the next section that may qualify this assumption). These storage lipids are the major source of animal-derived dietary fat and are found in the various types of adipose tissue as well as in other products, such as milk. Storage lipids are mostly made up of

triacylglycerols, they play no part in membrane function, and they, therefore, are subject to fewer constraints over their acyl composition than other classes of lipid. Hence, most efforts to manipulate the composition of animal lipids have been focused on the adipose and mammary tissues of domesticated mammals and, more recently, on some of the increasingly overexploited marine resources including fish, such as cod, salmon and tuna, and invertebrate seafood, such as prawns, molluscs and crabs. We will now examine some of the ways in which animal lipids are being manipulated in the three major classes of dietary product, namely the flesh of domesticated animals (including meat and fat), milk, and marine oils.

11.8.3.1 Animal flesh (see also Section 2.4)

Animal flesh is defined as the "soft tissue between the skin and bones," but excludes blood and other bodily fluids. The majority of the lipid in animal flesh is normally present in the storage adipose tissue, which may either be concentrated in specific fatty deposits or dispersed throughout the various organs. Smaller amounts of lipid, but still significant in terms of diet, are also present in other tissues, such as the heart, liver, striated muscle, and the various types of endocrine and nervous tissue. Lipids from the meat component of animal flesh can be a particularly important dietary component, especially in wealthier countries, where they typically make up about a quarter of total fat intake. The adverse effects of specific animal lipids, such as cholesterol and saturated fatty acids, have been much debated in both biomedical and popular forums. The perception that such dietary lipids may not have optimal compositions for long-term health has led to a great deal of interest in manipulating the amount and composition of adipose tissue in domesticated animals that are sources of edible flesh-derived products, such as meat, lard, offal and all the many derivatives thereof.

The amount and location of adipose deposits varies according to the genotype, diet, and lifestyle of the animal. The fatty acid composition can also vary to some extent and can be especially affected by diet, as shown in Table 11.26. Hence, the meat (i.e., muscle tissue) of a particular domesticated animal can be relatively lean or fatty, and can contain greater or lesser amounts of saturated fatty acids. These variations in acyl composition are generally determined by a combination of the genetics and environment for a particular animal. It is now becoming increasingly clear that adipose tissue, and especially individual adipocytes, can be highly dispersed among the cells of other tissues where the adipocytes may have more dynamic functions than as mere long-term lipid stores (Pond, 1999). For example, it has been shown that adipocytes interact closely with elements of the immune system, and especially lymph nodes (Pond, 2005). Such specialised adipocytes supply lipids to the immune system and buffer it from fluctuations in dietary lipid intake. This recently recognised and much more dynamic role for adipose tissue in animals may complicate some of the more radical efforts to modify its acyl composition, e.g., by expressing some of the very long-chain polyunsaturates (VLCPUFAs) that are normally found in marine oils. In the future, it will be important to clarify in more detail the role of nonmembrane lipid pools, including triacylglycerols, in the everyday function of all animal tissues and organs. This will then inform efforts to manipulate the acyl composition of such lipids without incurring unwanted physiological side effects in the animals concerned.

11.8.3.1.1 Dietary manipulation

Dietary manipulation can be used to modify the lipid composition of both the lean and the fatty tissue of animal flesh. It is well known that the inclusion of rations rich in ω-3 or ω-6 polyunsaturates can enhance the amount of VLCPUFAs in both membrane and storage lipids of cattle (Scollan et al., 2001). Hence, cattle fed on

TABLE 11.26 Fatty acid composition of depot (storage) fats of farm animals under different husbandry conditions (% w/w total fatty acids)

Fatty acid	Pig					Poultry		Cattle			Lamb	
	A	B	C	D	E	F	G	H	I	J	K	L
Myristic (14:0)	1.4	1.4	1.1	1.4	2.9	0.8	1.2	3.3	3.4	–	3.3	4.3
Palmitic (16:0)	28.7	28.5	20.9	21.0	27.7	26.9	21.8	26.0	23.9	19.9	20.9	18.7
Palmitoleic (16:1)	2.5	2.5	2.6	3.8	3.8	8.8	5.0	9.4	10.6	–	4.3	6.0
Stearic (18:0)	15.4	15.4	11.6	16.5	12.9	7.0	6.4	8.2	6.9	9.6	19.6	16.4
Oleic (18:1)	43.3	41.8	45.5	53.7	44.7	44.7	26.7	44.7	48.7	33.3	41.1	36.5
Linoleic (18:2)	9.3	10.7	16.2	3.1	8.9	11.2	35.2	2.1	1.5	23.1	5.1	11.5

Note: A: Low-fat cereal-based diet (Wiseman, 1984); B: Selected for lean carcass, low-fat cereal-based diet (Wiseman, 1984); C: High-fat diet containing soybean oil (Brooks, 1971); D: High-fat diet containing tallow (Brooks, 1971); E: Diet containing 250 ppm copper (Christie and Moore, 1969); F: Low-fat cereal-based diet (Bartov et al., 1974); G: High-fat diet containing soybean oil (Bartov et al., 1974); H: Diet based on hay (Wiseman, 1984); I: Diet based on barley (Wiseman, 1984); J: Diet containing "protected" safflower oil (Cook et al., 1972); K: Cereal-based concentrate diet (Astrup and Nedkvitne, 1975); L: Concentrate diet supplemented with "protected" soybean oil (Cook et al., 1972).

fresh grass or silage tend to accumulate more ω-3 or ω-6 polyunsaturates than do cattle fed on pelleted rations that have lower levels of these fatty acids. It is also possible to boost ω-3 or ω-6 polyunsaturate levels by supplementing rations with sources rich in ω-3 acids, such as linseed oil, or VLCPUFAs, such as marine oils or microalgae. On the other hand, levels of monounsaturates in muscle and fat tissue can be enhanced by feeding rations enriched in oleate and linoleate, e.g., from genotypes of sunflower or safflower that are specifically enriched in these fatty acids (Bolte et al., 2002; Hristov et al., 2005). Similar strategies of dietary manipulation have been used effectively in other domestic animals, including sheep and poultry (Boles et al., 2005; Lopez-Ferrer et al., 2001).

One of the problems of the dietary manipulation strategy, especially in ruminants, is the tendency for the dietary lipids to become biohydrogenated during their passage through the gut of the animal. This leads to the absorption and assimilation into muscle and adipose tissue of more saturated acyl lipids than were included in the original diet, which can negate the whole purpose of the exercise. This difficulty can be addressed to some extent by "protecting" the dietary fatty acids, e.g., by encapsulating the oil in formaldehyde-treated protein or a similar preparation (McDonald and Scott, 1977; Chilliard et al., 2003). It has also been found that, in the case of dietary VLCPUFA supplementation, biohydrogenation is less of a problem if marine algae are used as the source of these fatty acids in animal rations, rather than preparations made from fish oils (Cooper et al., 2004).

However, care must be taken in using diet to change the acyl composition of meat or adipose tissue in too radical a fashion, lest it result in unforeseen adverse consequences. It is fairly obvious that excessive changes to the acyl composition of the highly sensitive membrane phospholipids should be avoided. But it is also now recognised that storage lipids in adipose tissues sometimes respond to dietary manipulation in surprising and possibly undesirable ways. For example, recent research with animal models has shown that the inclusion of dietary sunflower seed oil led to an increase, while fish oil rations led to a decrease in numbers of dendritic cells in lymph nodes where adipose depots were associated with lymphatic tissue under chronic mild inflammation (Mattacks et al., 2004). The long-term consequences of such changes in dendritic cell behaviour are unknown and obviously merit further research, but in the meantime this example should highlight the possibility of unexpected side effects in what has often been assumed to be a relatively mundane and safe practice. In summary, the manipulation of dietary lipids is often used to alter the useful tissues from animal flesh. In the future, there may be more scope for further modifications by this method, although more research is needed before the practice can be made more precise and less empirical. In the meantime, this is a rapid and cost-effective technique that can be readily applied to virtually any species or domestic breed of animal.

11.8.3.1.2 Genetic manipulation

An alternative but longer term strategy for the production of modified lipids in animal flesh is to breed new genotypes that will accumulate the desired fatty acids without the need for some of the more expensive supplementary rations or free-range husbandry. This can be done by conventional breeding, providing there is sufficient variation for the traits of interest. But, in the many cases where there is not enough existing variation, more radical approaches, such as genetic engineering, are now possible (Maga, 2005). Perhaps surprisingly, the first example of the transfer of a gene to produce a new genetically engineered animal dates back over 2 decades to 1982, when a transgenic mouse with enhanced growth rates was created (Palmiter et al., 1982). During the past 2 decades or so, there has been much progress in the research underlying the formation of transgenic livestock, but this has yet to be translated into mainstream commercial reality, particularly in the case of lipid-related phenotypes. However, experimental results over the past few years suggest that such genetically engineered animals may be fast becoming a practical proposition.

For example, it appears that lipid composition of most bodily tissues, including meat and fat, can be modified by the insertion of one or a few transgenes. Hence, in the case of a mouse model, the transfer of a roundworm (*Caenorhabditis elegans*) gene encoding an ω-3 fatty acid desaturase can result in appreciable decreases in the ratios of ω-6: ω-3 fatty acids in all tissues and organs that were examined (Kang et al., 2004). Unfortunately, the actual fatty acid compositions of the transgenic animals were not reported in this case, so it cannot be ascertained whether the effects are dramatic enough to justify use of this strategy in commercial breeding. However, if the work can be extended to cattle, it may enable the future production of a whole range of animal products, including meat, milk, and eggs, that are enriched in desirable ω-3 fatty acids. This could be achieved by dietary supplementation using relatively inexpensive vegetable oils rather than the more costly microalgae or fish oils.

In addition to producing transgenic livestock, breeders are interested in regenerating exact clones of the best examples of a particular genotype. This would ensure high levels of uniformity and consistency in the flesh of such animals, which are characteristics that are highly desired by the food industry. Alongside the genetic engineering of livestock to carry novel genes, the most significant advance over recent years has been the production of genetically identical clones of adult animals. Plant breeders, horticulturalists, and domestic gardeners have taken advantage of clonal propagation for many centuries, but no adult animal was successfully cloned until the advent of Dolly the sheep in 1997 (Campbell et al., 1997). One of the concerns among scientists and public alike, concerning the cloning of domesticated livestock, is the potential for unforeseen

abnormalities. For example, there have been reports of premature aging and other undesirable side effects in some of the more high-profile cloned animals, including Dolly the sheep herself. In Dolly's case, these concerns were unfounded as it has been reported that the animal died from a relatively commonplace lung disorder rather than from any direct consequence of its origin as a clone (Rhind et al., 2004). More recently, a systematic study of more than 100 parameters relating to the meat and milk composition of commercially cloned beef and dairy cattle has shown no greater variability in these animals than normal industry standards from conventionally bred cattle (Tian et al., 2005). These data, and similar studies from other forms of livestock, give confidence that cloning *per se* will not introduce additional unwanted variation into meat and dairy composition. However, the challenge still remains in trying to engineer the desired lipid modification in the animal to be cloned in the first place.

11.8.3.2 Milk (see Section 2.4 and Section 2.7)

Bovine milk and its various derived products, such as yoghurt, cheese, and butter, are a major source of dietary lipid in many countries. Over the past 20 years, consumption of dairy products has declined somewhat, but they still form 20 to 30% of the fat intake of the average North European and North American diet. The typical acyl composition of milk fat deviates significantly from what is commonly regarded by nutritionists and the food industry as the ideal. For example, the ratio of saturates:monounsaturates:polyunsaturates is 60:35:5, whereas a more desirable ratio would be more like 8:82:10 (Beitz et al., 1995). One option is for individuals to attempt to reduce their consumption of dairy products, and indeed the average individual intake of direct milk products, such as butter and cheese, has declined appreciably over the past few decades. However, many dairy products are also used less visibly in the formulation of a host of other foods, and especially in processed food items, where the average consumer is less likely to be aware of their presence. It may not always be straightforward to find suitable substitutes for dairy products in many of these formulations. In addition, many countries have long-standing and efficient dairy industries that have well-established distribution chains and often wield considerable political/economic influence.

These commercial realities provide an incentive for researchers to attempt the manipulation of milk fat composition so as to better suit current nutritional standards, rather than seeking to reduce or even displace dairy products altogether from their current important place in the food chain. As with the manipulation of animal flesh, milk composition can be manipulated either by dietary or genetic methods. However, because milk fat is produced by mammals simply as a postpartum energy source for their offspring, its manipulation should, in principle, be more straightforward than that of the other forms of animal lipid considered above, where the lipids may have more direct roles in cellular structure and function.

11.8.3.2.1 Dietary manipulation

It is well known that the acyl composition of milk in all mammals, including humans, can be strongly influenced by dietary lipid intake in the lactating female. For example, the inclusion of 5% soybean oil in the rations of Holstein cattle in feedlots led to an increase in milk monounsaturates from 25.8 to 40.8% and a decrease in saturates from 66.8 to 51.4%, compared to nonsupplemented controls (Hippen et al., 1995). In addition, the dietary oil supplementation resulted in an overall reduction in the yield of fat in the milk. As discussed above, this is a desirable outcome given the global glut of milk fat and the demand from consumers for lower total fat, but an improved acyl composition in the remaining milk fat. Many other forms of dietary supplementation with lipid products have been tried, often with promising results. One potentially useful method being investigated is to use partially processed oilseeds in cattle rations, rather than the extracted oil. Although this is a comparatively cheap approach, it was not as effective in increasing unsaturated fatty acid levels in milk as the more expensive supplementation with plant oils, particularly if the latter were protected by a coating such as the modified protein formulation discussed in Section 11.8.3.1.1. In one case, cows fed protected lipid supplements containing linseed oil and safflower oil produced milk containing greater than 30% linoleic (ω-6) and 20% linolenic (ω-3) acids. In contrast, milk from control cows contained about 2% and 1%, respectively, of these fatty acids (Kennelly and Glimm, 1998).

The use of fish oils in cattle rations is one of the most expensive options, but it can have dramatic effects on both milk composition and fat yield. These effects are not only due to the assimilation of the ingested oils and their direct conversion into milk fat. It has been found that fish oils, particularly when supplied as glutaraldehyde-protected microcapsules, can cause changes in the expression of lipid-related genes in the mammary tissue. This depression of gene expression can cause a decrease in the synthesis of short-chain fatty acids and a decline in overall lipid accumulation. In one study, consumption of protected fish oil decreased the abundance of the mRNA of several key lipogenic enzymes in the mammary gland. Acetyl-CoA carboxylase, fatty acid synthase, and stearoyl-CoA desaturase mRNAs for cattle given 3% protected fish oil averaged only 30, 25, and 25% of control values, respectively (Ahnadi et al., 2002). The result of these changes in gene expression was a reduction of overall milk fat content from 36.0 g/kg in controls to only 20.4 g/kg in cows fed with the 3% fish oil supplement. The dietary significant components of fish oils are the VLCPUFAs, such as DHA and EPA. The use of a 3% fish oil supplement can increase such fatty acids (i.e., 20:4, 20:5, 22:5, and 22:6) from a control level of

< 0.5% to as much as 10% of milk fatty acids (Donovan et al., 2000).

In addition to the VLCPUFAs, other milk fatty acids, such as conjugated linoleic acids (CLA) and butyric acid, are now being recognised for their nutritional potential. The biologically active isomer of CLA is believed to be the *cis*-9, *trans*-11 form. This fatty acid has been associated with a variety of biological effects including anticarcinogenic activity, antiatherogenic activity, the ability to reduce the catabolic effects of immune stimulation, enhancement of growth promotion and ability to reduce body fat (Parodi, 1999). Butyric acid has been shown to inhibit cell proliferation and to induce differentiation in a broad spectrum of cancer cell lines including those of the breast and colon (Parodi, 1999). This has led to much interest in ways of enhancing the accumulation of such fatty acids in milk fat. Most attention has been focused on CLA and its possible manipulation via supplementation with dietary vegetable oil. Typical CLA amounts in bovine milk are in the range of 3 to 6 mg/g, although this can vary considerably in different breeds (Kelly and Bauman, 1996; Reil, 1963). However, addition of vegetable oils increased CLA levels to as much as 24.4 mg/g (Kelly et al., 1998). More nutritionally favourable acyl composition in milk lipids can also be obtained by open pasturage of dairy cattle, but this requires careful management of the forage system. For example, milk CLA levels may be affected by the grass cultivar grazed by the animal and also by the presence of other forage species, such as clover and lucerne. In the future, there is probably a place for all of these dietary manipulation strategies according to whether the stress is on low cost effectiveness at manipulating acyl quality, or long-term consistency of milk composition. The other option is to modify the dairy animals themselves in order to produce a more nutritionally desirable (for humans) form of milk.

11.8.3.2.2 Genetic manipulation

It is already well known that milk composition can be manipulated by genetic engineering. For example, in 2005, the creation of transgenic cattle resistant to mastitis, a serious disease of the mammary gland, was reported (Donovan et al., 2005). In this case, the cattle were engineered to express the antimicrobial peptide, lysostaphin, in their milk-producing epithelial cells. The peptide accumulated in their milk in sufficient quantity to kill the *Staphylococcus aureus* bacteria responsible for mastitis, but without compromising the nutritional quality of the milk. This and similar achievements have encouraged efforts to modify milk lipid composition by genetic methods, both transgenic and conventional.

There are two different targets for genetic manipulation of milk lipids. First to reduce the overall fat content of liquid milk and, second, to alter the acyl profile of milk fat to suit nutritional requirements of consumers, inasmuch as these are currently understood. The first target has come about due to the increasing problem of dealing with surplus milk fat in the dairy industry. The proliferation of low-fat or no-fat dairy products has created an unwanted amount of milk fat that cannot be absorbed by traditional outlets, such as cheese or butter production (Bobe et al., 1995). One of the challenges in trying to reduce milk fat content while maintaining the quality of the nonfat components is the strong genetic linkage between fat and protein yield in cattle. However, there are indications that by selecting against specific (and relatively undesirable) fatty acids, such as stearate, and towards the main protein (i.e., β-casein), this genetic linkage might be broken (Bobe et al., 1995). In this way, it may be possible to use conventional breeding methods, possibly supplemented by advanced tools, such as marker-assisted selection, to manipulate milk fat yields.

The manipulation of the acyl composition of milk fat may be addressed to some extent by conventional breeding, but there is also a great deal of interest in using transgenic techniques to create addition variation, rather than relying solely on existing variation in the relatively restricted gene pool of domesticated cattle. The technology for the insertion of transgenes into cattle is now well established (Reh et al., 2004) and, as discussed above, the production of clonal copies of single animals appears to raise no significant new safety concerns (Tian et al., 2005). The next potential hurdle is to establish whether the addition of single or small numbers of transgenes can actually result in a sufficiently large shift in acyl composition to make the technique commercially viable. This is by no means a trivial undertaking. In the case of oil crops, it has proven to be difficult to achieve commercially worthwhile levels of desired fatty acids because such traits appear to be regulated by numerous genes, i.e., acyl composition appears to be a quantitative character rather than a simple Mendelian trait (Voelker et al., 1996).

Fortunately for milk biotechnologists, data from preliminary experiments indicate that, at least in the case of stearoyl-CoA desaturase, the addition of a single transgene may lead to useful modifications to milk lipid composition. For example, the expression of a rat stearoyl-CoA desaturase gene under the control of a mammary gland-specific bovine β–lactoglobulin promoter led to the accumulation of elevated levels of monounsaturates in the milk of transgenic goats and mice (Reh, 2002; Reh et al., 2004). Another approach has been to over express genes encoding milk proteins, such as β-casein and κ-casein, in order to reduce the ratio of fat:protein in the final milk (Brophy et al., 2003). These studies encourage the view that it is likely to be feasible to use genetic engineering to alter the amount and quality of lipid in milk.

11.8.3.3 Fish oils

So-called "fish oils" are differentiated by relatively high levels of nutritionally desirable VLCPUFAs. As discussed above, there are numerous efforts to produce

VLCPUFA-enriched animal and plant lipids, either by dietary manipulation or by genetic modification (e.g., Donovan et al., 2000; Wu et al., 2005). Of course, such VLCPUFA-rich fish oils can also be obtained by including fish themselves in our diet. The current recommendation from the U.K. Food Standards Agency is that oily fish should be a regular part of the diet and that, apart from pregnant women, as many as four portions per week are beneficial to health (http://www.foodstandards.gov.uk). Oily fish include salmon, trout, mackerel, sardines, pilchards, herring, kipper, eel and whitebait. Due to overfishing and other factors, stocks of many of these fish are dwindling and they are becoming more expensive. This has led to the development of offshore and inland fish farms (Sargent and Tacon, 1999). One of the challenges to the fish farmer is to provide a sufficient supply of dietary VLCPUFA for his fish. If naturally occurring microalgae are not present in sufficient quantity, the fish diet will require supplementation with alternative rations, such as powdered microalgae or cyanobacteria.

Farmed Salmonidae are also frequently deficient in a class of dietary carotenoids, known as xanthins. The absence of xanthins can leave their flesh with a pallid and unattractive appearance. Because they are unable to forage for their normal xanthin-rich food, farmed fish are unable to accumulate xanthins, which are important for their growth and development (Torrissen and Christiansen, 1995). Xanthins and other carotenoids are useful nutrients in the human diet where they act as antioxidants and serve as sources of vitamin A (Miki, 1991). In order to ensure the adequate development of farmed Salmonidae, dietary supplementation with astaxanthin is required. This xanthin can be produced by biotechnological fermentation using the microalga *Haematococcus pluvialis* or the pink yeast *Xanthophyllomyces dendrorhous*, or it can be synthesised chemically (McCoy, 1999).

11.8.4 Microbial lipids

Although most of the economically important lipids are derived from plants and animals, microbial organisms are an increasingly useful source of a range of lipid-based feed stocks for both edible and nonedible applications. In most cases, microbial lipid production involves a biotechnological fermentation process in sealed environmentally controlled vessels, although some microbes, such as certain microalgae and fungi, can also be cultured in more open environments, e.g., in ponds or growth chambers. Metabolic manipulation and genetic modification of many microbial organisms is often more straightforward than the more complex plants and animals, and this has encouraged research into the alteration of lipid composition in some microbial species. As we shall see below, the major hurdle facing the exploitation of microbial lipids is the economic feasibility of their production, rather than technical hurdles in their manipulation.

11.8.4.1 Fungi and microalgae (see also Section 2.11 and Section 2.12)

The fungi include a huge range of organisms from the simplest unicellular yeasts and facultatively colonial slime moulds, to the more complex multicellular mushrooms. Most of the fungal and yeast species of interest in lipid production are unicellular species that are cultivated via controlled fermentation processes. For several decades there has been interest in growing either native or genetically engineered strains of various fungi as sources of useful lipid feed stocks. However, the high costs of such biotechnological production means that only relatively high value commodities can be manufactured by this route. One of the most potentially valuable products of such microbial biotechnology would be a cocoa butter substitute. Increasing quantities of chocolate products or chocolate-like coatings are now manufactured from a mixture of palmitate, oleate, and stearate derived from sources other than the cocoa fat from the plant, *Theobroma cacao*. During the early 1990s, there were several attempts to develop such a process using yeasts, such as *Yarrowia lipolytica*. Although they were technically successful, these efforts ended as commercial failures, mainly due to a sustained fall in the price of cocoa butter on the world market at the time (Ratledge, 1993). More recent efforts to produce cocoa butter substitutes have focused on using improved yeast strains and cheaper growth media (for example, Papanikolaou et al., 2001, 2003).

Other possible microbial lipid products include polyunsaturated fatty acids of both the ω-6 and ω-3 series; these can be generated by using either bacteria, fungi, or microalgae. Certain microorganisms also produce prostaglandin precursors, and even prostaglandins themselves, as well as cerebroside lipids and other unusual lipids that are not normally regarded as being of microbial origin. According to a recent text on microbial lipid production, "*The prospects of producing a complete range of PUFA* (are) *within our grasp*" (Cohen and Ratledge, 2005). Among some of the microbial systems that are under development in labs across the world using modern molecular genetic and biotechnological methods are the following:

- *Mortierella alpina* (a yeast) — to produce arachidonic acid
- *Schizochytrium* spp (marine algae) — to produce docosahexaenoic acid
- *Ulkenia* spp (marine algae) — to produce docosahexaenoic acid
- *Crypthecodinium cohnii* (a heterotrophic dinoflagellate) — to produce docosahexaenoic acid
- *Nitzschia laevis* (a marine alga) — to produce eicosapentaenoic acid (Wen and Chen, 2003)

- *Shewanella* spp (marine bacteria) — to produce eicosapentaenoic acid (Yazawa, 1996; Bowman et al., 1997)

These microbial production systems may well be a future source of high-value lipids, especially for the health-related market for so-called "nutraceuticals." In the case of cocoa butter substitutes, their success will depend largely on their ability to compete with other mainly plant-based sources of such fatty acids. In the case of VLCFUFA oils, the microbial products may be able to compete effectively with fish oils as the latter become more expensive as stocks become depleted. A further opportunity may arise from the rise of fish farming because such fish may require additional dietary supplementation with VLCPUFAs, as they do already in the case of xanthins. In the longer term, the main threat to microbial lipid production via fermentation may come from genetically engineered crops and livestock that will be able to accumulate VLCPUFAs as part of their normal development and without any dietary supplementation. However, this prospect may still be several decades off and, even then, might not prove to be commercially viable. In the meantime, therefore, microbes will merit continuing attention as potentially useful lipid production systems.

11.8.5 Herbicides that affect acyl lipid metabolism

Some of the most effective modern herbicides are those which interfere with carotenoid formation (Bramley, 1991). These chemicals kill plants because of the chlorophyll photooxidation induced by the lack of carotenoids. However, these "bleaching" herbicides will not be dealt with here, where our attention is focused on chemicals that are known to inhibit acyl lipid synthesis.

Substituted pyridazinoes (Figure 11.15) were originally developed as potential photosynthetic electron transport inhibitors. However, it was soon discovered that many of them also reduced the formation of thylakoid pigments (both chlorophylls and carotenoids). The mechanism of phytotoxicity has been discussed, as well as the conversion of the herbicide to more toxic metabolites (St. John, 1982). In some cases, it was noted that certain herbicides also inhibited fatty acid desaturation (e.g., Hilton et al., 1971). Sandoz 6706 and Sandoz 9785 were both noted to reduce α-linolenate levels, with a commensurate rise in linoleate (see Harwood, 1991b). This action suggested that substituted pyridazinones could inhibit Δ15-desaturation, and that proposal was confirmed by measurement of desaturase activity using radiolabelled precursors (Murphy et al., 1985; Davies and Harwood, 1983). Sandoz 9785 is notable in that its only effect seems to be on Δ15-desaturation and, then, at the level of monogalactosyldiacylglycerol (Norman and St John, 1987) rather than phosphatidylcholine (see

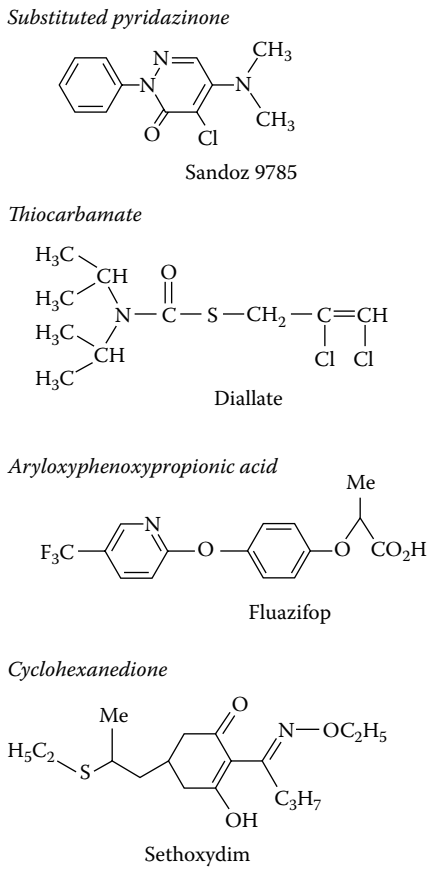


FIGURE 11.15 Structures of some herbicides that affect fatty acid synthesis.

Section 10.1.4). Other substituted pyridazinones affect additional metabolic activities and different plants show various susceptibilities (see Harwood, 1991a,b). In particular, algae do not show such clear-cut effects as higher plants (e.g., Murphy et al., 1985; Henderson et al., 1990). Nevertheless, substituted pyridazinones have been used to study pathways for the production of long-chain and very long-chain (>18C) polyunsaturates (see Khozin-Goldberg et al., 2002).

The carbamate herbicides, which are normally applied preemergence, exert their herbicidal action at early stages of seedling growth (Fuerst, 1987). Several thiocarbamates (see Figure 11.15), including EPTC, diallate, CDEC, and triallate, have been reported to inhibit lipid metabolism. These effects, which are summarised by Harwood (1991b), include alterations in cuticular wax formation, primary alcohol content and the level of very-long chain fatty acids. The action (and important herbicidal effect) of thiocarbamates on the surface layers of susceptible plants has a rationale in that these chemicals have been shown to be rather specific inhibitors of fatty acid elongation *in vivo*. Since very long-chain fatty acids are the precursors of many components of wax, cutin, and suberin (see Section 1.2.11, Section 1.2.12, and Section 2.5), it is easy to understand their action. Actually, work with subcellular

fractions from peas (Abulnaja and Harwood, 1991b) and with safening agents (Abulnaja et al., 1992) suggests that it is the herbicidally active sulfoxide derivative of thiocarbamates (Baldwin et al., 2003) that is the specific inhibitor of fatty acid elongation. Direct measurement of I_{50} values for specific elongation reactions confirmed this conclusion (Barrett and Harwood, 1993).

Another herbicide that has been reported to interfere with wax formation (Duncan et al., 1982) is ethofumesate. Experiments with this compound were also consistent with its action on waxes due to inhibition of fatty acid elongation (Abulnaja et al., 1992).

Two important classes of selective (grass-specific) herbicides are the aryloxyphenoxypropionates and the cyclohexanediones (see Figure 11.15). The action of these compounds on lipid metabolism has been reviewed thoroughly (Harwood, 1991a; Hoppe, 1989). The herbicides affect acetyl-CoA carboxylase in the *Graminae,* but are without action (except at exceptionally high concentrations) on the same enzyme in other monocotyledons or in dicotyledons (see Harwood, 1991b). Early experiments to investigate tolerance in some species found that detoxifying metabolic reactions were responsible. Thus, wheat is tolerant to diclofop because of hydroxylation of the herbicide (Walker et al., 1989; Hoppe, 1989). However, in the increasing number of cases of acquired resistance that are being found, mutation of the target enzyme, acetyl-CoA carboxylase, is usually involved (see Harwood 1999; Price et al., 2004).

Examination of the kinetics of acetyl-CoA carboxylase inhibition by aryloxyphenoxypropionates and cyclohexanediones has shown conclusively that it is the carboxyl transferase partial reaction (see Section 10.1.2) that is sensitive (Rendina et al., 1989). Competition experiments have shown that the two classes of herbicide bind to the same site on the carboxyltransferase (Rendina et al., 1989; Burton et al., 1991). The interaction of graminicide with acetyl-CoA carboxylase isoforms has been studied in some detail (e.g., Herbert et al., 1996). Only the chloroplast isoform of Gramineae appears significantly sensitive (Harwood, 1999) and neither the cytosolic isoform of plants in general or the chloroplast isoform of acetyl-CoA carboxylase in dicotyledons (Alban et al., 1994) is inhibited significantly. Studies with purified isoforms revealed a correlation between the binding of graminicides and the reaction kinetics in sensitive vs. insensitive enzymes (Price et al., 2003).

Several other herbicides have been reported to alter lipid metabolism (see Harwood, 1991b), although in most cases this is due to secondary effects following a more acute action elsewhere in metabolism.

11.8.6 Xenobiotic lipids

Xenobiotic (or foreign) compounds are a diverse group of chemicals that include drugs, pesticides, food additives, and a variety of environmental pollutants. Many xenobiotic compounds are lipophilic and may partition into the fatty tissue of an animal or plant, DDT being a well-known example (Morgan and Roan, 1974). The metabolism of such compounds is usually orchestrated in such a way as to make them less hydrophobic and, therefore, easier to excrete. The reactions that accomplish the transformation are classified into phase I, where reactive functional groups may be introduced or exposed, and phase II, where the product of phase I can be conjugated with another group, such as an amino acid, sugar, glucuronic acid or sulfate (Caldwell, 1986).

Not all xenobiotic compounds behave so conveniently all the time, however. An increasing number are now known to form xenobiotic lipids as a result of being recognized by an enzyme or set of enzymes involved in the biosynthesis of natural lipids. As a simple working definition, *xenobiotic lipids* result when xenobiotic compounds act as substrates for enzymes working in the direction of lipid biosynthesis. In other words, they are all covalently modified xenobiotic compounds that resemble naturally occurring lipids; simple physical association with a lipid phase does not qualify. This definition also excludes compounds that result from degradation by lipid-metabolizing enzymes (for instance, both ω-phenyl fatty acids and some herbicides are known to be degraded by enzymes of β-oxidation (Cole, 1983), but includes any esters formed by the action of a so-called reverse lipase. Modification of double-bonded lipids by free radicals also falls outside the definition and is covered elsewhere (see Section 8.1).

A number of general reviews cover the whole topic of xenobiotic lipids (Caldwell and Marsh, 1983; Caldwell, 1985; Fears et al., 1984; Hutson, 1982; Niemann, 1990; Quistad and Hutson, 1986). Further information may be obtained by reference to these reviews; additional references are only included where the topic is not covered adequately in the relevant review (see also Dodds, 1995).

11.8.6.1 Xenobiotic fatty acids

This simplest class of xenobiotic lipid occurs when a xenobiotic carboxylic acid is elongated, two carbons at a time, to form a xenobiotic fatty acid. There are a few examples in the literature of herbicides being so elongated by plants. The most dramatic example of an acid being elongated by more than two carbons is cyclopropanecarboxylic acid (itself a metabolite of the miticide cycloprate), which can be converted by rat tissues into xenobiotic fatty acids 10, 12 or 14 carbons longer than the precursor. The obvious mechanism for such elongation involves the enzyme fatty acid synthase (see Section 10.1.1), an enzyme that is known to accept a variety of primers. This supposition is borne out, to some extent, by the products of chain elongation. For example, the elongated metabolite of benzoic acid has a hydroxyl group in the 3 (or β) position; 5-(4′-chlorobutyl)-picolinic acid forms enoyl and keto acid derivatives as well as the new

saturated acid. All these metabolites represent the predicted intermediates in the fatty acid synthase pathway. There has been no account of toxicity resulting from xenobiotic fatty acids and some would appear to undergo normal conjugation, to glycine, for instance. Only in the case of the ω-cyclopropyl acids do more complex xenobiotic lipids appear to result. Figure 11.16 shows some of the compounds involved and further examples can be found in the reviews by Hutson (1982) and Caldwell (1985).

11.8.6.2 Fatty acid esters of xenobiotic alcohols

It is tempting to refer to this group as "xenobiotic wax esters" because of their analogy to one class of natural waxes (see Section 1.2.11 and Section 2.5). In general terms, these conjugates occur when the hydroxyl group of a xenobiotic compound, or its phase I metabolite, reacts with a natural fatty acid to form an ester. Probably the simplest examples of the class are the ethyl esters (at least, in as much as ethanol may be regarded as xenobiotic), which have been detected in brain tissue from humans (Laposta et al., 1987). There are several other much more elaborate xenobiotic alcohols now known to form esters of this sort; they are recorded in the general reviews on xenobiotic lipids (see above) and in one specialised review (Chang et al., 1986).

Apart from ethanol, there have been reports of fatty acid esters of a number of halogenated pollutants, including 2-chloro-, 2-bromo-, and trichloroethanol, pentachlorophenol, and 3-chloro-1,2-propanediol (glycerol chloride). Diacyl derivatives of the latter are particularly interesting because of their similarity to natural glycerolipids. Codeine, 7-OH-tetrahydrocannobinol, phencyclidine, dipyridimol, etofenamate, plaunotol, chlorambucil and metabolites of ivermectin and DDT are all commonly known as widely used (or abused) compounds that have been shown to form fatty acid esters in animal tissues *in vivo* or *in vitro*. Figure 11.17 shows the structures of a few of the compounds involved. Even the tumour-promoting phorbol diester, 12-tetradecanoylphorbol-13-acetate, can be further acylated at the 20 position (Roeser et al., 1991). This is a phenomenon that is not restricted to animal tissues; the best documented case occurring in plants is 2-naphthylethanol (a metabolite of an acaricide), which forms esters with several fatty acids in foliage and fruit from apple trees.

Reports of palmitate esters are more numerous than those of other fatty acids, but when more exhaustive analysis of metabolites formed *in vivo* or of specificity *in vitro* has been carried out, a wide variety of fatty acid esters have been found. Neither chain length nor degree of unsaturation appears to have much bearing. Many of the fatty acid esters have been detected *in vitro* using a microsomal preparation enriched with coenzyme A, ATP, and Mg^{2+} (Chang et al., 1986), strongly suggesting the involvement of an acyl-CoA acyltransferase, possibly acyl-CoA:cholesterol acyltransferase, with the xenobiotic alcohol substituting for cholesterol. Other reports have shown that cholesterol ester hydrolase (cholesterol esterase) is capable of esterifying the 2-haloethanols (Bhat and Ansari, 1990) and plaunotol. There have been various reports of ethanol acyltransferase activity in the literature (Polokoff and Bell, 1978; Mogelson and Lange, 1984), and these enzymes would also be candidates to esterify at least some of the xenobiotic alcohols.

A number of attempts have been made to determine whether fatty acid esters of xenobiotic alcohols have any pharmacological or toxic properties over and above those of the parent xenobiotic compound. Fatty acid ethyl esters can be found in membranes and they have been shown, by fluorescence anisotropy, to disturb the structure of those membranes (Hungund et al., 1988). Palmitoylpentachlorophenol causes selective tissue damage to the exocrine pancreas (Ansari et al, 1987). However, most of the studies conducted suggest that the properties of the conjugate are similar to those of the parent compound (codeine, 7-OH-tetrahydrocannabinol, hydroxy-DDT) or that formation of the conjugate deactivates the parent compound (phorbol diesters). If most of the conjugates produce effects similar to the precursor, they usually do so in a more protracted manner. The results are consistent with the compounds exerting their effects only after they have been released by hydrolysis of the conjugate. The effect of the conjugation is to render the compound more lipophilic, more likely to be stored in adipose tissue and, in consequence, slower to exert its effects and slower to be excreted. It is this tendency to be stored and even

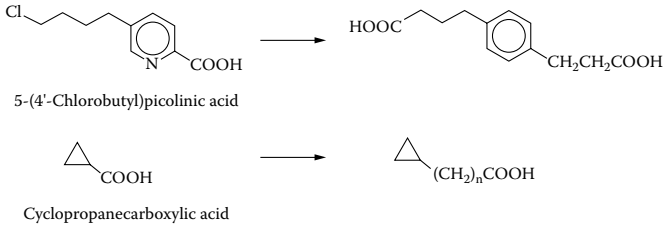
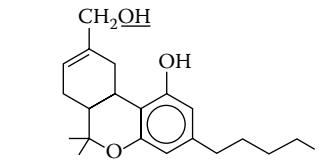
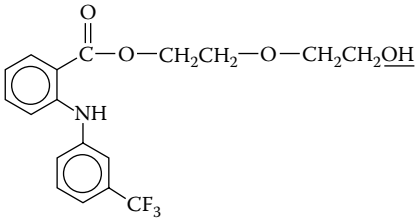


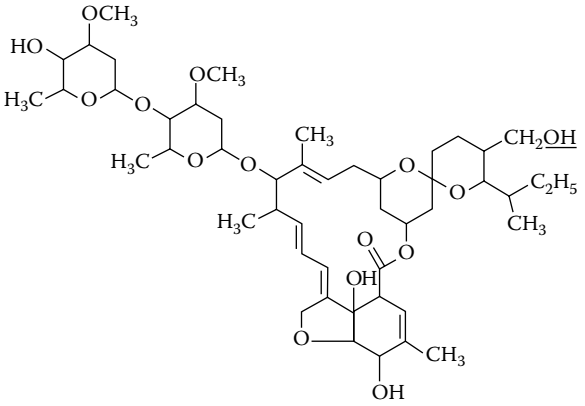
FIGURE 11.16 Examples of xenobiotic acids that undergo chain elongation. Chlorobutylpicolinic acid is elongated by two carbons at the 2-carboxylic acid group. The alkyl chlorine also undergoes oxidative metabolism to form the corresponding 5-(3′-carboxypropyl) side chain. Other metabolites are the 2-(2′-unsaturated) acid and the 2-(3′-keto) acid. Cyclopropanecarboxylic acid is elongated by the addition of several two-carbon fragments: n = 10, 12, or 14.



11-Hydroxy-Δ^8-tetrahydrocannabinol



Etofenamate



Hydroxy metabolite of ivermectin

FIGURE 11.17 Some xenobiotic alcohols that form esters with fatty acids. The alcohol group involved is shown underlined. 1-hydroxytetrahydrocannabinol forms esters with palmitic, stearic, oleic, and linoleic acids. Etofenamate forms esters with all of these and palmitoleic, myristic, and lauric acids as well. Oleic acid and others of shorter chain length have been found esterified with the ivermectin metabolite.

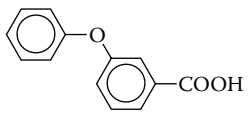
concentrated in adipose tissue that causes most general concern among toxicologists.

11.8.6.3 Esters of xenobiotic carboxylic acids

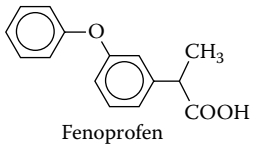
Analogues of natural lipids, mostly cholesterol esters or neutral acylglycerols, are formed when a xenobiotic acid is esterified to a free hydroxyl group on a natural acyl acceptor. Most of the published claims that over 38 different xenobiotic carboxylic acids can form such lipids are documented in a specialized review (Dodds, 1991), which also examines the evidence to support the claims. Hypolipidaemic drugs, anti-inflammatory drugs, various pesticides, food additives, and some environmental pollutants are all represented in the 38, and a sample of their structures in Figure 11.18.

11.8.6.3.1 Cholesterol esters

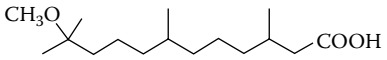
Although reports of xenobiotic cholesterol esters are less numerous than those of the following group, there are a



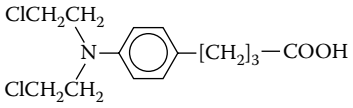
3-Phenoxybenzoic acid



Fenoprofen



11-Methoxy-3, 7, 11-trimethyldodecanoic acid



Chlorambucil

FIGURE 11.18 Three xenobiotic acids that form xenobiotic lipids: 3-phenoxybenzoic acid (a metabolite of pyrethroid insecticides) is incorporated into di- and triacylglycerol analogues; fenoprofen (a nonsteroidal antiinflammatory drug) also forms triacylglycerol analogues; and 11-methoxy-3,7,11-trimethyldodecanoic acid, a metabolite of the insect growth regulator, methoprene, is incorporated into di- and triacylglycerols as well as cholesterol ester analogues. Chlorambucil is a cytotoxic drug that forms cholesterol esters. Further examples are shown in Dodds (1991).

variety of structures that are involved. These include substituted benzoic acids, aryl-substituted short-chain acids, analogues of phytanic acid and halogenated acids. The enzymatic mechanism for the synthesis of the cholesterol esters has not been widely studied in spite of a tacit assumption that acyl-CoA:cholesterol acyltransferase may be responsible. In the two cases where studies have been reported, the acyl donor has not been the *S*-acyl coenzyme A derivative, but the precursor *O*-acyl ester. For chlorambucil, the precursor was its ester with prednisalone (itself an analogue of cholesterol), and there is evidence that the enzyme involved was lecithin:cholesterol acyltransferase. The acyl donor for the synthesis of the cholesterol ester of 3-(4-chlorophenyl) isovaleric acid is its precursor, fulvalinate. The enzyme responsible for the transfer appears to be highly specific *in vitro* for the acyl donor, if not the acyl acceptor.

The relatively high melting point of a number of xenobiotic cholesterol esters and consequent low fluidity appear to make them poor substrates for hydrolysis. This results in the accumulation of the ester of 1-(4-carboxyphenoxy)-10-(4-chloro-phenoxy)decane at its site of synthesis, an occurrence that has been implicated in its mode of action as a hypocholesterolaemic compound. Similary, the cholesterol ester of 3-(4-chlorophenyl)isovaleric acid forms crystalline structures in a number of tissues. The crystalline structures appear to be the focus for microgranuloma to form and constitute the first "xenobiotic-lipid

storage disease" to be reported. In the light of these findings, the formation of any xenobiotic cholesterol ester must be viewed with concern until such time as its mobilization can be demonstrated.

11.8.6.3.2 Neutral acylglycerols

Reports of xenobiotic acids being incorporated into analogues of triacylglycerol or diacylglycerol easily outnumber those of the other classes. The same general types of xenobiotic acids as encountered among the xenobiotic cholesterol esters are found in this group. One of the most interesting groups is the 2-substituted propanoic acids, which includes both the "profen" nonsteroidal, anti-inflammatory drugs and the "fop" herbicides (Section 11.8.5). Here, again, the reports derive from a number of different experimental approaches, including analysis of feral tissues, dosing of animals *in vivo,* incubation of whole cells with the acid *in vitro,* and enzyme assays using subcellular preparations.

There is still a shortage of information on the mechanism of synthesis of the xenobiotic acylglycerols. There are a variety of acyltransferase enzymes that are candidates, but, surprisingly, good evidence only for the involvement of monoacylglycerol acyltransferase has been forthcoming. When the acid was 3-phenoxybenzoic acid (3PBA), then activity was shown to require the prior formation of the CoA ester to be specific for 2-(as opposed to 1-) monoacylglycerol as acyl acceptor, and to show a similar distribution among tissues and species as the activity measured with a natural precursor including the very large increase in hepatic activity found during the suckling period in rats. There is also published evidence that diacylglycerol acyltransferase can be active with some xenobiotic acyl-CoAs or with some xenobiotic diacylglycerols; taken with the established evidence for enzymatic synthesis of xenobiotic acyl-CoA, the entire monoacylglycerol pathway would appear to be operational with at least some xenobiotic acids.

Although there have been no reports that other acyltransferases are involved in the synthesis of xenobiotic triacylglycerols, it seems probable that some of the others are involved in, at least, some cases. For instance, dodecylthioacetic acid is incorporated into both triacylglycerols and phospholipids at rates comparable to its natural counterpart (Skerde et al., 1989), and it would appear likely that glycerol 3-phosphate acyltransferase is the enzyme responsible, especially when, as here, the xenobiotic acid bears a strong structural resemblance to the natural acid. Furthermore, there have been reports of xenobiotic acids occurring in the *sn*-2 position of the triacylglycerol, a position normally accessible to neither monoacylglycerol or diacylglycerol acyltransferase. The involvement here, as elsewhere, of lipolytic or other hydrolytic enzymes working "in reverse" cannot be ruled out.

Further metabolism of triacylglycerol is normally expected to result in their secretion in lipoprotein or their degradation by lipolytic enzymes. Certainly, there are two reports of xenobiotic triacylglycerols occurring in chylomicrons, and there is circumstantial evidence that 4-benzyloxybenzoic acid is transferred as a triacylglycerol component of VLDL from liver to adipose tissue of rat. The reports of attempts to measure lipolysis of xenobiotic acylglycerols all suggest that the enzymes (both lipoprotein lipase and pancreatic lipase have been used) are considerably less active with xenobiotic substrates than with, say, trioleoylglycerol. The ester bond between the xenobiotic acid and glycerol appears to be particularly resistant; lipolysis of the xenobiotic triacylglycerol may result in a corresponding xenobiotic partial glyceride by the removal of the nonxenobiotic fatty acids.

There does not appear to be any evidence of toxic effects mediated by xenobiotic acylglycerols, but speculation that xenobiotic diacylglycerols may be able to mimic the second messenger activities of the natural form were borne out when it was shown that *sn*-1-acyl-2-(3PBA)glycerol was able to stimulate protein kinase C. The further demonstration that the compound was resistant to degradation by lipase suggested a potential to act like the tumour promoting phorbol diesters — a potential that has yet to be tested in a cell-transformation assay.

On the other hand, it is noticeable that a large number of the compounds involved are either hypolipidaemic agents or have hypolipidaemic properties in addition to their intended properties (e.g., the "profen" anti-inflammatory drugs and 3PBA). Fears (1985) has shown a direct correlation between the ability of the profens to act as hypolipidaemic agents and their activity as substrates for xenobiotic triacylglycerol synthesis. It was suggested that both effects are mediated by the formation of the corresponding acyl-CoA.

11.8.6.3.3 Glycerophospholipids

There are reports of at least seven xenobiotic acids being incorporated into glycerophospholipids, mostly phosphatidylcholine and phosphatidylethanolamine. All the reports, which are substantiated by reasonable experimental evidence, refer to acids that are close analogues of natural acids. These findings are consistent with the belief that the structures of membrane phospholipids are more tightly controlled than those of the storage lipids.

11.8.6.3.4 Other lipids

ω-Cyclohexyl fatty acids and 2-fluoropalmitate have been shown to form sphingomyelin analogues, and the latter can also be incorporated into ceramide and various glycosphingolipids. These examples differ from the others in the class by being *N*-acyl, rather than *O*-acyl, compounds (see Section 1.2.4).

11.8.6.4 Xenobiotic modifications to the polar groups of phospholipids

Phospholipids in which the polar head group has been replaced with, or modified by, a xenobiotic compound

represent a fourth classification of xenobiotic lipids. It remains a classification, the only firmly established member of which results from the *N*-acylation of phosphatidylethanolamine by monochloroacetic acid in tissues of the rat (Bhat et al., 1990). *N*-acyl esters of phosphatidylethanolamine are found in significant quantities in a number of seeds (see Section 2.3), but are rare in animal tissues. Nevertheless, the reaction with chloroacetic acid can be demonstrated in rat liver microsomes and requires coenzyme A and ATP for full activity. In view of the physiological activity of *N*-acyl phosphatidylethanolamine and its endocannabinoid derivatives (see Chapman, 2004; Kokotos, 2004), this is, potentially, an important area.

11.8.6.5 Other related conjugates

Coenzyme A esters, as well as being the substrates for many reactions in lipid biochemistry, are also intermediates in the metabolism of numerous xenobiotic acids, resulting in the formation of amino acid conjugates, for instance. Several enzymes have been implicated in the biosynthesis of xenobiotic acyl-CoA and their role is reviewed elsewhere (Huckle and Millburn, 1982). It is of interest that many xenobiotic acyl-CoAs appear to inhibit fatty acid synthesis, and that this property may be implicated in their action as hypolipidaemic agents or as herbicides (Kemal and Casida, 1992).

Carnitine esters of small aliphatic acids, including cyclopropanecarboxylic acid and benzoic acid, have been reported in the tissues and urine of a number of species. Although the conjugate is water-soluble, cyclopropanoylcarnitine was stored in considerable quantities in the muscle of dogs; furthermore, a link between its formation and lipid deposition in the heart muscle, mediated by carnitine depletion, has been suggested (Quistad et al., 1986). The same group also detected that the aniline acid of fluvalinate can be conjugated to bile acids and bile salts. These conjugates appear to be excreted with bile and have not caused more concern; however, little is known about their effects on enterohepatic circulation (Quistad et al., 1982).

11.8.6.6 Concluding comments

Although the compounds known to form xenobiotic lipids of some description probably still number less than 100, one has only to look through a chemical catalogue or the Merck Index to find hundreds more likely candidates. Until more work is done with a wide variety of potential xenobiotic precursors, it will not be possible to infer the rules that will allow us to predict which xenobiotic compounds will be incorporated into which xenobiotic lipids and in what quantities.

Similarly, evidence that xenobiotic lipids mediate toxic effects is rare, and the conversion of the active compound to an inert lipid may be considered a benign or even a protective event. However, it is this very inertia, leading to persistence, storage and concentration of the compound, that causes most general concern. The potential for subsequent release of the concentrated compound under the action of a lipolytic hormone — in a sort of "flashback" — is one that does not appear to have been investigated. In addition, there are a growing number of cases where the formation of a xenobiotic lipid has physiological or toxic consequences. A short review of the potential toxic implications of xenobiotic lipid formation has appeared (Hutson et al., 1985). Further investigations into the mechanism of synthesis, the rate of degradation, and the physiological implications of xenobiotic lipids are required before we can decide whether they are an important 'new' route of metabolism or merely an interesting but inconsequential phenomenon.

References

Abbadi, A. et al. (2004). Biosynthesis of very-long-chain polyunsaturated fatty acids in transgenic oilseeds: constraints on their accumulation. *Plant Cell*, **16**, 2734–2748.

Abulnaja, K.O. and Harwood, J.L. (1991a). Effect of a safener towards thiocarbamates on metabolism. *Z. Naturforsch.*, **46**, 931–933.

Abulnaja, K.O. and Harwood, J.L. (1991b). Interaction of thiocarbamate herbicides with fatty acid synthesis in germinating peas and their microsomal fractions. *Phytochemistry*, **30**, 2883–2887.

Abulnaja, K.O. et al. (1992). Inhibition of fatty acid elongation provides a basis for the action of the herbicide, ethofumesate, on surface wax formation. *Phytochemistry,* **31**, 1155–1159.

AgCanada (2004). The United States Canola Industry: Situation and Outlook, *Agriculture and Agri-Food Canada Bi-weekly Bulletin*, February 27, 2004 vol. 17, no. 4, available on the Web: http://www.agr.gc.ca/mad-dam/e/bulletine/v17e/v17n04_e.htm.

Ahnadi, C.E. et al. (2002). Feeding fish oil to dairy cows: II. Effects on milk fat and gene expression of mammary lipogenic enzymes. *J. Dairy Res.* **69**, 521–531.

Alban, C. et al. (1994). Localisation and characterisation of two structurally different forms of ACCase in young pea leaves, of which one is sensitive to aryloxyphenoxypropionate herbicides. *Biochem. J.* **300**, 557–565.

Ansari, G.A. et al. (1987). Selective pancreatic toxicity of palmitoylpentachlorophenol. *Toxicology*, **46**, 57–63.

Astrup, H.N. and Nedkvitne, J.J. (1975). The improved production of unsaturated lamb meat. *Acta Agric. Scand.,* **25**, 49–52.

Baldwin, A. et al. (2003). Fatty acid elongation is important in the activity of thiocarbamate herbicides and in safening by dichlormid. *J. Exptl. Botany,* **54**, 1289–1294.

Barrett, P.B. and Harwood, J.L. (1993). The inhibition of fatty acid elongation by a thiocarbamate herbicide and its sulphoxide. *Biochem. Soc. Trans.*, **21**, 184.

Bartov, I. et al. (1974). Differential effects of dietary acidulated soybean oil soapstock, cottonseed oil soapstock and tallow on broiler carcass fat characteristics of poultry. *Poultry Sci.*, **53**, 115–124.

Basri, M.W. et al. (2004). Oil palm — achievements and potential, In: *New Directions for a Diverse Planet*, Proceedings

of the 4th International Crop Science Congress, 26 Sept.–1 Oct. 2004, University of Brisbane, Australia.

Beitz, D.C. et al. (1995). *Genetic Manipulation of Mammary Cells to Improve Fatty Acid Composition of Milk, Iowa State University Dairy Report 59,* www.extension.iastate.edu/Pages/dairy/report95/products/dsl-59.pdf.

Benatti, P. et al. (2004). Polyunsaturated fatty acids: biochemical, nutritional and epigenetic properties, *J. Am. Coll. Nutr.* **23**, 281–302.

Bhat, H.K. and Ansari, G.A. (1990). Cholesterol ester hydrolase mediated conjugation of haloethanols with fatty acids. *Chem. Res. Toxicol.*, **3**, 311–317.

Bhat, H.K. et al. (1990). Toxicokinetics of monochloroacetic acid: a whole-body autoradiography study. *Toxicology*, **63**, 35–43.

Bobe, G. et al. (1995). *New Selection Criteria to Alter Milk Composition, Iowa State University Dairy Report 58,* http://www.extension.iastate.edu/Pages/dairy/report95/products/dsl-58.pdf.

Boles, J.A. et al. (2005). Supplemental safflower oil affects the fatty acid profile, including conjugated linoleic acid, of lamb. *J. Animal Sci*. **83**, 2175–2181.

Bolte, M.R. et al. (2002). Feeding lambs high-oleate or high-linoleate safflower seeds differentially influences carcass fatty acid composition. *J Animal Sci.* **80**, 609–616.

Bowman, J.P. et al. (1997). *Shewanella gelidimarina* sp. nov. and *Shewanella frigidimarina* sp. nov., novel Antarctic species with the ability to produce eicosapentaenoic acid (20:5 omega 3) and grow anaerobically by dissimilatory Fe(III) reduction. *Int. J. Sys. Bacteriol*., **47**, 1040–1047.

Bramley, P.M. (1991). Carotenoid biosynthesis. In *Target Sites for Herbicide Action,* Ed. R.C. Kirkwood, Plenum, New York, pp. 95–122.

Brooks, C.C. (1971). Fatty acid composition of pork lipids as affected by basal diet, fat source and fat level. *J. Animal Sci.,* **33**, 1224–1231.

Brophy, B. et al. (2003). Cloned transgenic cattle produce milk with higher levels of beta-casein and kappa-casein. *Nat. Biotechnol.,* **21**, 157–62.

Broun, P. et al. (1999). Genetic engineering of plant lipids. *Ann. Rev. Nutr.,* **19**, 197–216.

Budzisewski, G.J. et al. (1996). Uses of biotechnology in modifying plant lipids, *Lipids,* **31**, 557–569.

Burton, J.D. et al. (1991). Kinetics of inhibition of acetyl-coenzyme-A carboxylase by sethoxydim and haloxyfop. *Pesticide Biochem. Phys.,* **39**, 100–109.

Cahoon, E.B. (2003). Genetic enhancement of soybean oil for industrial uses: prospects and challenges. *AgBioForum,* **6**, 11–13. Available on the Web: http://www.agbioforum.org.

Cahoon, E.B. et al. (2003). Metabolic redesign of vitamin E biosynthesis in plants for tocotrienol production and increased antioxidant content. *Nat. Biotechnol.,* **21**, 1082–1087.

Caldwell, J. (1985). Novel xenobiotic-lipid conjugates. *Biochem. Soc. Trans.*, **13**, 852–854.

Caldwell, J. (1986). Conjugation mechanisms of xenobiotic metabolism: mammalian aspects. In *Xenobiotic Conjugation Chemistry, ACS Symposium Series* 299, Eds. G.D. Paulson et al., American Chemical Society, Washington, D.C., pp. 2–28.

Caldwell, J. and Marsh, M.V. (1983). Interrelationships between xenobiotic metabolism and lipid biosynthesis. *Biochem. Pharmacol*., **32**, 1667–1672.

Campbell, K.H. et al. (1997). Sheep cloned by nuclear transfer from a cultured cell line. *Nature,* **380**, 64–66.

Chang, M.J.W. et al. (1986). Acylation of xenobiotic alcohols: a metabolic pathway for drug retention. In *Xenobiotic Conjugation Chemistry, ACS Symp. Ser.* 299, Eds. G.D. Paulson et al., American Chemical Society, Washington, D.C., pp. 214–220.

Chapman, K.D. (2004). Occurrence, metabolism and prospective functions of *N*-acylethanolamines in plants. *Prog. Lipid Res.,* **43**, 302–327.

Chilliard, Y. et al. (2003). A review of nutritional and physiological factors affecting goat milk lipid synthesis and lipolysis. *J. Dairy Sci*., **86**, 1751–1770.

Christie, W.W. and Moore, J.H. (1969). The effect of dietary copper on the structure and physical properties of adipose tissue triglycerides in pigs. *Lipids*, **4**, 345–349.

Cohen, Z. and Ratledge, C. (2005). *Single Cell Oils*, AOCS, Champaign–Urbana, IL.

Cole, D. (1983). Oxidation of xenobiotics in plants. In *Progress in Pesticide Biochemistry and Toxicology*, vol. 3, Eds. D.H. Hutson and T.R. Roberts, John Wiley & Sons, New York, pp. 199–254.

Cook, L.J. et al. (1972). Fatty acid interrelationships in plasma, liver, muscle and adipose tissues of cattle fed safflower oil protected from ruminal hydrogenation. *Lipids*, **7,** 83–89.

Cooper, S.L. et al. (2004). Manipulation of the n-3 polyunsaturated fatty acid content of muscle and adipose tissue in lambs. *J. Animal Sci.,* **82**, 1461–1470.

Corbin, D.R. et al. (2001). Expression and chloroplast targeting of cholesterol oxidase in transgenic tobacco plants. *Plant Physiol.,* **126**, 1116–1128.

Corley, H. (2000). New technologies for plantation crop improvement, *Cranfield University Server*, Available on the Web: http://www.taa.org.uk/WestCountry/corley.html.

Crawford, M.A. et al. (1997). Are deficits of arachidonic and docosahexaenoic acids responsible for the neural and vascular complications of preterm babies? *Am. J. Clin. Nutr.,* **66**, 1032S–1041S.

Davies, A.O. and Harwood, J.L. (1983). Effect of substituted pyridazinones on chloroplast structure and lipid-metabolism in greening barley leaves. *J. Exp. Bot.*, **34**, 1089–1100.

Dodds, P.F. (1991). Incorporation of xenobiotic carboxylic acids into lipids. *Life Sci*., **49**, 629–649.

Dodds, P.F. (1995). Xenobiotic lipids: the inclusion of xenobiotic compounds in pathways of lipid biosynthesis. *Prog. Lipid Res.,* **34**, 219–247.

Donovan, D.C. et al. (2000). Influence of dietary fish oil on conjugated linoleic acid and other fatty acids in milk fat from lactating dairy cows. *J. Dairy Sci*., **83**, 2620–2628.

Donovan, D.M. et al. (2005). Engineering disease resistant cattle. *Transgenic Res.,* **14**, 563–567.

Downey, R.K. and Craig, B.M. (1964). Genetic control of fatty acid biosynthesis in rapeseed (*Brassica napus* L.). *J. Amer. Oil Chem. Soc.,* **41**, 475–478.

Duncan, D.N. et al. (1982). Basis for increased activity from herbicide combinations with ethofumesate applied on sugarbeet (*Beta vulgaris*). *Weed Sci.*, **30**, 195–200.

Fears, R. (1985). Lipophilic xenobiotic conjugates: the pharmacological and toxicological consequences of the participation of drugs and other foreign compounds as substrates in lipid biosynthesis. *Prog. Lipid. Res.*, **24**, 177–195.

Fears, R. et al. (1984). Hyperalphalipoproteinaemic activity of BRL 26314–II. Inhibition of atherosclerosis in rabbits. *Biochem. Pharmacol.*, **33**, 219–228.

Forster, B.P. et al. (2000). The development and application of molecular markers for abiotic stress tolerance in barley. *J. Exp. Bot.*, **51**, 19–27.

Fourmann, M. et al. (1998). The two genes homologous to Arabidopsis FAE1 co-segregate with the two loci governing erucic acid content in *Brassica napus*. *Theor. Appl. Genet.,* **96**, 852–858.

Fuerst, E.P. (1987). Understanding the mode of action of the chloroacetamide and thiocarbamate herbicides. *Weed Technol.*, **1**, 270–277.

Funk, C.D. (2001). Prostaglandins and leukotrienes: advances in eicosanoid biology. *Science,* **294**, 1871–1875.

Gill, P. et al. (1985). Forensic application of DNA "finger prints." *Nature,* **318**, 577–579.

Gurr, M.I. (1983). The nutritional significance of lipids. In *Developments in Dairy Chemistry 2: Lipids,* Ed. P.F. Fox, Applied Science, London, pp. 365–417.

Han, N.M. et al. (2004). Isolation of palm tocols using supercritical fluid chromatography. *J. Chromatogr. Sci.,* **42**, 536–539.

Harwood, J.L. (1991a). Herbicides affecting chloroplast lipid synthesis. In *Herbicides,* Eds. N.R. Baker and M.P. Percival, Elsevier, Amsterdam, pp. 209–246.

Harwood, J.L. (1991b). Lipid synthesis. In *Target Sites for Herbicide Action*., Ed. R.C. Kirkwood, Plenum, New York, pp. 57–94.

Harwood, J.L. (1999). Graminicides that inhibit lipid synthesis. *Pestic. Outl.,* **10**, 154–158.

Hazel, J.R. (1995). Thermal adaptation in biological membranes: is homeoviscous adaptation the explanation? *Annu. Rev. Physiol.,* **57**, 19–42.

Henderson, R.J. et al. (1990). Differential effects of the substituted pyridazinone herbicide Sandoz 9785 on lipid composition and biosynthesis in photosynthetic and nonphotosynthetic marine microalgae. 2. Fatty acid composition. *J. Exp. Bot.*, **41**, 729–736.

Herbert, D. et al. (1996). Kinetic studies on two isoforms of acetyl-CoA carboxylase from maize leaves. *Biochem. J.,* **318**, 997–1006.

Hilton, J.L. et al. (1971). Interactions of lipoidal materials and a pyridazinone inhibitor of chloroplast development. *Plant Physiol.*, **48**, 171–177.

Hippen, A.R. et al. (1995). *Redesigning milk fat from dairy cows, Iowa State University Dairy Report 60,* http://www.extension.iastate.edu/Pages/dairy/report95/products/dsl-60.pdf.

Hong, S. et al. (2003). Novel docosatrienes and 17S-resolvins generated from docosahexaenoic acid in murine brain, human blood, and glial cells. Autacoids in anti-inflammation. *J. Biol. Chem.,* **278**, 14677–14687.

Hoppe, H.H. (1989). Fatty acid biosynthesis — a target site of herbicide action. In *Target Sites of Herbicide Action*, Eds. P. Böger and G. Sandmann, CRC Press, Boca Raton, FL, pp. 65–83.

Hristov, A.N. et al. (2005). Effect of diets containing linoleic acid- or oleic acid-rich oils on ruminal fermentation and nutrient digestibility, and performance and fatty acid composition of adipose and muscle tissues of finishing cattle. *J. Animal Sci.,* **83**, 1312–1321.

Huckle, K.R. and Millburn, P. (1982). Biochemical mechanisms of amino acid conjugation of xenobiotics. In *Progress in Pesticide Biochemistry,* vol. 2, Eds. D.H. Hutson and T.R. Roberts, Wiley, Chichester, U.K., pp. 127–169.

Hungund, B.L. et al. (1988) Formation of fatty acid ethyl esters during chronic ethanol treatment in mice. *Biochem. Pharmacol.*, **37**, 3001–3004.

Hutson, D.H. (1982). Formation of lipophilic conjugates of pesticides and other xenobiotic compounds. In *Progress in Pesticide Biochemistry,* vol. 2, Eds. D.H. Hutson and T.R. Roberts, Wiley, Chichester, U.K., pp. 171–184.

James, C. (2004). Global status of commercialized biotech/GM crops: 2004, *ISAAA Briefs* No 32, ISAAA, Ithaca, New York, available online: http://www.isaaa.org.

Kang, J.X. et al. (2004). Fat-1 mice convert n-6 to n-3 fatty acids. *Nature,* **427**, 504.

Kelly, M.L. and Bauman, D.E. (1996). Conjugated linoleic acid: a potent anticarcinogen found in milk fat. In: *Proc. Cornell Nutr. Conf. Feed Manuf.*, Cornell University, Ithaca, NY, pp. 68–74.

Kelly, M.L. et al. (1998). Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating dairy cows. *J. Nutr.,* **128**, 881–885.

Kemal, C. and Casida, J.E. (1992). Coenzyme A esters of 2-aryloxyphenoxypropionate herbicides and 2-arylpropionate antiinflammatory drugs are potent and stereoselective inhibitors of rat liver acetyl-CoA carboxylase. *Life Sci.*, **50**, 533–540.

Kennelly, J.J. and Glimm, D.R. (1998). The biological potential to alter the composition of milk, Lennoxville Conference on Milk Production. *Can. J. Anim. Sci.,* **78** (Suppl.), 23–56.

Khozin-Goldberg, L. et al. (2002). Biosynthesis of eicosapentaenoic acid (EPA) in the freshwater eustigmatophyte *Monodus subterraneus*. *J. Phycol.,* **38**, 745–756.

Kleingartner, L.W. (2002). NuSun sunflower oil: redirection of an industry. *Trends in New Crops and New Uses,* Eds. J. Janick and A. Whipkey, ASHS Press, Alexandria, VA, pp. 135–138.

Kokotos, G. (2004). Endocannabinoids. In *Bioactive Lipids,* Eds. A. Nicolaou and G. Kokotos, The Oily Press, Bridgwater, U.K., pp. 245–264.

Laposata, E.A. et al. (1987). Metabolism of ethanol by human brain to fatty acid ethyl esters. *J. Biol. Chem.,* **262**, 4653–4657.

Lopez-Ferrer, S. et al. (2001). n-3 Enrichment of chicken meat. 1. Use of very long-chain fatty acids in chicken diets and their influence on meat quality: fish oil, *Poult. Sci.*, **80**, 741752.

Maga, E.A. (2005). Genetically engineered livestock: closer than we think? *Trends Biotechnol.,* **23**, 533–535.

Mattacks, C.A. et al. (2004). The effects of dietary lipids on dendritic cells in perinodal adipose tissue during chronic mild inflammation. *Br. J. Nutr.,* **91**, 883–892.

McCoy, M. (1999). Astaxanthin market a hard one to crack. *Chem.& Eng. News*, **77**, 15–17.

McDonald, I.W. and Scott, T.W. (1977). Foods of ruminant origin with elevated content of polyunsaturated fatty acids. *World Rev. Nutr. Diet.,* **26**, 144–207.

Miki, W. (1991). Biological functions and activities of animal carotenoids. *Pure Appl. Chem.,* **63**, 141–146.

Millar, A. et al. (2000). All fatty acids are not equal: discrimination in plant membrane lipids. *Trends Plant Sci.,* **5**, 95–101.

Mogelson, S. and Lange, L.G. (1984). Nonoxidative ethanol metabolism in rabbit myocardium: purification to homogeneity of fatty acyl ethyl ester synthase. *Biochemistry*, **23**, 4075–4081.

Morgan, D.P. and Roan, C.C. (1974). The metabolism of DDT in man. In *Essays in Toxicology*, vol. 5, Ed. W.J. Hayes, Academic Press, New York, pp. 39–97.

Murphy, D.J. Ed. (1994). *Designer Oil Crops*, VCH Press, Weinheim, Germany.

Murphy, D.J. (1999). Production of novel oils in plants. *Curr. Op. Biotechnol.,* **10**, 175–180.

Murphy, D.J. (2001). The biogenesis and functions of lipid bodies in animals, plants and microorganisms. *Prog. Lipid Res.,* **40**, 325–438.

Murphy, D.J. (2004). Biotechnology, its impact and future prospects. In *From Molecular to Global Photosynthesis*. Eds. J. Barber and M. Archer, Imperial College Press, London.

Murphy, D.J. Ed. (2005). *Plant Lipids: Structure, Biogenesis and Utilisation*, Blackwell, Oxford, U.K.

Murphy, D.J. (2006). *Plant Breeding and Biotechnology: Societal Context and the Future of Agriculture*, Cambridge University Press, Cambridge, U.K.

Murphy, D.J. et al. (1985). Differential responses of a range of photosynthetic tissues to a substituted pyridazinone, Sandoz 9785. Specific effects on fatty acid desaturation. *Phytochemistry*, **24**, 1923–1929.

Niemann, R. (1990). The effects of xenobiotics on hepatic lipid and lipo-protein metabolism. *Exp. Pathol*., **39**, 213–232.

Norman, H.A. and St. John, J.B. (1987). Differential effects of a substituted pyridazinone, BASF 13-338, on pathways of monogalactosyldiacylglycerol synthesis in Arabidopsis. *Plant Physiol*., **85**, 684–688.

Oil World (2005). vol. **48**, 1 April 2005, ISTA Mielke GmbH, Hamburg.

Paine, J.A. et al. (2005). Improving the nutritional value of Golden Rice through increased pro-vitamin A content. *Nat. Biotechnol.,* **23**, 482–487.

Palmiter, R.D. et al. (1982). Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion genes. *Nature,* **300**, 611–615.

Papanikolaou, S. et al. (2001). Kinetic profile of the cellular lipid composition in an oleaginous *Yarrowia lipolytica* capable of producing a cocoa-butter substitute from industrial fats. *Ant. Leeuw.*. **80**, 215–224.

Papanikolaou, S. et al. (2003). Accumulation of a cocoa-butter-like lipid by *Yarrowia lipolytica* cultivated on agro-industrial residues. *Curr. Microbiol*., **46**, 124–130.

Parodi, P.W. (1999). Conjugated linoleic acid and other anticarcinogenic agents in bovine milk. *J. Dairy Sci.,* **82**, 1339–1349.

Phillips, R.L. (1993). Plant genetics: out with the old, in with the new? *Am. J. Clin. Nutr*., **58**, 259S–263S.

Plat, J. and Mensink, R.P. (2001). Effects of plant sterols and stanols on lipid metabolism and cardiovascular risk. *Nutr. Metab. Cardiovasc. Dis.,* **11**, 31–40.

Polokoff, M.A. and Bell, R.M. (1978). Limited palmitoyl-CoA penetration into microsomal vesicles as evidenced by a highly latent ethanol acyltransferase activity. *J. Biol. Chem.*, **253**, 7173–7178.

Pond, C.M. (1999). Physiological specialisation of adipose tissue. *Prog. Lipid Res.,* **38**, 225–248.

Pond, C.M. (2005). Adipose tissue and the immune system. *Prostagland. Leukot. Ess. Fatty Acids,* 73, 17–30.

Price, L.J. et al. (2003). Graminicide insensitivity correlates with herbicide-binding co-operatively on acetyl-CoA carboxylase isoforms. *Biochem. J.,* **375**, 415–423.

Price, L.J. et al. (2004). Graminicide resistance in a blackgrass (*Alopecurus myosuroides*) population correlates with insensitivity of acetyl-CoA carboxylase. *Plant Cell Environ.,* **27**, 15–26.

Qi, B. et al. (2004). Production of very long chain polyunsaturated omega-3 and omega-6 fatty acids in plants. *Nat. Biotechnol.,* **22**, 739–745.

Quiros, C.F. and Paterson, A.H. (2004). Genome mapping and analysis. In: *Biotechnology in Agriculture and Forestry,* Eds. E.C. Pua and C.J. Douglas, vol. 54, *Brassica*, Springer-Verlag, Berlin, pp. 31–64.

Quistad, G.B. and Hutson, D.H. (1986). Lipophilic xenobiotic conjugates. In *Xenobiotic Conjugation Chemistry, ACS Symp. Ser.* 299, Eds. G.D. Paulson et al., American Chemical Society, Washington, D.C., pp. 204–213.

Quistad, G.B. et al. (1982). Xenobiotic conjugation: a novel role for bile acids. *Nature*, **296**, 462–464.

Quistad, G.B. et al. (1986). The role of carnitine in the conjugation of acidic xenobiotics. *Drug Metab. Dispos.*, **14**, 521–525.

Rahman, S.M. et al. (2001). Combining ability in loci for high oleic and low linolenic acids in soybean. *Crop Sci.,* **41**, 26–29.

Rahman, S.M. et al. (2003). A novel soybean germplasm with elevated saturated fatty acids. *Crop Sci.,* **43**, 527–531.

Ratledge, C. (1993). Single cell oils — have they a biotechnological future? *Trends Biotechnol.,* **11**, 278–84.

Reh, W.A. (2002). Alteration of Milk Fat Composition and Transgene Silencing in Mice Carrying a Bovine ß-Lactoglobulin-Rat Stearoyl-CoA Desaturase Transgene. Master's thesis, University of California, Davis.

Reh, W.A. et al. (2004). Hot topic: using a stearoyl-CoA desaturase transgene to alter milk fatty acid composition. *J. Dairy Sci.,* **87**, 3510–3514.

Reil, R.R. (1963). Physico-chemical characteristics of Canadian milk fat: unsaturated fatty acids. *J. Dairy Sci.,* **46**, 102–106.

Rendina, A.R. et al. (1989). Kinetics of inhibition of acetyl-coenzyme A carboxylase by the aryloxy phenoxypropionate and cyclohexanedione graminicides. In *Proceeding of Brighton Crop Protection Conference — Weeds,* pp. 163–173.

Rhind, S. et al. (2004). Dolly: a final report, reproduction, *Fertil. Develop.,* **16**, 156.

Roeser, H. et al. (1991). Toxicokinetics of tumour promoters of mouse skin. II. Metabolism of the tumour promoter 12-O-tetradecanoylphorbol-13-acetate in mouse skin and biological activities of metabolites. *Carcinogenesis*, **12**, 1563–1570.

Sargent, J.R. and Tacon, A.G. (1999). Development of farmed fish: a nutritionally necessary alternative to meat. *Proc. Nutr. Soc.,* **58**, 377–383.

Scislowski, V. et al. (2004). Linoleate supplementation in steers modifies lipid composition of plasma lipoproteins but does not alter their fluidity. *Br. J. Nutr.,* **91**, 575–584.

Scollan, N.D. et al. (2001). Manipulating the fatty acid composition of muscle and adipose tissue in beef cattle. *Br. J. Nutr.,* **85**, 115–124.

Sinensky, M. (1974). Homeoviscous adaptation — a homeostatic process that regulates the viscosity of membrane lipids in *Escherichia coli. Proc. Natl. Acad. Sci. USA,* **71**, 522–525.

Skrede, S. et al. (1989). The effects of alkylthioacetic acids (3-thia fatty acids) on fatty acid metabolism in isolated hepatocytes. *Biochim. Biophys. Acta*., **1005**, 296–302.

Spector, A. (1999). Essentiality of fatty acids, *Lipids,* **34**, 1–3.

St. John, J.B. (1982). Effects of herbicides on the lipid composition of plant membranes. In *Biochemical Responses Induced by Herbicides*, Eds. D.E. Moreland et al., American Chemical Society, Washington, D.C., pp. 97–109.

Thelen, J.J. and Ohlrogge, J.B. (2002). Metabolic engineering of fatty acid biosynthesis in plants. *Metab. Eng.,* **4**, 12–21.

Thompson, G.A. and Li, C. (1997). Altered fatty acid composition of membrane lipids in seeds and seedling tissues of high-saturate canolas. In: *Physiology, Biochemistry and Molecular Biology of Plant Lipids,* Eds. J.P. Williams et al., Kluwer, Dordrecht, The Netherlands, pp. 313–315.

Tian, X.C. et al. (2005). Milk and milk compositions of bovine clones. *Proc. Natl. Acad. Sci. USA,* **102**, 6261–6266.

Torrissen, O.J. and Christiansen, R. (1995). Requirements for carotenoids in fish diets. *J. Appl. Ichthyol.,* **11**, 225–230.

Vergroesen, A.J. Ed. (1975). *The Role of Fats in Human Nutrition*. Academic Press, London.

Voelker, T.A. et al. (1996). Genetic engineering of a quantitative trait: metabolic and genetic parameters influencing the accumulation of laurate in rapeseed. *Plant J.,* **9**, 229–241.

Walker, K.A. et al. (1989). Action of aryloxy-phenoxy carboxylic acids on lipid metabolism. *Rev. Weed Sci.*, **4**, 71–84.

Wen, Z.Y. and Chen, F. (2003). Heterotrophic production of eicosapentaenoic acid by microalgae. *Biotechnol. Adv.,* **21**, 273–294.

Wiseman, J. Ed. (1984). *Fats in Animal Nutrition,* Butterworths, London.

Wu, G. et al. (2005). Stepwise metabolic engineering of economically significant amounts of very long chain polyunsaturated fatty acids in seeds of *Brassica juncea*. *Nat. Biotechnol.,* **23**, 1013–1017.

Yazawa, K. (1996). Production of eicosapentaenoic acid from marine bacteria. *Lipids* **31**, Suppl. S297–300.

Ye, X. et al. (2000). Engineering the provitamin A (β-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. *Science,* **287**, 303–305.

11.8.7 Databases and other resources for plant lipids

(Much of this information comes from the Lipid Analysis Unit site, as listed below.)

General lipids

The American Oil Chemists' Society — the largest global society of oils and fats chemists, technologists, and biologists with much useful information of nutrition and labelling (www.aocs.org). Of particular interest to the general reader is their monthly magazine, *Inform* (http://www.aocs.org/press/inform/) and the journals *JAOCS* and *Lipids*.

The European Federation for the Science and Technology of Lipids (http://www.eurofedlipid.org/index.htm) publishes the *European Journal of Lipid Science and Technology* (www.ejlst.de) and organises conferences.

Cyberlipid — a website containing much useful information on lipid chemistry, biochemistry and analysis: (http://www.cyberlipid.org/).

Lipid Nomenclature — this is the IUPAC guide: http://www.chem.qmw.ac.uk/iupac/lipid

Conjugated Linoleic acid — Wisconsin Food Research Institute: (http://www.wisc.edu/fri/clarefs.htm). These pages give a comprehensive list of references to papers dealing with CLA.

Compilation of trivial names of fatty acids (by R.O. Adlof and F.D. Gunstone): (http://www.aocs.org/member/division/analytic/fanames.htm)

Lipidat — a relational database of thermodynamic and associated information on lipid mesophase and crystal polymorphic transitions, including lipid molecular structures (glycero- and sphingolipids): http://www.lipidat.chemistry.ohio-state.edu/

Lipid Bank for Web — a database of information on lipid structures and properties with thousands of references: (http://lipid.bio.m.u-tokyo.ac.jp/)

Lipid Analysis Unit — This site is a general information resource on lipids, supported by the Lipid Analysis Unit at the Scottish Crop Research Institute: (http://www.lipid.co.uk)

Plant lipids

Online chemical database for new seed crops produced by the New Crop Research Unit at NCAUR, Peoria, IL: (http://www.ncaur.usda.gov/nc/ncdb/search.html-ssi/) (see Abbot et al. (1997) *J. Am. Oil Chem Soc*., **74**, 723–726 and correction on p. 1181 for instructions) — chromatographic, physical chemical, and spectroscopic information on oil seeds.

Similar, but more extensive, database to the previous provided by BAGKF (Institute for Chemistry and Physics of Lipids), Munster, Germany — SOFA (Seed Oil Fatty Acids) — www.bagkf.de/sofa — (see Aitzetmuller et al. (2003) *Eur. J. Lipid Sci. Technol.,* **105**, 92–103).

A catalogue of genes for plant lipid biosynthesis at Michigan State University: (http://www.canr.msu.edu/lgc/index.html)

NPLC (National Plant Lipid Cooperative): (http://www.msu.edu/user/ohlrogge/). A further source of links to web-based lipid information, includes: NPLC Direc-

tory of Plant Lipid Scientists, The NPLC Electronic Mailing List, The NPLC Database of Plant Lipid Literature.

The Plant Lipid Home Page: (http://blue.butler.edu/~kschmid/lipids.html). Maintained by Katherine Schmid, this page contains many useful lipid-related links.

The Malaysian Palm Oil Board (MPOB) — website devoted to all aspects of oil palm biology, technology, food and nonfood uses and commercial matters.:(http://www.mpob.gov.my)

Food and industry related

Loders Croklaan, once a subsidiary of Unilever Ltd, is now part of the IOI group in Malaysia. The site covers science, technology, and nutrition related to lipids in general and to oil palm products in particular: (www.croklaan.com)

ITERG. French Research Institute dealing with oils and fats research and technology: (www.iterg.com)

Natural of Norway. Manufacturers of conjugated linoleic acid for health food and other applications: (www.natural.no)

The website of the British Nutrition Foundation carries information on lipids in addition to other food components: (www.nutrition.org.uk)

USDA — U.S. government site with information on fat-related nutritional advice: (http://www.nalusda.gov/fnic/dga/dga95/lowfat.html)

CTVO — a European group devoted to the chemical and technological utilization of vegetable oils: (www.danet.de/fnr/ctvo)

IENICA — an Interactive European Network for Industrial Crops and their Applications: (www.csl.gov.uk/ienica/)

ACTIN — a UK group devoted to nonfood uses of oils and fats: (www.actin.co.uk)

European website for the American Soybean Association: (www.asa-europe.org)

International Food Science and Technology — contains information on various food problems, including those involving lipids: (www.ifst.org)

Oil World is a German company producing data on a weekly basis for oilseeds, oils, and fats, and oil meals and covers production, imports, exports, and disappearance. Information is based on different commodity oils and fats and is presented on the basis of individual countries: (www.oilworld.de)

FFA Sciences is a company manufacturing probes to measure free fatty acid levels in oils and clinical samples: (www.ffasciences.com)

Britannia Foods has some articles "By Invitation Only" of interest mainly to lipid technologists: (www.britannia-food.com)

Peter Lapinskas, consultant to the Oils & Fats Industry; some interesting data on unusual seed oils: (www.lapinskas.com)

Plant Lipids — an Indian company specialising in products derived from a range of plant lipids: (http://www.plantlipids.com)

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