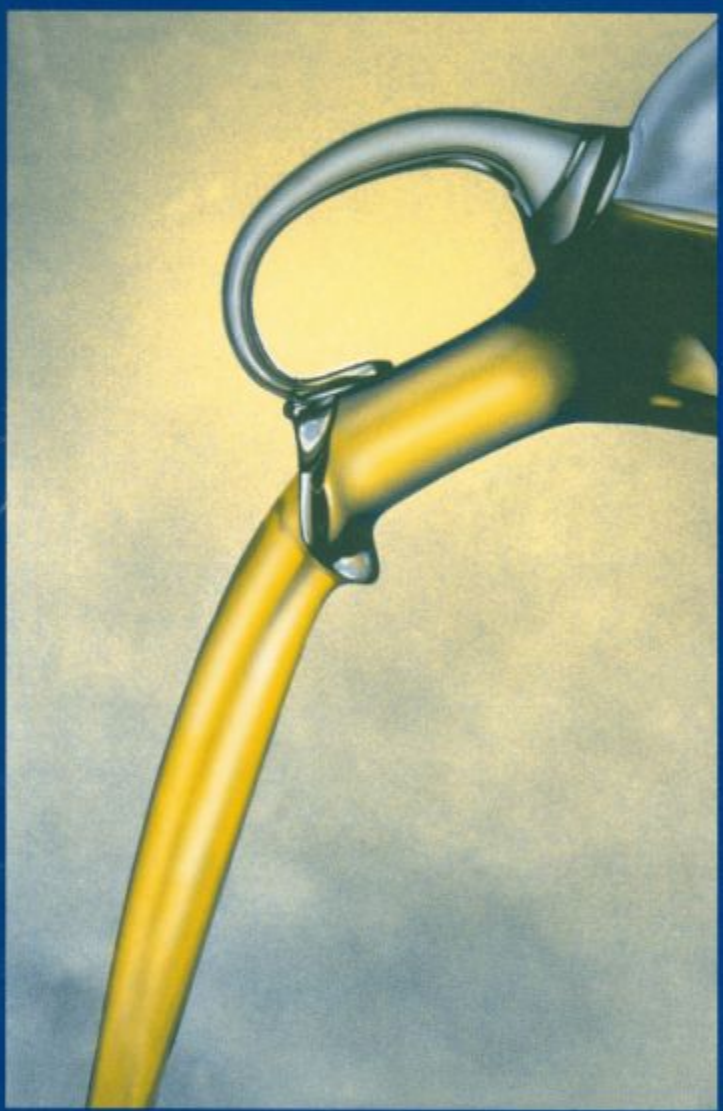
THE CHEMISTRY OF OILS AND FATS

Sources, Composition, Properties and Uses

Frank D. Gunstone



Blackwell Publishing

CRC Press

THE CHEMISTRY OF OILS AND FATS
Sources, Composition, Properties and Uses

FRANK D. GUNSTONE
Professor Emeritus
University of St Andrews
and
Honorary Research Professor
Scottish Crop Research Institute
Dundee, UK

Blackwell
Publishing

CRC Press

The Chemistry of Oils and Fats

Editorial offices:
Blackwell Publishing Ltd, 9600 Garsington Road, Oxford OX4 2DQ, UK
Tel: +44 (0) 1865 776868
Blackwell Publishing Asia Pty Ltd, 550 Swanston Street, Carlton South, Victoria 3053, Australia
Tel: +61 (0)3 8539 1011

ISBN 1-4051-1626-9

Published in the USA and Canada (only) by
CRC Press LLC, 2000 Corporate Blvd., N.W., Boca Raton, FL 33431, USA
Orders from the USA and Canada (only) to
CRC Press LLC

USA and Canada only:
ISBN: 0-8493-2373-8

First published 2004

Library of Congress Cataloging-in-Publication Data:
A catalog record for this title is available from the Library of Congress

British Library Cataloguing-in-Publication Data:
A catalogue record for this title is available from the British Library

Set in 10/12 Times
by MHL Production Services Ltd,
Coventry, UK
Printed and bound in Great Britain by
MPG Books Ltd, Bodmin, Cornwall

The publisher's policy is to use permanent paper from mills that operate a sustainable forestry policy, and which has been manufacturing from pulp processed using acid-free and elementary chlorine-free practices. Furthermore, the publisher ensures that the text paper and cover board used have met acceptable environment accreditation standards.

For further information on Blackwell Publishing, visit our website:
www.blackwellpublishing.com

Contents

Preface

The three major macronutrients are proteins, carbohydrates and lipids (oils and fats). This book is devoted to lipids, which are an important part of life for all of us. They are a significant part of all living systems, and of our diet, and they furnish the functional constituents of all personal care products and those materials by which we keep our homes and environment clean and wholesome. What are these materials in molecular terms? Where do they come from? What happens to them between the harvesting of crops and the appearance of the oils and fats in different products in the supermarket? How does nature produce these molecules and can we act on nature to modify the materials to increase their beneficial properties? How important are the minor products present in the fats that we consume? Since oils and fats vary, how can we analyse them? What are their physical, chemical and nutritional properties? How does the fat we consume affect our health and well-being in both quantitative and qualitative terms? What are their major food and non-food uses? This book addresses all these questions.

The book is designed for 'beginners' – but who are they? They may be graduate students in many disciplines, whose studies require a knowledge of lipids; graduates and others in later life coming afresh to an industry using oils and fats; they may be interested in nutrition, or they may be technologists in the food industry. All of these people may find their knowledge of lipids to be inadequate and require a more thorough introduction to the topic. This book is a good place to start.

In recent years I have edited several texts on various aspects of lipid science and I have tried to incorporate some of the information from these texts into this broad-ranging book. I have not provided references for every statement made but the Bibliography and References attached to each chapter contain some key references cited in the text and some general references where more detailed information may be sought.

I acknowledge the assistance of my colleagues. Charlie Scrimgeour has read the whole manuscript and has saved me from several trivial errors and a few more serious ones. Bill Christie has given me sound advice on the analytical sections of the book. They and others have allowed to me to work in an environment where lipid problems are discussed regularly. I also

acknowledge the generous help and advice that I have received from Graeme MacKintosh and David McDade of Blackwell Publishing.

Frank Gunstone
St Andrews

Abbreviations

The following is a list of the major abbreviations used in the text.

AA	arachidonic acid
ALA	alpha-linolenic acid
AMF	anhydrous milk fat
APCI	atmospheric pressure chemical ionisation
APG	alkyl polyglycosides
CBE	cocoa butter equivalent
CLA	conjugated linoleic acid
DCL	double-chain length
DHA	docosahexaenoic acid
DMOX	dimethyloxazolines
EDTA	ethylenediamine tetra-acetic acid
EPA	eicosapentaenoic acid
ESR	electron spin resonance
FTIR	Fourier transform infrared
GLA	gamma-linolenic acid
HDL	high density lipoproteins
HPLC	high performance liquid chromatography
L	linoleic acid or ester
La	lauric acid or ester
LCPUFA	long-chain polyunsaturated fatty acids
LDL	low density lipoproteins
Ln	linolenic acid or ester
MCT	medium-chain triglycerides
MUFA	monounsaturated fatty acids
NIR	near infrared
NMR	nuclear magnetic resonance
P	palmitic acid or ester
PAF	platelet activating factor
PAH	polycyclic aromatic hydrocarbons
PC	phosphatidylcholine
PE	phosphatidylethanolamine
PFAD	palm fatty acid distillate

PI	phosphatidylinositol
PMF	palm mid-fraction
PU	polyurethane
PUFA	polyunsaturated fatty acids
O	oleic acid or ester
S	saturated acids or esters
St	stearic acid or ester
TBA	thiobarbituric acid
TCL	triple-chain length
TMP	trimethylolpropane
UV	ultraviolet
VLDL	very low density lipoproteins

Triacylglycerols are frequently represented by three letters such as POL. This symbol stands for all six of the triacylglycerols having palmitic, oleic and linoleic acid attached to glycerol. There will be three triacylglycerols of the type POO and only one for triacylglycerols such as OOO. Other three letter groupings are to be interpreted similarly.

1 Oils and fats: sources and constituents

1.1 Introduction

The oils and fats of commerce are mixtures of lipids. They are mainly triacylglycerols (generally >95%) accompanied by diacylglycerols, monoacylglycerols and free fatty acids, but they may also contain phospholipids, free sterols and sterol esters, tocols (tocopherols and tocotrienols), triterpene alcohols, hydrocarbons and fat-soluble vitamins. This refers to crude oils when first extracted. During refining (Chapter 2) some of the minor components are removed, wholly or in part, and useful materials may be recovered. For example, phospholipids are separated during degumming, and deodoriser distillates contain fatty acids, along with valuable sterols, sterol esters, tocols, etc. Throughout this chapter there will be references to names, structures and ways of representing fatty acids and triacylglycerols. Readers who are not familiar with these concepts should perhaps read Chapter 3 before continuing beyond this point.

1.2 Specifications

Major oils and fats are usually named by their biological source (such as soybean oil or butter fat), but each oil/fat has a range of physical, chemical and compositional parameters by which it can be recognised. Traditional physical properties include density, measures of melting behaviour (if solid), refractive index and viscosity. To these must now be added several chromatographic and spectroscopic properties. Chemical properties include:

- iodine value (a measure of average unsaturation),
- saponification value or saponification equivalent (a measure of average acyl chain length),
- acetyl value (a measure of free hydroxyl groups),
- acid value (an indicator of quality, measuring free or unesterified acids),
- peroxide value, etc. (indicators of quality measuring oxidative deterioration).

Compositional parameters include quantitative assessment of:

- component acids,
- component triacylglycerols,
- minor components as groups or as individual compounds.

Some values are given later in this chapter and the analytical methods are described in Chapter 5. Specifications for refined oils may differ from those of crude oils. Some commodity oils now exist in differing forms, produced either by traditional breeding methods or by genetic engineering. It is likely that their number will grow as efforts are made to produce oils considered to be healthier according to the current nostrum. To extend their range of use, commodity oils may be modified by fractionation, partial hydrogenation, or interesterification (sections 1.3.18 and 2.3.2–2.3.5). There are legal definitions of several oils and fats, such as butter and olive oil, and of fat-containing products such as margarine, spreads and chocolate. The *Codex Alimentarius* is a source of specifications for many commodity oils and has been described by Stewart in Jee (2002).

1.3 Major oils and fats

One market analyst (ISTA Mielke of Hamburg) recognises 17 commodity oils and fats and produces market data for these on a weekly basis (Oil World) and on an annual basis (Oil World Annual). These comprise 13 vegetable oils and four animal fats (butter fat, lard, tallow and fish oil). The vegetable oils may be further subdivided into three categories:

1. By-products, where the crop is grown for another purpose other than seed oil: cotton (fabric), corn (grain), soybean (protein-rich meal).
2. Tree crops, which are generally slow to mature but then produce crops regularly for many years: palm, palmkernel, coconut, olive.
3. Crops, which have to be replanted each year to produce an annual harvest and where decisions about cultivation are made each sowing season by a large number of individual farmers: rape/canola, sunflower, groundnut, linseed, sesame, castor.

Other sources of minor oils and fats not included in this categorisation are discussed in section 1.4.

To appreciate the role of commodity oils and fats in daily life, it is useful to have a basic knowledge of their major uses. These are detailed in Chapters 10 and 11 and it is sufficient to note here that about 80 per cent of world production is used as human food, a further 6 per cent in animal feed (providing further food for humans), and the remaining 14 per cent is the basis of the oleochemical industry (Gunstone & Hamilton, 2001). In contrast to the much larger petrochemical industry, the oleochemical industry uses oils and fats as its major starting material (Chapter 11).

More detailed information about many of the oils in the following sections is to be found in Gunstone (2002) where separate chapters are devoted to individual oils.

1.3.1 Castor oil

Castor oil is derived from the plant *Ricinus communis,* grown mainly in India, Brazil and China at a world production level of 400 000 to 500 000 tonnes of oil. Castor oil differs from all other commercial oils in being rich in ricinoleic acid (~90%, 12-hydroxyoleic). Compared with common vegetable oils, castor oil is more viscous, less soluble in hexane, and more soluble in ethanol, all as a consequence of the presence of the hydroxy acid. This hydroxy acid has several interesting properties and is converted to a range of useful products (section 11.9).

$CH_3(CH_2)_5CHOHCH_2CH{=}CH(CH_2)_7COOH$
ricinoleic acid (12-hydroxyoleic acid)

1.3.2 Cocoa butter

The cocoa bean (*Theobroma cacao*) is the source of two important ingredients of chocolate: cocoa powder and a solid fat, cocoa butter. The usefulness of cocoa butter for this purpose is related to its fatty acid (Table 1.1) and triacylglycerol composition. The major triacylglycerols are symmetrically-disaturated oleic glycerol esters of the type SOS and include POP (18–23%), POSt (36–41%), and StOSt (23–31%). Cocoa butter generally commands a premium price and cheaper alternatives have been developed. These are cocoa butter alternatives, cocoa butter equivalents, cocoa butter improvers, cocoa butter replacers and cocoa butter substitutes. They may or may not have similar chemical composition to cocoa butter but they must display similar melting behaviour (section 1.4.1). Further information on chocolate is given in section 10.6.

1.3.3 Coconut oil

Coconut oil from the coconut palm (*Cocus nucifera*) is one of two important lauric oils (see also palmkernel oil). Grown mainly in Indonesia and the Philippines, annual production of coconut oil exceeds 3 million tonnes. The oil is characterised by its high level of lauric acid (12:0) accompanied by the C_8–C_{14} short- and medium-chain acids. A detailed fatty acid composition is given in Table 1.2. The oil is used in the food industry and in the oleochemical industry. In the latter case it is used mainly as a derivative of the corresponding alcohols (dodecanol or coco alcohol). For more details see Pantzaris in Gunstone (2002).

Table 1.1 Typical fatty acid composition (wt%) of selected vegetable oils and fats

	16:0	18:0	18:1	18:2	18:3
Cocoa butter	26	34	35	–	–
Corn	13	3	31	52	1
Cottonseed	23	2	17	56	–
Groundnut[a]	7–12	3	46–71	14–35	–
Linseed	6	3	17	14	60
Linola[b]			16	72	2
Olive	10	2	78	7	1
Palm	44	4	39	11	–
Palm olein	41	4	31	12	–
Palm stearin	47–74	4–6	16–37	3–10	–
Palm mid-fraction	41–55	5–7	32–41	4–11	–
Rape (high erucic)[c]	3	1	16	14	10
Rape (low erucic)	4	2	62	22	10
Rice bran oil	20	2	42	32	–
Safflower (regular)	7	3	14	75	–
Safflower (high oleic)	6	2	74	16	–
Sesame	9	6	41	43	–
Soybean	11	4	23	53	8
Sunflower (regular)	6	5	20	60	–
Sunflower (Sunola)	4	5	81	8	–
Sunflower (NuSun)	4	5	65	26	–

– indicates values of 0–1.
[a] also C_{20}–C_{24} saturated and unsaturated acids 4–7%.
[b] also saturated acids 10%.
[c] also 20:1 6% and 22:1 50%.

1.3.4 Corn oil

Corn oil, with an annual production of around 2 million tonnes, is obtained from corn or maize (*Zea mays*) by wet milling, particularly in the United States. The major acids are palmitic (9–17%), oleic (20–42%) and linoleic (39–66%) (Table 1.1) and the major triacylglycerols are typically: LLL (25%), LLO (21%), LLP (15%), LOO (11%), and LOP (10%). Despite its high unsaturation, the oil has good oxidative stability. The refined oil is used as frying oil, salad oil and in the production of spreads after partial hydrogenation. It contains an unusually high level of sterols (section 1.6.3). For more details see Moreau in Gunstone (2002).

Table 1.2 Typical fatty acid composition (%wt) of the two major lauric oils

	8:0	10:0	12:0	14:0	16:0	18:0	18:1	18:2
Coconut	8	7	48	16	9	2	7	2
Palmkernel	3	4	45	18	9	3	15	2

1.3.5 Cottonseed oil

The cotton plant (*Gossypium hirsutum* or *G. barbadenseis*) is grown for its fibre. The oil is a by-product with about 12 per cent of the gross value of the total product. Cottonseed oil was once the major vegetable oil competing with the more widely-used animal fats. Today, it occupies ninth place in production tables after five vegetable oils (soybean, palm, rape/canola, sunflower and groundnut) and three land animal fats (tallow, lard and butter) (section 1.7). Cottonseed oil is unusual among commodity vegetable oils in that it contains a relatively high level of palmitic acid (typically 23%) along with oleic acid (17%) and linoleic acid (56%). Linolenic acid is virtually absent (Table 1.1). Low levels of malvalic and sterculic acids (cyclopropene acids, section 3.8) are removed during refining. The major triacylglycerols reported in one analysis were PLL (26%), LLL (16%), POL (14%), LLO (13%) and PLP (9%). For more details see O'Brien in Gunstone (2002).

Gossypol is a toxic phenolic C_{40} compound present in cotton boll cavities. When the seed is extracted, the gossypol adheres to the protein meal and only a small proportion remains in the crude oil giving it a red-brown colour. This is largely removed during refining – especially through caustic refining and bleaching when it falls to safe levels, not exceeding 1–5 ppm.

1.3.6 Groundnut oil

Groundnut oil, obtained from the legume *Arachis hypogea,* is also known as peanut oil, monkeynut oil or arachis oil. The plant is grown widely, especially in India, China and the United States. Many of the nuts are consumed as snacks, but crushing still yields about 5 million tonnes of oil each year. Its major acids are palmitic (7–12%), oleic (41–67%) and linoleic (14–35%) along with 4–7 per cent of C_{20}, C_{22} and C_{24} saturated and monoene acids (Table 1.1). The major triacylglycerols in one sample of oil were reported to be LLL (6%), LLO (26%), LLS (8%), LOO (21%), LOS (13%), OOO (5%), OOS (16%) and others (5%). The oil shows high oxidative stability and is considered to have a desirable nutty flavour. There is also a high-oleic variety with 76 per cent of oleic acid (section 1.3.18). For more details see Sanders in Gunstone (2002).

1.3.7 Linseed (flaxseed, linola)

Different varieties of flax (*Linum usitatissimum*) are grown for fibre and oil. Linseed oil is one of the most unsaturated vegetable oils, resulting from its high level of linolenic acid (50–60%, Table 1.1). As a consequence of this, it oxidises and polymerises very readily and this property is exploited in its use in paints, varnishes and inks, in the production of linoleum and as a sealant for

concrete. These uses diminished with the appearance of alternative petroleum-based products, but the natural oil is returning to favour on environmental grounds.

With recognition of the importance of n-3 acids in the diet, the oil and seed – under the name of flaxseed – are used increasingly in food products both for humans (cereals and breads) and for animals. This is independent of the growing use of linola oil (solin).

Using chemical mutation, plant breeders in Australia developed a variety of linseed with a low level of linolenic acid (~2%) and a high level of linoleic acid. This is called linola and is a linoleic-rich oil-like sunflower (Table 1.1). Solin is the generic name given to a Canadian flaxseed oil with <5 per cent of linolenic acid. To distinguish these from traditional linseed the seeds must be coloured yellow. They can be grown in the same temperate zones as rapeseed (canola) and the oil is used as an alternative to sunflower seed oil in the production of spreads rich in EFA. It is being grown in Australia, Canada and Europe. For more details see Kochar in Gunstone (2002).

1.3.8 Olive oil

Olive oil is a major vegetable oil obtained from the mesocarp of the fruits of the olive tree (*Oleo europaea*). Annual production is 2.5–2.8 million tonnes, with commercial growth of the tree confined almost entirely to the Mediterranean countries of Italy, Greece, Spain, Turkey and Tunisia. It is an important part of the Mediterranean diet. Virgin olive oil is produced from the first pressing and other grades of lower quality are produced subsequently. The oil is characterised by a high level of oleic acid with *Codex* ranges of 8–20 per cent for palmitic acid, 55–83 per cent for oleic acid, and 4–21 per cent for linoleic acid (Table 1.1). The major triacylglycerols are: OOO (40–59%), LOO (12–20%), POO (12–20%) and POL (6–7%) and the oil is characterised by a range of unsaponifiable constituents which confer high oxidative stability. The oil contains squalene at a higher level (150–170 mg/100ml) than other vegetable oils (usually only 5–50 mg/100ml) and this can be recovered from deodoriser distillate (section 2.2.4). The oil contains many other minor components. Several of these show antioxidant properties and add to the nutritional value of this oil. For more details see Boskou in Gunstone (2002).

1.3.9 Palm oil

The oil palm (*Elaies guinensis*) produces two distinct oils – palm oil from the fleshy endosperm and palmkernel oil from the kernels. The tree grows in the tropical regions of Asia, Africa and the Americas, and predominantly in Malaysia and Indonesia. At an average of about 4 tonnes per hectare of the

two oils combined on well-managed plantations, the oil palm outcrops all other oil crops. Fruit bunches of 4–20 kg contain 200–2000 individual fruits which furnish palm oil (20–24%) and palmkernel oil (2–4%). Through seed breeding, palm trees are being developed which are smaller to make harvesting easier, give higher oil yields, have a more unsaturated oil and contain a higher proportion of the more valuable kernel.

The oil contains almost equal proportions of saturated (palmitic ~44% and stearic ~4%) and unsaturated acids (oleic ~39% and linoleic ~10%). The major triacylglycerols are POP (27–31%), POO (20–26%), PLO (9–11%) and PLP (6–11%). The oil is used mainly for food purposes but finds some non-food uses as it is a source of valuable by-products such as carotene (500–700 ppm), and tocopherols and tocotrienols (vitamin E, combined total 710–1140 ppm). Red palm oil, prepared so that it retains around 80 per cent of the carotenes present in the crude oil, is a valuable dietary source of these important compounds.

Palm oil with a melting range 21–27ºC can be crystallised to give solid (palm stearin, 25–35%, melting between 48 and 50ºC) and liquid fractions (palm olein, 65–70%, melting between 18 and 20ºC) thereby extending the range of usefulness of this oil. With modern filtration procedures, the yield of olein is usually in the range 71–78 per cent. The olein has a cloud point of 7–10ºC and can be crystallised again to give yet more unsaturated oleins and palm mid-fraction. Palm olein is used as a high quality, highly stable, frying oil and the export of palm oil from Malaysia is mainly in the form of palm olein. Palm stearin is the less valuable component, but it can be used as a hard fat in the production of spreads and as a vegetable alternative to tallow in the oleochemical industry. Fatty acid composition of palm oil and its fractions is given in Table 1.1. For more details see Siew Wai Lin in Gunstone (2002).

1.3.10 Palmkernel oil

Palmkernel oil is produced from the kernels of the oil palm, usually by solvent extraction, and is an important lauric oil (see also coconut oil, section 1.3.3). Its fatty acid composition is detailed in Table 1.2. Annual production is now around 3 million tonnes. The kernels originate mainly in the oil palm growing areas of Malaysia and Indonesia and are crushed almost entirely in the country of origin. The oil can be hardened, fractionated or interesterified with a non-lauric oil to make it more appropriate for certain end uses. For more details see Pantzaris in Gunstone (2002).

1.3.11 Rapeseed oil (also called canola oil)

The seed oil of *Brassica napus* or *B. campestris* was typically rich in erucic acid (22:1) (Table 1.1) and the seed meal had an undesirably high level of

glucosinolates. These components reduced the value of both the oil and the protein meal but they have been bred out of modern rapeseed which is now known as double zero or canola. Rapeseed (of all kinds) is now the third largest source of oil, at 13–14 million tonnes a year, after soybean oil and palm oil. It is grown mainly in Western Europe, China, India, and Canada (where the canola varieties were developed). Typically, it contains palmitic (4%), stearic (2%), oleic (62%), linoleic (22%) and linolenic (10%) acids and has less saturated acids than any other commodity oil. In one example, its major triacylglycerols were LnLO (8%), LLO (9%), LnOO (10%), LOO (22%), LOP (6%), OOO (22%) and POO (5%).

Rapeseed oil lends itself to genetic modification and rapeseed varieties having oils with modified fatty acid composition have been developed, though it is still not clear how many of these will be economically viable. Rapeseed oils with less linolenic acid, or enhanced levels of lauric acid, stearic acid, oleic acid, or with unusual acids such as γ-linolenic acid, ricinoleic acid, or vernolic acid have all been developed for commercial exploitation. An oleic-rich variety developed in Australia, called Monola, contains about 78 per cent oleic acid (section 1.3.18). For more details see Mag & Przyblski in Gunstone (2002) and Gunstone (2004a).

1.3.12 Rice bran oil

Rice (*Oryza sativa*) is an important cereal with an annual production of about 600 million tonnes. To produce white rice, the hull is removed and the bran layer is abraded giving 8–10 per cent of the rice grain. The bran contains the testa, cross cells, aleurone cells, part of the aleurone layer and the germ, and includes almost all the oil of the rice coreopsis. There is probably a potential for over 5 million tonnes of rice bran oil per annum, but present production is only about 700 000 tonnes and not all this is suitable for human consumption. India (500 000 tonnes), China (120 000 tonnes) and Japan (80 000 tonnes) are the major countries producing rice bran oil.

Lipases, liberated from the testa and the cross cells, promote rapid hydrolysis of the oil and therefore it should be extracted within hours of milling. The major acids in rice bran oil are palmitic (12–28%, typically 20%) oleic (35–50%, typically 42%) and linoleic acid (29–45%, typically 32%) (Table 1.1). The oil contains phospholipids (~5%), a wax which may be removed for industrial use and unsaponifiable material.

Refined rice bran oil is an excellent salad and frying oil with high oxidative stability resulting from its high level of tocopherols and tocotrienols (~860 ppm) and from the presence of the oryzanols (ferulic acid esters of sterols and triterpene alcohols, ferulic acid is 3-methoxy-4-hydroxycinnamic acid). The oxidative stability of this oil is exploited in a frying oil based on oleic-rich sunflower oil with up to six per cent of added rice bran and/or

sesame oil to confer high oxidative stability. Rice bran oil also has several non-food uses.

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. This effect does not seem to be related to fatty acid or triacylglycerol composition, but to the unsaponifiable fraction (4.2% of the oil) and in particular to the oryzanols (1.5–2%). These can be isolated in concentrated form from rice bran oil soapstock but have not yet been accepted for food use. For more details see Kochar in Gunstone (2002).

1.3.13 Safflower oil

Safflower seed oil is obtained from the seed of *Carthamus tinctorius,* grown particularly in India as a source of a valuable red-yellow or orange dye. Annual production of seed has declined from 770 000 tonnes in 1999/2000 to only 410 000 tonnes in 2002/03. Oil is exported from Mexico and the United States and imported by Germany, the United States and Japan. Normally it is a linoleic-rich oil (~75% linoleic acid) with LLL (47%), LLO (19%) and LLSt (18%) as the major triacylglycerols (Table 1.1). An oleic-rich variety (~74% oleic acid) has been developed and designated saffola (section 1.3.18). The oil has been described in detail by Smith (1996).

1.3.14 Sesame oil

Sesame oil comes from the plant *Sesamum indicum*. This is grown mainly in India and China and also in Myanmar, Sudan and Mexico with a total annual production of oil of around 800 000 tonnes. The seed has 40–60 per cent of oil with almost equal levels of oleic (range 33–50%, typically 41%) and linoleic acids (range 33–50%, typically 43%) and some palmitic acid (range 7–12%, typically 9%) and stearic acid (range 3–6%, typically 6%) (Table 1.1). The oil contains sesamin (0.1–1.1%) and sesamolin (0.1–0.6%) which together give the oil high oxidative stability. It may be added to other oils to enhance oxidative stability such as in the preparation of frying oils. For more details see Kochar in Gunstone (2002).

1.3.15 Soybean oil

At almost 30 million tonnes per annum (section 1.7), soybean oil is produced in greater amounts than any other oil. Soybeans (*Glycine max*) are grown mainly in the United States, Brazil, Argentina and China. When extracted, the beans provide oil (18%) and a high quality protein meal (79%) which is used both as animal feed and in many processed foods for humans. Oil is the minor product in terms of quantity but in terms of value, oil and meal are more

evenly balanced though the meal generally has the greater value. There is considerable trade in soybean oil and in the beans that are then extracted in the importing country. The main producers of soybean oil are the United States, Brazil, Argentina, China (using locally produced and imported beans) and EU-15 (using mainly beans imported from North and South America).

Soybean oil is characterised by the presence of linoleic (53%), oleic (23%), palmitic (11%), linolenic (8%) and stearic acids (4%), and is an unsaturated oil containing useful proportions of the two essential fatty acids – linoleic and linolenic (Table 1.1). Because of its high level of linoleic acid (>50%) over half its triacylglycerols contain two or three linoleic chains. Most of the remainder have one linoleic chain. In a typical analysis, triacylglycerols exceeding four per cent were LLL (17.6%), LLO (15.3%), LLP (10.2%), LLLn (7.9%), LLSt (4.2%), PLO (6.9%), OLO (6.3%), LnLO (4.8%) and others (26.8%).

The usefulness of this oil is extended by brush hydrogenation to selectively reduce the level of linolenic acid and so enhance oxidative stability, or by partial hydrogenation which further reduces the level of unsaturation, thereby producing the solid acids (saturated or *trans* 18:1) required to make a good quality spread. The presence of linolenic acid in any oil raises some interesting questions. This acid is oxidised twice as quickly as linoleic acid and produces short-chain aldehydes with flavours that are even stronger and less acceptable than those produced from linoleic acid. Food products containing linolenic acid therefore have shorter shelf-life than those from which this acid is absent. Food producers want to maximise shelf-life, but against this is a growing awareness that on nutritional grounds, we should reduce our intake of n-6 acids and increase our intake of n-3 acids (section 9.3). Despite this, pressure to reduce the content of linolenic acid in soybean oil remains and this is being achieved slowly by breeding programmes or more quickly by brush hydrogenation (section 2.3.3).

Attempts are being made to modify the fatty acid composition of this oil in order to enhance its usefulness. Oils with less or more saturated acid, with less linolenic acid, and with high levels of oleic acid are in various stages of development. Some, but not all of these, result from biotechnology (section 1.3.18).

Crude soybean oil contains several valuable minor components that can be recovered in some measure during refining. Degumming produces a fraction enriched in phospholipids (lecithin) which is the main industrial source of these materials (sections 1.6.2 and 2.2.1). Deodoriser distillate contains tocopherols (vitamin E) (0.15–0.21% in the oil raised to ~15% in the distillate) and phytosterols, (~0.33% in the oil raised to ~18% in the distillate). Both of these are valuable byproducts (section 2.2.4). For more details see Wang in Gunstone (2002).

1.3.16 Sunflower oil

Sunflower seed oil is obtained from *Helianthus annuus* grown mainly in Russia and Ukraine, Argentina, Western and Eastern Europe, China and the United States. The oil normally contains 60–75 per cent of linoleic acid with >90 per cent of oleic and linoleic acids combined and virtually no linolenic acid. Its major triacylglycerols are typically LLL (14%), LLO (39%), LLS (14%), LOO (19%), LOS (11%) and others (3%). It is widely used as a cooking oil and is valued as an important component of soft spreads. High oleic varieties have been developed (section 1.3.18). Sunola (Highsun) comes from a high-oleic variety and has about 85 per cent oleic acid (some samples reach 90%). It is used to meet the growing demand for high oleic oils. NuSun with around 60 per cent oleic acid has been developed in the United States and it is hoped that it will replace regular sunflower oil in that country (Table 1.1). For more details see Gupta in Gunstone (2002).

1.3.17 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 when pine wood chips are digested, under pressure, with an alkaline solution of sodium sulfate or an acidic solution of sodium sulfite. Tall oil is produced mainly in North America (~250 000 tonnes) and Scandinavia (~90 000 tonnes), but the products from these two sources differ in composition because of the differences in wood species being pulped. The crude extract is distilled to separate fatty acids (with less than 2 per cent of resin acids) from resin acids (with less than 2 per cent of fatty acids). The former is a good and cheap source of an oleic-linoleic acid mixture (75–80%) (Table 1.3). 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 compounds. They are used instead to prepare dimer acids, alkyds and coatings, detergents and lubricants, and are being examined for use as solvents, in inks and for biodiesel production. Tall oil pitch is a valuable source of phytosterols. These are hydrogenated and acylated for use in cholesterol-lowering spreads (section 9.8.2).

Table 1.3 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

(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.

1.3.18 Oils with modified fatty acid composition

Fatty acid composition can be modified through genetic manipulation. This has been achieved in the past by traditional seed breeding procedures and now also by more modern methods of biotechnology. Reference has already been made to modifications of:

- rapeseed oil to reduce the content of erucic acid (section 1.3.11) and to increase the level of oleic acid (Monola, section 1.3.11),
- groundnut (section 1.3.6), safflower (section 1.3.13) and sunflower oils (section 1.3.16) to increase the level of oleic acid,
- linseed oil to change the proportions of linolenic and linoleic acids (section 1.3.7).

Attempts are being made to develop oils with improved nutritional or technical properties through modification of fatty acid composition or by changing the levels of minor components, such as tocopherol. Many such examples have been reported but it is still not clear how many are likely to be commercially viable. Fatty acid changes include:

- lower levels of saturated acids on nutritional grounds and to avoid undesirable crystallisation in salad oils, frying oils and oils used as lubricants and biofuels,
- increased levels of saturated acids, so that the oils contain solids and do not have to be subjected to partial hydrogenation, thereby avoiding undesirable acids with *trans* configuration in spreads and cooking fats,
- increased levels of oleic acid on nutritional grounds and to increase their value for the oleochemical industry (Baoru *et al*. in Gunstone, 2003),
- reduced levels of α-linolenic acid, since these oxidise readily and reduce shelf-life,
- incorporation of less common acids such as medium chain acids, γ-linolenic acid, hydroxy acids and epoxy acids into commodity oils to make these acids more readily available.

It would be beneficial to have a vegetable source of long-chain polyunsaturated fatty acids such as arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid. These are important dietary acids and it would be beneficial if plants could provide a convenient source. Efforts are being made to incorporate the necessary desaturase and elongase enzymes into plant systems to achieve this. Proof of concept has been achieved but a field crop is still some way off.

1.4 Minor vegetable oils

In addition to the major oils described in section 1.3 there are several minor vegetable oils worthy of mention. These are generally of interest because they

contain a fatty acid or other component which gives the oil interesting dietary or technical properties or they are oils available in modest quantities that can be used in a niche market or have an unusual composition.

1.4.1 Cocoa butter alternatives

As already indicated (section 1.3.2), cocoa butter is an important commodity which carries a premium price. Cheaper alternatives with similar physical properties may be employed, such as materials derived from lauric oils. Such products cannot be called chocolate and are generally described as confectionery fats. However, in some European countries, up to one third of the cocoa butter can be replaced with fats taken from a prescribed list and the product can still be designated chocolate. These include palm mid-fraction (section 1.3.9) and the five tropical fats listed in Table 1.4 (Timms, 2003).

1.4.2 Oils containing γ-linolenic acid and/or stearidonic acid

γ-Linolenic acid (6,9,12-18:3, GLA) is now recognised as 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 ailments. 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 Δ^6-desaturation, elongation and Δ^5-desaturation, of which, the first step is rate-determining (section 4.5.4).

9,12-18:2 → 6,9,12-18:3 → 8,11,14-20:3 → 5,8,11,14-20:4
(linoleic) (arachidonic)

Table 1.4 Tropical fats which may partially replace cocoa butter in some countries

Common name	Botanical name	Major triacylglycerols (%)[a]		
		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	*Mangifer ndica*	2	13	55
Sal stearin	*Shorea robusta*	1	10	57
Shea stearin	*Butyrospermim parkii*	1	7	71

[a] Major SOS triacylglycerols are shown as typical values.

Table 1.5 Component acids of oils containing γ-linolenic acid and stearidonic acid (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]

[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).

A similar sequence of changes converts α-linolenic acid to eicosapentaenoic acid and docosahexaenoic acid (section 4.5.4) with the first metabolite being stearidonic acid (6,9,12,15–18:4).

GLA is present in a number of seed oils, of which three are commercially available. Echium oils serve as a source of stearidonic acid. The fatty acid composition of some of these oils is given in Table 1.5 (Clough in Gunstone, 2001 and Baoru *et al*. in Gunstone, 2003).

1.4.3 Avocado oil (Persea americana)

The avocado grows in tropical and subtropical regions between 40ºN and 40ºS and is available particularly from California, Florida, Israel, New Zealand and South Africa. Like the palm and the olive, lipid is concentrated in the fruit pulp (4–25%) from which it can be pressed. There is very little oil in the seed (2%). Avocado oil is used widely in cosmetic products as it is easily absorbed by the skin and its unsaponifiable material is reported to provide some protection from the sun. It is also available as a high-oleic speciality oil for food use and is incorporated into spreads. It is rich in chlorophyll, making it green before processing. It contains palmitic (10–20%), oleic (60–70%) and linoleic acid (10–15%) as its major fatty acids.

1.4.4 Chinese vegetable tallow and stillingia oil (Sapium sebiferum *and* Stillingia sebifera)

This seed is unusual in that it yields lipids of differing composition from its outer seed coating (Chinese vegetable tallow, 20–30%) and from its kernel (stillingia oil, 10–17%). The former, with around 75 per cent palmitic acid and 20–25 per cent oleic acid, is mainly a mixture of PPP (~70%) and POP (20–25%) triacylglycerols and is a potential confectionery fat. However, it is difficult to obtain the fat free from stillingia oil (the kernel oil), which is considered to be nutritionally unacceptable. Stillingia oil is quite different, with oleic (13%), linoleic (23%), linolenic acids (47%) and 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:

Glyc-OCOCH=C=CH$(CH_2)_4$OCOCH=CHCH=CH$(CH_2)_4CH_3$

1.4.5 Crambe (Crambe abyssinica *and* C. hispanica)

Present interest in this oil, particularly in North Dakota and in the Netherlands, depends on the fact that it is a potential source of erucic acid (50–55%) which finds several industrial uses. This was once the major acid in rapeseed oil but modern varieties of this seed produce a low-erucic oil (such as canola) suitable for food use. High-erucic rapeseed oil is still grown for industrial purposes and attempts are being made to increase the level of this C_{22} acid from around 50 per cent to over 65 per cent and even to 90 per cent by genetic engineering.

1.4.6 Cuphea

Cuphea plants furnish seeds with oils that may be rich in C_8, C_{10}, C_{12} or C_{14} saturated acids. They generally contain >30 per cent of oil and are expected to produce a commercial crop in the period 2005–2010. 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. The oil (17–29%) from *Cupea procumbens*, for example, contains 89–95 per cent of decanoic acid.

1.4.7 Dimorphotheca

The seed of *Dimorphotheca pluvialis* is not very rich in oil (13–28%, 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. This acid is unstable and easily dehydrates to a mixture of conjugated 18:3 acids.

$CH_3(CH_2)_4CH=CHCH=CHCH(OH)(CH_2)_7COOH$
Dimorphecolic acid (9-OH 10*t*12*c*-18:2)

1.4.8 Hemp (*Marijuana,* Cannabis sativa)

Hemp seed oil has an interesting fatty acid composition. One report gives the following values: palmitic (4–9%), stearic (2–4%), oleic (8–15%), linoleic (53–60%), α-linolenic (15–25%), γ-linolenic (0–5%) and stearidonic acid (0–3%). The oil is being used in cosmetic formulations. There is evidence that dietary consumption of hemp seed oil leads to increased levels of γ-linolenic acid in blood serum. The growing of hemp is banned or regulated in most developed countries.

1.4.9 Gold of Pleasure (Camelina sativa*, also called false flax*)

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 also be grown on poorer soils and is reported to show better gross margins than either rape or linseed. The seed yield is in the range 1.5–3.0 tonnes per hectare and the oil content between 36 and 47 per cent. The oil has an unusual fatty-acid composition. It contains significant levels of oleic acid (10–20%), linoleic acid (16–24%), linolenic acid (30–40%) and C_{20} and C_{22} acids, especially eicosenoic (15–23%). Another set of values are 30–38 per cent oil containing oleic (14–20%), linoleic (19–24%), linolenic (27–35%), eicosenoic (12–15%) and other acids (12–20%) along with a range of tocols (5–22 mg/100 g, mean 17). Despite its high level of unsaturation, the oil shows reasonable oxidative stability. Attempts are being made to optimise the agronomy. Its use in paints, varnishes, inks, cosmetics and even as a food oil is being examined and developed. This vegetable oil is unusual in that it contains cholesterol at a level of 188 ppm which is remarkably high for a vegetable source.

1.4.10 Hazelnut (Corylus avellana*, also called filberts*)

Hazelnut oil is rich in oleic acid (65–75%) and also contains linoleic acid (16–22%). The level of saturated acids is low. Grown in New Zealand, the nuts produce 55–63 per cent of oil with saturated acids (6–8%), monoene acids (74–80%) and linoleic acid (6–8%). This fatty-acid composition is very similar to that of olive oil and hazelnut is sometimes added as an adulterant to the more costly olive oil.

1.4.11 Honesty (Lunaria annua)

This seed oil contains significant levels of erucic (22:1, 41%) and nervonic acids (24:1, 22%) 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.

1.4.12 Lesquerella oils

The only oil of commercial significance with a hydroxy acid is castor oil (section 1.3.1), but among the new crops being seriously developed are two containing hydroxy acids. Lesquerella oils have some resemblance to castor oil but *Dimorphotheca pluvialis* seed oil contains a different type of hydroxy acid (section 1.4.7). 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 9-18:1
densipolic acid	12-OH 9,15-18:2
lesquerolic acid	14-OH 11-20:1
auricolic acid	14-OH 11,17-20:2

A typical analysis of *L. fendleri* seed oil showed the presence of palmitic (1%), stearic (2%), oleic (15%), linoleic (7%), linolenic (14%), lesquerolic (54%) and auricolic (4%) acids. Since 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. For example, pyrolysis should give heptanal and 13-tridecenoic acid (in place of 11-undecenoic acid). 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. This C_{12} dibasic acid is already available from petrochemical products and has a number of applications.

1.4.13 Marigold (Calendula officinalis)

Interest in this seed oil is based on the fact that it contains significant levels of calendic acid (53–62%) along with linoleic acid (28–34%). Calendic acid (8*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 replacing volatile organic compounds.

1.4.14 Meadowfoam (Limnanthes alba)

This oil is unusual in that over 95 per cent of its component acids are C_{20} or C_{22} compounds and include 5–20:1 (63–67%), 5–22:1 (2–4%), 13–22:1 (16–18%) and 5,13–22:2 (5–9%). It is being grown in the United States 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. Potential uses of this oil include cosmetic applications, production of dimer acid, as a lubricant and through a wide range of novel derivatives based on reaction at the $\Delta 5$ double bond (section 8.3.6, Isbell in Gunstone, 1998).

1.4.15 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. Several health benefits are claimed

for this oil which is now available in encapsulated form and is being incorporated into functional foods. The oil is rich in sterols, carotenoids and tocopherols. The seed oil is rich in oleic, linoleic and linolenic acids, but the berry oil contains significant levels of palmitoleic acid (16–22%) (Baoru in Gunstone, 2003).

1.4.16 Tung oil (Aleurites fordii)

This oil comes mainly from China, which explains 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 non-conjugated 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 and Paraguay (30–40 000 tonnes) and is imported mainly by Japan, South Korea, Taiwan and the United States.

1.4.17 Walnut (Juglans regia)

Walnut oil is an unsaturated oil containing both linoleic (50–60%) and linolenic acids (13–15%) and is rich in tocopherols (~1500 mg/kg of oil). It is used as a gourmet oil in Japan, France and other countries.

1.4.18 Wheatgerm (Triticum aestivum)

This oil is highly unsaturated with linoleic (~60%) and some linolenic acid (~5%). It is valued for its high tocopherol levels (up to 2500 mg/kg of oil).

1.5 Animal fats

1.5.1 Butter fat

Cow milk fat is consumed mainly as milk, butter or cheese 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, including those with *trans* unsaturation, present at low levels (Table 1.6). Attempts to extend the usefulness of butter fat by fractionation and to find alternative uses for the fat and its fractions are hampered by the high cost of the starting material compared with that of vegetable oils and by legislation which strictly defines the term butter.

The production of butter declined during the 1990s but is predicted to rise again. With a world total of 6.3 million tonnes in 2002, the major consuming

Table 1.6 Typical fatty acid composition (%wt) of the major animal fats

	14:0	16:0	16:1	18:0	18:1[a]	18:2
Butter[b]	12	26	3	11	28	2
Lard	2	26	5	11	44	11
Beef tallow	3	27	11	7	48	2
Mutton tallow	6	27	2	32	31	2

[a] including *trans* isomers.
[b] also 4:0 (3%), 6:0 (2%), 8:0 (1%), 10:0 (3%), 12:0 (4%).
Adapted from Gunstone in Hamm & Hamilton (2000).

countries are EU-15 (1.52 million tonnes), India (1.57 million tonnes), former USSR (600 000), USA (520 000), Pakistan (490 000) and Central Europe (200 000). Consumption in EU-15 is mainly in France (430 000) and Germany (410 000) at levels comparable to the United States. Since there is very little trade across national borders (~11 per cent of total production) these consumption figures also reflect production levels. Exceptionally, New Zealand exports over 90 per cent of its butter production and almost 50 per cent of all traded butter comes from New Zealand.

Milk fat is mainly triacylglycerols (97–98%) along with some free acids, monoacylglycerols and diacylglycerols. Also present are cholesterol (0.2–0.4%), phospholipids (0.2–1.0%) traces of carotenoids, squalene and vitamins A and D (section 10.1.1).

The fatty acid composition of milk (Table 1.7) depends on the diet of the cow so that in many countries there is a difference in composition of milk fat produced during the winter when cattle are fed indoors and that produced in the summer when cattle are pasture fed. Milk fat composition can be further modified by appropriate additions to the diet.

Cow milk fat contains over 500 different fatty acids. Most of these are present only at exceedingly low levels but some are important, such as the lactones which provide important flavour notes. Among the many fatty acids are the following:

- saturated acids in the range C_4 to C_{18} including some odd-chain members,
- low levels of iso-, anteiso- and other branched-chain acids,
- a significant level of monoene acids (28–31%) which is mainly oleic but includes other isomers, some with *trans* configuration,
- *trans* acids, produced by biohydrogenation of dietary lipids, are significant components of all ruminant milk fats (about 4–8%) and are mainly C_{16} and C_{18} monoene acids of which vaccenic acid (11*t*-18:1) is the major component,
- very low levels of polyene fatty acids and even those cited as linoleic or linolenic are not entirely the all-*cis* isomers,
- trace amounts of oxo (keto) and hydroxy acids and lactones.

Table 1.7 Major fatty acids (%wt) in cow milk fat

	4:0	6:0	8:0	10:0	12:0	14:0	16:0	18:0	20:0	18:1	18:2	others
June	4.2	2.5	2.3	2.2	2.4	9.0	22.1	14.3	2.6	30.4	1.2	6.8
December	3.5	2.2	1.1	2.6	2.8	10.6	26.0	11.6	2.3	24.8	2.8	9.7
Average	3.6	2.2	1.2	2.8	2.8	10.1	25.0	12.1	2.1	27.1	2.4	8.6

The level of butyric acid at around four per cent by weight may be considered insignificant, but it should be recognised that this is equivalent to about 8.5 per cent 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.

An exciting development in lipid science in recent years has been the recognition of the importance of certain octadecadienoic acids 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 (sections 3.7 and 9.6).

1.5.2 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 1.6). Differences in the fatty acid composition of tallow and lard reflect the fact that tallow (section 1.5.3) is a ruminant fat and dietary lipids are subject to bio-hydrogenation in the rumen, whereas the pig is monogastric and its depot fats more closely resemble the dietary intake. An unusual feature about lard is the fact that it contains high levels of palmitic acid in the *sn*-2 position in its triacylglycerols (72 per cent of the acids in this position are palmitic acid). In this respect, it resembles human milk fat. As a consequence of this unusual feature, its physical properties (especially melting behaviour) are changed markedly when the fat is randomised (section 2.3.4) and it can then be used as an effective shortening. Compared with vegetable oils, animal fats are rich in cholesterol (section 1.6.3) 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.

1.5.3 Tallow

Tallow is mainly fat from cattle. It may contain some fat from sheep but should be free of pig fat (lard). Production of tallow now exceeds 8 million tonnes. About one quarter of this is exported/imported and the balance is used in the country/region in which it is produced. Tallow is traded in six or more

grades depending on levels of free acid, colour of bleached oil, content of moisture and dirt and of unsaponifiable material. Only the highest grades are fit for human consumption. The major fatty acids in the higher quality edible grades include myristic (1–8%), palmitic (17–37%), stearic (6–40%) and oleic (26–50 per cent, including isomeric acids with *trans* unsaturation), but the fat also contains acids with an odd number of carbon atoms, branched-chain acids, and linoleic acid (up to 5%) (Table 1.6).

Tallow is used as a food in spreads and in frying oils, as an energy-rich component of animal feed and in the oleochemical industry. Tallow derivatives (acids, esters, alcohols, soaps and *N*-compounds) are used in personal care products, cosmetics, emulsifiers, etc. From a nutritional viewpoint tallow is perceived as having several disadvantages based on the following:

- It is an animal fat and therefore neither acceptable to vegetarians nor to some religious or cultural groups. This objection extends from food to personal care products. For example, stearic acid incorporated into such materials for use by some groups must be derived from vegetable sources. Associated with this is the overall concern in some communities over animal welfare.
- Almost half of the fatty acids in tallow are saturated and include myristic acid, the saturated acid with greatest effect on raising blood plasma cholesterol levels (section 9.5).
- The level of essential fatty acids is low.
- It contains acids with *trans* unsaturation (~5%).
- It contains cholesterol (~1000 ppm) at levels higher than those found in vegetable oils (negligible) though lower than those in dairy products (2000–3000 ppm).
- It does not contain any 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 (sections 3.7 and 9.6).

1.5.4 Fish oils

For those fortunate to live near rivers, lakes and the sea, fish have been part of their diet for many centuries and trade in dried fish has a long history. The important fishing industry developed when fishermen learned to fish over wider areas of the seas and when improvements in freezing facilities allowed storage at sea and subsequent distribution to urban dwellers. For many, fresh fish and fried fish are now standard parts of their diet. Today's fishing industry is organised to produce fish for consumption as fresh fish and in various processed forms. In addition, some industrial fishing is carried out primarily to produce fish meal (protein) with fish oil only a by-product.

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 provide an additional source of both meal and oil. Annual production of fish oils during the last ten years has been between 1.0 and 1.4 million tonnes with fluctuations in production mainly due to the climatic phenomenon, El Niño. This changes conditions in the ocean, particularly along the coasts of Peru and Chile, causing the fish to move to deeper waters. In many seas and oceans, over-fishing has seriously reduced stocks.

The leading producing countries of fish oil are Peru and Chile, followed by Denmark, the United States, Iceland and Norway. For the South American fishing nations of Peru and Chile, the major fish species used in commercial production of fish meal and oil are anchovy, jack mackerel, Pacific mackerel and sardine. In the fishing grounds of western European fleets (Iceland, Norway, Denmark, UK and Spain) capelin, Atlantic horse mackerel, sandeel, Norway pout, sprat, herring and blue whiting are important. Menhaden and pollack are popular among US fishermen. 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 n-3 acids (Gunstone, 2004b).

Typical fatty acid data for some commercial fish oils are given in Table 1.8. Such oils are rich in saturated acids (mainly myristic and palmitic acids), monounsaturated acids covering the range hexadecenoic through docosenoic, and the *n*-3 C_{20} and C_{22} polyunsaturated fatty acids which are characteristic of fish oils and of which there is no more convenient source (section 9.3).

As shown in Table 1.9 there have been significant changes in the applications of fish oil in the last ten years. Ten years ago, fish oils were used mainly (after partial hydrogenation) for the production of margarine and

Table 1.8 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

Adapted from Haraldsson & Hjaltason in Gunstone (2001).

Table 1.9 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 & Barlow (2000, p. 58).

spreads but this has declined considerably on dietary grounds. In its place, the use of fish oil for aquaculture feed has increased rapidly from 16 to 55 per cent over this period. Fish oil in the feed provides an additional source of energy and the required n-3 acids. Use for industrial purposes remains around six per cent. Pharmaceutical and dietary use of fish oils without partial hydrogenation is based on the very important higher unsaturated fatty acids, vitamins and other materials present in such sources. This use is growing rapidly but remains very small. Refined fish oils or preparations made from these are available in encapsulated form or they may be incorporated into bread, drinks and into infant formulas as a source of long-chain poly-unsaturated fatty acids.

Cod liver oil (annual production ~ 10 000 tonnes) has long been marketed, first as a source of vitamins A and D and now also as a source of the long-chain n-3 fatty acids – EPA and DHA. Shark liver oil (mainly from SE Asia, particularly China) is a source of vitamin A, squalene and alkoxy glycerols.

1.6 Major and minor components

1.6.1 Fatty acids, glycerol esters and waxes

Over 1000 natural fatty acids have been identified (sections 3.2–3.10). These vary in chain length, degree of unsaturation and the presence or absence of other functional groups. Only a limited number of these – perhaps 25–50 at most – are likely to be important to most lipid scientists and technologists. Some members of this small group are detailed in Table 1.10. These common acids may be divided into four categories: saturated acids, monounsaturated acids, polyunsaturated acids of the n-6 family and polyunsaturated acids of the n-3 family (the terms n-6 and n-3 relate to the position of the first double bond with respect to the methyl end of the chain). Within this limited range of acids, unsaturation is confined to olefinic systems with *cis* configuration and the most important polyunsaturated acids have methylene-interrupted patterns of unsaturation. The (largely unnatural) *trans* acids differ from the *cis* isomers in their physical properties (especially melting points) and their nutritional properties. There is sufficient concern about the latter for most food

Table 1.10 Structures of the most common fatty acids

Common name	Symbol	Unsaturation (if any)
Saturated		
Lauric	12:0	–
Myristic	14:0	–
Palmitic	16:0	–
Stearic	18:0	–
Monounsaturated		
Oleic	18:1	9c
Petroselinic	18:1	6c
Erucic	22:1	13c
Polyunsaturated (non-conjugated)		
Linoleic	18:2	9c12c
Linolenic (α)	18:3 (n-3)	9c12c15c
Linolenic (γ)	18:3 (n-6)	6c9c12c
Arachidonic	20:4 (n-6)	5c8c11c14c
Eicosapentaenoic	20:5 (n-3)	5c8c11c14c17c
Docosahexaenoic	22:6 (n-3)	4c7c10c13c16c19c
Polyunsaturated (conjugated)		
Eleostearic	18:3	9c11t13t
Calendic	18:3	8t10t12c
Oxygenated		
Ricinoleic	18:1	12-OH 9c
Vernolic	18:1	*cis*-12,13-epoxy 9c

processors to keep the levels of *trans* acids as low as possible and to advertise that fact when this has been achieved. The common fatty acids are easily recognised and separated from each other by gas chromatography and this technique is a standard analytical procedure in quality control laboratories (section 5.3.4).

A crude oil or fat will usually contain at least 95 per cent of triacylglycerols. After refining, this will rise to 97–99 per cent, depending mainly on the level of (unsaponifiable) material insoluble in aqueous alkali after hydrolysis. Triacylglycerols are fatty acid esters of the trihydric alcohol glycerol and contain three acyl chains in each molecule, usually from two or three types of acid. In vegetable oils, bio-acylation of glycerol phosphate is promoted by enzymes and the fatty acids are distributed in a non-random manner. If the natural mixtures are randomised, the resulting material has the same total fatty acids but different triacylglycerols and consequently different melting behaviour (section 6.1.2). In vegetable oils, the *sn*-2 hydroxyl group is esterified almost entirely with unsaturated acids while saturated acids and the remaining unsaturated acids are in the *sn*-1(3) positions (section 3.12).

Accompanying the triacylglycerols are lower levels of diacylglycerols, monoacylglycerols and free acids. These may result from incomplete triacylglycerol biosynthesis in immature seeds or from post-harvest lipolysis. Almost all the free acid and most of the monoacylglycerol will be removed by refining, but diacylglycerols tend to remain in the product. These are usually in the range 0–2 per cent but refined palm oil contains up to six per cent of diacylglycerols.

After conventional refining, some oils such as rape/canola, corn, ricebran and sunflower contain high melting material that slowly crystallises when the oil is stored at sub-ambient temperature. This causes a haze in the oil which does not find favour with the users of salad oils and frying oils even though it is acceptable from a nutritional standpoint, This is caused mainly by wax esters which can be removed by holding the oil at about 5ºC for several hours and then filtering (at a slightly higher temperature to reduce viscosity) with the assistance of a filter aid. Levels of waxes sometimes rise during breeding programmes.

1.6.2 Phospholipids

Crude oils generally contain phospholipids, which are removed during refining at the degumming stage. These valuable byproducts are the basis of the phospholipid industry and are used extensively in food products, in animal feeds and industrial processes. The major components are phosphatidylcholines (PC), phosphatidylethanolamines (PE) and phosphatidylinositides (PI) accompanied by smaller proportions of other phospholipids. Soybean oil (3.2%), rapeseed oil (2.5%), and sunflower seed oil (1.5%) contain the proportions of total phospholipids indicated in parenthesis and are the main sources of commercial lecithins with palm oil containing little or no phospholipids.

The structures of phospholipids are detailed in section 3.3.15. The major phospholipids in crude lecithin depend, in part, on the source. Crude lecithin recovered from the degummimg of soybean oil contains PC (10–15%), PE (9–12%), and PI (8–10%) along with other minor phospholipids and triacylglycerols. This product can be treated in various ways to give increasingly pure fractions. The triacylglycerols can be washed out with acetone – a process called de-oiling – to give a product with 50–80 per cent phospholipids. Further purification by fractionation with an alcohol followed by chromatogaphy can provide fractions with >90 per cent of a single phospholipid class. Lecithin is used in animal feed, in chocolate/confectionery and in a range of other food products (Gunstone in Gunstone, 2001). Useful information on phospholipid structure and composition has been provided by Christie (website).

1.6.3 Sterols

Most vegetable oils contain 1000–5000 mg/kg of sterols, partly as free sterols and partly as esterified sterols. Higher levels are present in rapeseed oil (5–11 g/kg, mean ~7.5) and in corn oil (8–22 g/kg, mean 14). Sitosterol is generally the major phytosterol (50–80 per cent of total sterols) with campesterol, stigmasterol and Δ^5-avenasterol frequently attaining significant levels. Brassicasterol is virtually absent from the major seed oils except for rapeseed oil where it comprises 10 per cent of the total sterol (see Przybylski & Mag in Gunstone, 2002).

Table 1.11 contains results of detailed sterol analyses of most of the major oils in their crude state. Total sterols range from 60–910 mg/kg, with palm oil and the two lauric oils being lowest (60–80 mg/kg) and corn, rape and cottonseed oil having the highest levels at 500–900 mg/kg. The ratio of esterified to free sterol also varies with the free sterols (41–83%) generally predominating.

Sterols can be recovered from deodoriser distillate along with other compounds. Deodorisation is essentially a steam-distillation carried out between 200 and 260ºC (though preferably below 245ºC) at $3–9\times10^{-3}$ bar. In the process the following are removed: free acids, aldehydes, ketones and other short-chain compounds resulting from oxidation, tocopherols (vitamin E), sterols, carotene degradation products, nitrosamines, residual extraction solvent, organo-chlorine pesticides and volatile sulfur compounds. The sterols are used as precursors of many pharmaceutical products.

Cholesterol is generally considered to be a zoosterol and is not present in plant systems at any significant level. The normal value of 20–50 ppm in vegetable oils compares with the much higher levels reported for animal fats (up to 1000 ppm), fish oils (up to 7000 ppm), dairy fats (2000–3000 ppm) and egg yolks (12 500 ppm). Phytosterol esters are now being added to margarines at significant levels (up to 10%) because they are considered to reduce cholesterol levels (see Salo *et al*. in Gunstone, 2003 and section 9.8.2).

1.6.4 Tocols

Tocol extracts are mixtures of up to eight compounds (Fig. 1.1). There are four tocopherols with a saturated branched C_{16} side chain and four tocotrienols which are analogous compounds with three double bonds in the side chain. The tocotrienols are significant in palm oil but are generally less common than the tocopherols and much less is known about their biological properties. The four tocopherols differ in the number of methyl groups attached to the heterocyclic moiety. They are designated α (5,7,8-trimethyl), β (5,7-dimethyl), γ (7,8-dimethyl) and δ (8-methyl).

The tocols have two valuable properties: they show vitamin E activity and they are powerful antioxidants. These two properties are not identical. The tocols

Table 1.11 Content of sterols and sterol esters (mg/100 g) in selected vegetable oils

Oil	Esterified sterols (%)	Free sterols (%)	Total sterols	Camp	Stigma	Sito	Δ^{5-} Avena
Coconut (crude)	26 (39)	41 (61)	67	8	13	49	–
Corn (crude)	423 (47)	485 (53)	908	200	68	646	10
Cotton (degummed)	87 (18)	402 (82)	489	33	5	402	19
Olive (cold pressed)	31 (17)	151 (83)	182	2	–	130	44
Palm (crude)	16 (25)	49 (75)	65	14	10	43	3
Palm olein (crude)	23 (29)	56 (71)	79	19	10	51	–
Peanut (refined)	73 (35)	133 (65)	206	38	22	169	–
Rapeseed (crude)[a]	475 (59)	336 (41)	811	293	–	420	111
Soybean (crude)	79 (25)	239 (75)	318	57	58	173	13
Sunflower (bleached)	114 (29)	285 (71)	399	41	34	265	43
Walnut (refined)	57 (42)	81 (58)	139	8	–	136	–

[a] also brassicasterol 86

Camp = campesterol, Stigma = stigmasterol, Sito = sitosterol, Avena = avenasterol

This Table is adapted from Verleyen *et al.* (2002). Crude oils have been selected where these are listed in the original paper. The paper contains additional information on other samples of these oils. Since sterol contents vary, the above values must be taken only as representative. Other figures are available in Gunstone (2002).

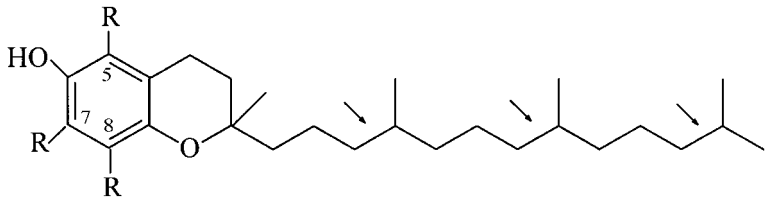


Figure 1.1 Tocopherols and tocotrienols. Tocopherols have a saturated C_{16} side chain, tocotrienols have three double bonds at the positions indicated by the arrows, R=H or CH_3, α = 5,7,8-trimethyltocol, β = 5,8-dimethyltocol, γ = 7,8-dimethyltocol, δ = 8-methyltocol.

differ in their vitamin E activity and total vitamin activity is often presented as α-tocopherol equivalents (mg α-tocopherol/mg compound) based on equivalence values for tocopherols α (1.0), β (0.5), γ (0.1) and δ (0.03), and tocotrienols α (0.3) and β (0.05). An alternative proposal that only α-tocopherol should be counted is now being debated. Some countries classify refined vegetable oils as being an excellent source, a good source, or merely as a source of vitamin E. Typically, sunflower and cottonseed oils are excellent sources, corn and rapeseed (canola) are good sources and groundnut, palm and soybean are sources. If less of the tocopherols were removed during deodorisation some of these descriptions could be raised by one grade.

Some typical levels are given in Table 1.12. Among readily available oils, palm and sunflower (as well as walnut and wheatgerm oils) are good sources of vitamin E because of the high level of the α compound, whereas soybean tocopherols are effective antioxidants by virtue of the high levels of γ and δ compounds. These compounds are recovered from refinery by-products such as PFAD (palm fatty acid distillate) and soybean deodoriser distillate. The latter contain up to 10 per cent of mixed tocopherols. The effectiveness of the tocotrienols is not fully known.

The annual production of vitamin E is about 20 000 tonnes made up mainly (90%) of synthetic vitamin E. This is produced from trimethylhydroquinone and (all-*rac*-)phytol and contains all eight possible racemic forms of α-tocopherol. The balance is natural material, mainly from soybean. The latter is an excellent antioxidant but its vitamin E activity is limited because of the low proportion of the α compound. This can be raised by a methylation procedure which converts the β, γ and δ compounds with one or two methyl groups to α-tocopherol with three methyl groups. These materials are used in animal feed, food for human consumption and in the cosmetic and pharmaceutical industries. Further details are given by Netscher in Gunstone (1999).

Crude palm oil contains around 700–1100 ppm of tocols of which α-tocopherol represents 22 per cent and β-, γ- and δ-tocatrienol represent 20, 46 and 12 per cent, respectively. About 70 per cent of this mixture remains in the

Table 1.12 Content of tocopherols and tocotrienols (ppm equivalent to mg/kg) in selected vegetable oils

Oil	α-toc	β-toc	γ-toc	δ–toc	α-T3	γ-T3	δ–T3	Total
Canola	272	–	423		–	–	–	770
Coconut	3	1	1	–	4	–	–	10
Corn	191	–	942	42	–	–	–	1175
Cottonseed	–	–	–	–	–	–	–	1000
Groundnut	–	–	–	–	–	–	–	650
Linseed	8	–	500	6	–	–	–	514
Linola	20	–	471	16	–	–		507
Olive	–	–	–	–	–	–	–	200
Palm	189	–	–	–	207	405	99	900
Palmkernel	2	21	9	–	–	2	–	34
Rice bran	347	–	89	42	126	301	10	915
Sesame	–	–	335	–	–	–	–	
Soybean	144	16	870	342	–	–	–	1370
Sunflower	608	17	11	–	–	–	–	
Walnut	563	–	595	450	–	–	–	
Wheatgerm	1330	71	260	271	26	18	–	1976

– not detected or not reported
Information taken from appropriate chapters in Gunstone (2002).
Other sources provide different figures so these should only be taken as typical. They refer to crude oils and levels will probably be reduced in refined oils.

oil after refining and the remainder is concentrated in the PFAD at a level 5–10 times higher than in the original oil. This fraction is used as a source of palm vitamin E which is 95 per cent tocols rich in tocotrienols (>60%).

Natural tocopherol mixtures are used as antioxidants at levels up to 500 ppm along with ascorbyl palmitate which extends the antioxidant activity. At higher levels (>1000 ppm) α-tocopherol is considered to act as a pro-oxidant. Since vegetable oils contain tocols at 200–800 ppm further additions show only a limited effect. The tocols themselves are very sensitive to oxidation and are more stable in esterified form where the all-important hydroxyl group is not free. However, such compounds do not show antioxidant activity until they have been hydrolysed *in vivo* to the free phenolic form (sections 7.2.7 to 7.2.9).

1.6.5 Fat soluble vitamins

Lipids are valuable dietary sources of fat-soluble vitamins. These include vitamins:

- A (retinol) involved in growth and differentiation of tissue and in good vision,
- D (cholecalciferol) required for calcium absorption and retention in bone,

- E (tocopherol) which is a powerful antioxidant, and
- K (menaquinones) involved in blood clotting.

There are alternative sources of vitamins D and K but fats provide the only natural dietary source of vitamin A as a precursor carotene (section 1.6.7) and of vitamin E (section 1.6.4). The level of these components may be reduced during refining processes and vitamin K is converted to its dihydro derivative through fat hydrogenation.

1.6.6 Chlorophyll

Chlorophyll and its magnesium-free derivative (pheaphytin) are not wanted in refined oils because they produce an undesirable green hue in the oil and act as sensitisers for photo-oxidation (section 7.2.3). No general listing of chlorophyll/pheaphytin levels has been discovered but the following information has been gleaned from a range of sources. Levels cited for chlorophyll in the following statements include pheaphytin:

- Olive oil: chlorophyll levels vary with the maturity of the olive and with the method of extraction. Unrefined oils contain 1–20 ppm of chlorophyll.
- Canola oil: levels of chlorophyll in crude oils (5–35 ppm) are much reduced (<50 ppb) by alkali-refining and bleaching.
- Soybean: low levels of chlorophyll in crude oil (1–1.5 ppm) are reduced to around 15 ppb after refining.
- Sunflower: refined oil contains <30 ppb of chlorophyll.
- Palm: crude palm oil contains 250–1800 ppb of chlorophyll (mean value 900 ppb). The level falls with increasing maturity of the palm fruit.

1.6.7 Hydrocarbons

Hydrocarbons are minor components of oils and fats but they are of dietary and legislative interest. They include alkanes, alkenes (such as squalene and carotenes) and polycyclic aromatic hydrocarbons (PAH).

1.6.7.1 Alkanes

Many studies of alkanes ignore the more volatile compounds (up to C_{12}) because of analytical difficulties. They are not likely to be significant in refined oils that have been submitted to high-temperature deodorisation. Levels of C_{13}–C_{33} alkanes in crude oils are usually between 40 and 100 mg/kg (ppm) with lower values for refined oils. Typical values for samples purchased from local retail outlets in the UK are: olive (30–100), sunflower (100–170), corn (25–35) and groundnut oil (25–35). There is a preponderance of odd carbon molecules.

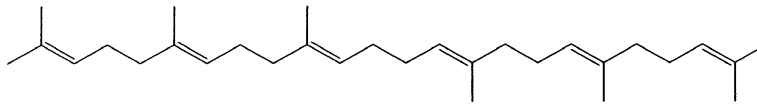


Figure 1.2 Structure of squalene. Copied with permission from Gunstone & Herslof (2000).

1.6.7.2 Squalene ($C_{30}H_{50}$)

This is a highly unsaturated open-chain triterpene used in the cosmetic industry, usually after hydrogenation to squalane ($C_{30}H_{62}$) (Fig. 1.2). The most abundant source of squalene is the liver oil of the deep sea dog fish (*Squalus acanthus* – hence the name squalene) and some other marine species. Vegetable sources of potential interest include olive oil (section 1.3.8) and amaranthus. Squalene levels of 100–1200 mg/100 ml of oil have been reported in olive oil with most samples containing 200–500 mg/100 ml. This rises to 10–30 per cent in the deodoriser distillate. Amaranthus oil contains 6–8 per cent squalene and this concentration can be raised 10-fold after short-path high vacuum distillation.

1.6.7.3 Carotenes

These are minor components in many vegetable oils and particularly in palm oil (Fig. 1.3). They contain a long chain of conjugated unsaturation and are yellow/orange/red in colour. It has been reported recently that pure β-carotene is black but that it reacts immediately with oxygen and assumes its familiar red/orange colour. Crude palm oil normally contains 500–700 ppm of carotenes. These are mainly α- and β-carotene (at 30–40% and 55–60% of total carotene respectively) with low levels of several other carotenes. Carotenes are also present in palm leaves and in pressed fibre remaining when oil has been expressed from palm fruits. This fibre still contains 5–6 per cent of oil which is very rich in carotenes (4000–6000 ppm). When palm oil is refined, bleached and deodorised, the carotenes are completely destroyed.

Some carotenes are a source of vitamin A and act as powerful antioxidants against both autoxidation and photo-oxygenation (section 7.2.8), and show anti-cancer activity. Attempts have therefore been made to retain these valuable materials in refined palm oil or to recover them in concentrated form. Products such as red palm oil retain most of the original carotene when

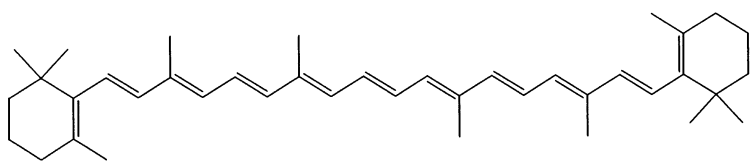


Figure 1.3 β-carotene. Other carotenes vary in the nature of the cyclic end groups. Copied with permission from Gunstone & Herslof (2000).

deodorisation is effected at temperatures below 150ºC (Che Man & Chin-Ping Tan in Gunstone, 2003).

Carotenes can be recovered from palm methyl esters, prepared by methanolysis of palm oil and produced in large quantities for use as biodiesel, as a solvent and for conversion to alcohols, etc. Enhancement of carotene content is achieved by chromatography in an open column or by molecular distillation. The latter gives a carotene concentrate (8%) which can be purified (>90%) by chromatography. Various methods of obtaining carotene from palm oil have been reviewed by Thyrion (in Gunstone, 1999). He also reports that in 1989 the annual production of carotenes was about 300 tonnes of which about 90 per cent was synthetic in origin and the rest of natural origin from palm oil or from algal sources. The natural product is more expensive at 2–3 times the price of the synthetic product. It is likely that total production and the proportion from natural sources have both increased since 1989. The concentrates are used as food-dyes, as a vitamin additive and by the pharmaceutical and cosmetic industries.

1.6.7.4 Polycyclic aromatic hydrocarbons

These are present at levels up to about 150 μg/kg (ppb) in most crude vegetable oils, though slightly less after refining (<80 ppb). They are removed only to a small extent during bleaching and somewhat more during deodorisation. This holds particularly for the more volatile tri and tetracyclic compounds. The pentacyclic and other less volatile compounds are best removed when activated charcoal is added to the earth during bleaching. These low values do not hold for crude coconut oil. This is dried with combustion gases and values around 3000 ppb are routinely recorded.

1.7 Oils and fats in the market place

This chapter ends with some details of the annual production of 17 commodity oils and fats. Table 1.13 gives average annual figures for selected five-year periods between 1976 and 1980 and 2006 and 2010 forecasts. Details are given for total production and exports/imports, for the four major vegetable oils (soybean, palm, rapeseed and sunflower), for nine minor vegetable oils, and for four animal fats. The final column shows the expected increase over the thirty-year period covered in the Table and emphasises the importance of the four major vegetable oils. In a period when total production increased by 280 per cent (and exports/imports by 410 per cent) both the minor vegetable oils and the animal fats rose by smaller values of 200 per cent and 150 per cent respectively so that although production levels increased, these two groups of oils have lost market share and are expected to continue to do so. In contrast, the major oils have more than maintained

Table 1.13 Average annual production of oils and fats (million tonnes) over the period 1976–1980 to 2006–2010 (forecasts)

	76–80	86–90	96–00	06–10	Increase[a]
Production	52.6	75.7	105.1	146.7	2.8
Exports[b]	12.4	20.9	32.9	50.8	4.1
Soybean	11.2	15.3	23.1	33.6	3.0
Palm	3.7	9.2	18.7	31.4	8.5
Rapeseed	3.0	7.5	12.6	17.7	5.9
Sunflowerseed	4.2	7.2	9.1	12.4	2.9
Other veg oils	13.3	16.7	20.3	26.2	2.0
Animal fats	17.2	19.8	21.3	25.4	1.5

Source: The Revised Oil World 2020, ISTA Mielke GmbH, Hamburg (2002)
[a] increase (fold) between 1976–1980 and 2006–2010.
[b] imports are virtually the same as exports.

market share and rapeseed oil (590 per cent) and palm oil (850 per cent) particularly have increased considerably.

Table 1.14 provides details of production levels for the 17 commodity oils and fats over the five years 1998 to 2002 along with the latest predictions for 2003. These figures show that despite overall increases, changes vary from year to year and individual commodities may even show a decline in particular years. In the four years between 1998 and 2002 total production has increased by 17.5 million tonnes but annual increases have varied markedly at 6.9, 4.8, 3.1 and 2.7 million tonnes. The smaller increases in production have even been below the annual increases in consumption. Increased production has depended strongly on soybean oil and palm oil, while rapeseed and sunflowerseed oils have declined from peak levels recorded in 2000. The minor vegetable oils show small overall increases with fluctuations up and down with the exception of palmkernel oil which has increased steadily, based on its association with palm oil production. It is interesting to note that the three land animal fats (tallow, lard and butter) occupy rankings 5, 6 and 7 after the four major vegetable oils. Coconut and palmkernel oil occupy positions 10 and 11, after groundnut and cottonseed oils, but together these two important lauric oils exceed 6 million tonnes and at this level would come eighth in the rankings. Sesame, linseed and castor oils are each produced at well below 1 million tonnes each year.

The uneven changes from year to year result in variations in the relation between supply and demand and consequently affect stocks. When stocks rise, prices fall, demand increases and production declines because it becomes less profitable. On the other hand, when stocks fall, prices rise, demand is stifled, but production then increases as profit margins grow. In this way the market takes care of changes in agricultural production arising both from man-made decisions and from changes in climate.

Table 1.14 Annual production of oils and fats (million tonnes) in the years 1998–2002

Oil	1998	1999	2000	2001	2002
Four major oils					
Soybean	24.01	24.78	25.53	27.79	29.75
Palm	17.15	20.62	21.87	23.92	25.03
Rapeseed	12.29	13.21	14.47	13.69	13.33
Sunflower	8.41	9.29	9.70	8.14	7.61
Nine minor vegetable oils					
Cottonseed	4.06	3.90	3.87	4.05	4.18
Groundnut	4.50	4.70	4.55	5.06	5.30
Sesame	0.71	0.69	0.71	0.74	0.83
Corn	1.87	1.93	1.97	1.96	2.02
Olive	2.59	2.47	2.54	2.76	2.66
Palmkernel	2.19	2.56	2.69	2.93	3.00
Coconut	3.15	2.40	3.28	3.51	3.11
Linseed	0.69	0.73	0.70	0.65	0.63
Castor	0.44	0.43	0.50	0.51	0.44
Four animal oils and fats					
Butter	5.76	5.92	6.04	6.10	6.30
Lard	6.52	6.62	6.67	6.72	6.91
Tallow	7.81	8.17	8.19	8.15	8.40
Fish	0.89	1.41	1.42	1.13	0.97
Total	103.0	109.9	114.7	117.8	120.5

Source: Oil World Annual 2003, ISTA Mielke GmbH, Hamburg (2003).
Forecasts for 2003/2004: total 128.7, soybean 32.8, palm 27.5, rapeseed 13.3 and sunflowerseed 9.2 million tonnes.
Total exports for these five years are 33.2, 35.0, 36.5, 38.7 and 41.3 million tonnes, imports are virtually the same as exports.

Bibliography and references

Christie, W.W. www.lipid.co.uk
Gunstone, F.D. (2000) Composition and properties of edible oils. In: *Edible Oil Processing*, (eds W. Hamm & R. J. Hamilton), The Oily Press, Bridgwater, England. pp. 1–33.
Gunstone, F.D. (ed.) (2001) *Structured and Modified Lipids*, Marcel Dekker, New York.
Gunstone, F.D. (ed.) (2002) *Vegetable Oils in Food Technology*, Blackwell Publishing, Oxford.
Gunstone, F.D. (ed.) (2003) *Lipids for Functional Foods and Nutraceuticals*, The Oily Press. Bridgwater.
Gunstone, F.D. (ed.) (2004a), *Rapeseed (Canola) Oil*, Blackwell Publishing, Oxford.
Gunstone, F.D. (2004b) in fish oils (LFRA), in press.
Gunstone, F.D. & Hamilton, R.J. (eds) (2001) *Oleochemical Manufacture and Applications*, Sheffield Academic Press, Sheffield.
Gunstone, F.D. & Herslof, B.G. (2000) *Lipid Glossary – 2*. The Oily Press, Bridgwater.
Pike, I.H. & Barlow, S.M. (2000) The fats of life – the role of fish, *Lipid Technology*, **12**, 58.
Rossell, B. (ed.) (2001) *Oils and Fats Volume 2 Animal Carcass Fats,* Leatherhead Publishing, Leatherhead.
Smith, J.R. (1996), *Safflower*, AOCS Press, Champaign, Illinois.

Stewart, C. (2002) Authenticity of edible oils and fats: the legal position. In: *Oils and Fats Authentication*, (ed M. Jee), Blackwell Publishing, Oxford.

Stewart, I.M. & Timms, R.E. Fats for chocolate and sugar confectionery. In: *Fats in Food Technology*, (ed K.K. Rajah), The Oily Press, Bridgwater.

Timms, R.E. (2003) *Confectionery Fats Handbook*, The Oily Press, Bridgwater.

Verleyen, T., Forcades, M. & Vehre, R., Dewetlinck, K., Huyghebaert, A. & De Greyt, W. (2002) Analysis of free and esterified sterols and vegetable oils, *Journal of American Oil Chemical Society*, **79**, 112–122.

2 Extraction, refining and processing

This chapter is devoted to the processes whereby fats and oils of appropriate quality can be obtained from the natural sources in which they occur. These may be tissues from land animals containing 60–90 per cent fat, whole fish with 10–20 per cent oil, or vegetable seeds and soft plant tissue with 20–70 per cent oil. In most cases (though not all) the crude oil will subsequently be refined to an acceptable quality and finally processed to make it more suitable for its end use in terms of its physical, chemical, and nutritional properties. During all these processes, as well as during storage and transport, the oil must be protected against deterioration, which is likely to be mainly hydrolytic or oxidative.

2.1 Extraction

Animal fats, from whole animals, depot fats, or viscera, contain active enzymes, so the fat should be extracted as quickly as possible. Oils to be extracted from whole fish or from fish waste are subject to the same problems and the highly unsaturated nature of fish oils also make them liable to rapid oxidation. Vegetable fruits, such as those from the oil palm, have a high moisture content and here also the oil should be extracted as quickly as possible to minimise enzymic hydrolysis. Vegetable seeds are drier and can be safely stored or transported prior to extraction at moisture levels between 7 and 13 per cent. Soybeans, for example, are normally traded at 12 per cent moisture content at which level storage life is 6–9 months. At a moisture level of 10 per cent they can be safely stored for up to 4 years.

Fat is recovered from land animals by reduction to suitably sized pieces (hashing) followed by steaming. The membranes of animal cells are weaker than those of plant cells and burst as the fat expands through heating. Treated with wet steam, the temperature is raised to around 100ºC and the mixture agitated. After settling, the oil layer is first decanted and then centrifuged. Alternatively, after heating with dry steam or indirect steam, the treated product is put through a screw press and the oil/water mixture is separated with a decanter and a centrifuge. Oil is removed from fish by a similar process of dry rendering. At these temperatures enzymes are deactivated.

Palm fruits are first sterilised at around 130ºC to loosen individual fruits in the fruit bunch and to deactivate hydrolytic enzymes (lipases). The fruits are then broken up in a digester to produce liquid (oil and water) and wet solids, and these are separated in a screw press. Oil is freed from water by centrifugation and by vacuum drying.

Seeds are pre-treated in a series of processes involving cleaning, dehulling or decorticating, size-reduction if necessary, cooking at 90–115ºC and flaking to about 0.3–0.4 mm thickness to rupture the cell. The resulting material can then be put through a screw press (expeller) at 3.5 kg/mm^2 to squeeze out the oil and leave protein meal still containing 3–6 per cent of oil. If the seed cake is to be solvent extracted, it is sufficient to operate the screw press at about 2 kg/mm^2. The residual cake, still containing 18–29 per cent oil, is then extracted by percolation or immersion in solvent and finally contains only 0.7–1.5 per cent of oil.

When the seeds are not very rich in oil, pre-pressing is not undertaken and the seeds, suitably adjusted to size and moisture content, are extracted directly with solvent. This is generally 'hexane' which is a mixture of hexane and methylpentane. The solvent is recovered for re-use, but there is a loss of 8–10 litres/tonne of meal and 2 litres/tonne of oil. The oil yields of some common vegetable sources are given in Table 2.1.

The US Environmental Protection Agency has ruled that from April 2004, any facility in that country emitting more than 10 tonnes per year of n-hexane will be subject to new rules and that any new facility will have to incorporate the most efficient technology for hexane emissions. It is expected that most plants will change over from n-hexane to methylpentane (also called iso-hexane). This is a more expensive solvent, but has a lower boiling point (60.3 compared to 69ºC) so there will be compensating savings. It is also less toxic than hexane. Ethanol and isopropanol are other possible solvents but with higher boiling points and higher latent heats there will be higher energy costs.

Table 2.1 Yields (%) of oil and of meal obtained by extraction of the major oilseeds

Oilseed	Oil	Meal
Soybean	18.3	79.5
Cottonseed	15.1	57.4
Groundnut	40.3	57.2
Sunflower	40.9	46.9
Rapeseed	38.6	60.3
Palmkernel	44.6	54.0
Copra	62.4	35.4
Linseed	33.3	64.2

Source: Figures are based on world crushing data for the year 2000/2001 given in OW Annual 2001 published by ISTA Mielke GmbH, Hamburg, Germany.

Oils from palm fruits and from olive are obtained by pressing without the involvement of any solvent. Cold pressing (in which the material being extracted is not heated by external means even though the pressing process itself generates considerable heat) is favoured for the production of niche products where it is desirable that solvents should not be used. The recovery of oil under these conditions is less efficient and will be reflected in the price. However, after recovery of cold-pressed oil, it is possible to get further (lower grade) oil by solvent extraction.

Supercritical carbon dioxide can be used as an extraction solvent. It has some environmental advantages compared with more traditional solvents. The oil extracted in this way differs from conventional solvent-extracted oil in the proportion of more polar and less polar lipids present. It may be used for some gourmet oils but has not been used on a large scale for commodity oilseeds.

2.2 Refining

There are several refining processes and the purpose of these is to convert the crude oil or fat into a product more suitable for its end purpose. This will involve the removal of undesirable components and will usually result in a product with minimal colour and flavour. The processes have been devised to minimise changes in the triacylglycerols and in the levels of those minor components which confer nutritional benefit. If the oil is to be processed with the aid of a catalyst (hydrogenation, interesterification, etc.), it will also be important to remove or minimise poisons that reduce catalyst efficiency. The compounds removed by refining include phospholipids, free acids, mono- and di-acylglycerols, colour, trace metals, oxidation products and environmental contaminants. The processes are summarised in Table 2.2 and discussed briefly below. Some of the 'impurities' removed in the refining processes are valuable by-products that can be recovered and used.

Oils may be refined by a series of processes that can be grouped together as 'chemical refining' or 'physical refining'. The former involves degumming, chemical neutralisation, bleaching and deodorisation, while the latter requires only bleaching and steam distillation (deodorisation). Physical refining is preferred in that it is a more economical process requiring less chemicals, producing less waste and giving higher oil yields. The advantages are particularly apparent with oils (such as palm oil) that have high levels of free acid and low levels of phospholipids. The chemical method is preferred for oils with high phospholipid levels and for cottonseed oil which contains gossypol that can only be conveniently removed by alkali treatment.

A fully-refined oil will have the following characteristics which represent maximum values unless indicated otherwise: free acid (0.05%), *trans* acids for

Table 2.2 Refining processes

Process	Procedure	Impurities removed or reduced
Degumming	H_3PO_4, H_2O, 70–80ºC	Phospholipids, trace metals, carbohydrates, proteins
Neutralisation	NaOH or other alkali	Fatty acids. phospholipids, pigments, trace metals, sulfur compounds
Washing	Water	Soap
Drying	–	Water
Bleaching	bentonite, etc.	Pigments, oxidation products, trace metals, sulfur compounds, phospholipids, traces of soap
Filtration	–	Spent bleaching earth
Deodorisation or physical refining	Steam at reduced pressure	Fatty acids, mono- and di-acylglycerols, oxidation products, pigment decomposition products, pesticides
Polishing	–	Traces of oil insolubles

oils free of linolenic acid (0.5%) and oils containing linolenic acid, such as soybean and rapeseed oils (1.0%), moisture (0.05%), peroxide (0.5%), total metals (0.1 ppm), PAH (25 ppb) and smoke point (220ºC minimum) as a consequence of the low level of free acid.

2.2.1 Degumming

Degumming is the treatment of crude oils with water or dilute acid (phosphoric or citric) to remove phospholipids. These are powerful emulsifying agents that increase refining losses if not removed. They also tend to carry associated metals which act as pro-oxidants. Degumming should be carried out as soon as possible after extraction and is usually linked with extraction rather than with the later refining processes. The phospholipid-rich fraction may be added to the meal destined to be used as animal feed or it (lecithin) may be used as the source for a range of phospholipid preparations (section 1.6.2). Crude oils contain up to 1200 ppm of phosphorus, equivalent to three per cent phospholipids. After degumming, this level should be reduced to 20–50 ppm. For physical refining, the phosphorus level has to be even lower than this and can be achieved with oils such as palm oil where the crude oil has only 15–30 ppm or by more advanced methods of degummimg.

2.2.2 Neutralisation

This is effected by treatment with alkali and should be carried out with minimum loss of neutral oil. The resulting soapstock is separated and can be acidified to give fatty acids. A similar result can be achieved by steam

distillation (physical refining), but this can only be applied to oils of low phospholipid level (section 2.2.4). Soapstock contains free acids as sodium salts (usually) contaminated with triacylglycerols and phospholipids. Traditionally, this has been acidified with sulfuric acid to provide crude fatty acids for soap making or as additives to animal feeds. This process also produces large volumes of acidic water which, in these environmentally-conscious days, has become an embarrassment and has increased the popularity of physical refining. It has been suggested that replacement of sodium hydroxide by potassium hydroxide and neutralisation of the sulfuric liquor by ammonia or ammonium hydroxide will trap these ions as valuable fertilisers (potassium and ammonium sulfate). Chemical and enzymatic methods of converting soapstock (as a cheap starting material) to methyl esters for use as biodiesel have also been described.

2.2.3 Bleaching

Bleaching is carried out mainly to reduce colour (carotenes, chlorophyll) and involves heating the oil at 80–180ºC (mainly at 90–120ºC) in the absence of oxygen with a bleaching earth such as bentonite, fullers' earth, activated carbon, or amorphous silica. The bleaching earth is usually activated by heating and by treatment with sulfuric or hydrochloric acid. The acid-treated earth will decompose hydroperoxides and dehydrate free sterols to steradienes. It also catalyses the formation of *trans* acids (up to 0.1%) and formation of diacylglycerols. The level of bleaching agent is 0.2–2 per cent of the weight of oil. Palm oil can be heat-bleached at high temperatures in the absence of bleaching earth through thermal reactions of carotenes. Bleaching is particularly important in physical refining since it provides the last opportunity in the process to remove, in addition to colour, the remaining traces of phospholipids, soaps, metals and oxidation products. Activated carbon is particularly important for the removal of pigments and of polycyclic aromatic hydrocarbons (PAH).

2.2.4 Deodorisation and physical refining

These final steps in the refining process are designed to produce an oil with bland flavour and odour and good shelf life (section 7.2). This requires, particularly, the removal of oxidation products responsible for off-flavours and is achieved by heating at temperatures between 170 and 250ºC under reduced pressure and using steam as a sparge for about one hour. With higher temperatures (especially > 220ºC) there is some danger of stereomutation (conversion of *cis* to *trans* isomers). This is greater with linolenic acid than with linoleic or oleic acid and is, therefore, particularly of concern with soy-bean and rapeseed oils which contain up to 10 per cent of this acid. Physical

refining requires harsher conditions than chemical refining because of the need to volatilise free acids. This can be achieved by using higher temperatures, but as these promote isomerisation it is better to reduce the pressure from three mbar to two mbar and to use more steam.

Deodoriser distillate is a useful source of sterols and tocopherols (sections 1.6.3 and 1.6.4). Its composition differs between physical and chemical refining by reason of the nature of the material being submitted to deodorisation and of the conditions employed. The main components are neutral oil, fatty acids, and unsaponifiable material. In chemical refining these have ranges of 25–33, 33–50 and 25–33 per cent respectively and in physical refining 5–10, 80–85 and 5–10 per cent. The distillate from physical refining is a useful source of fatty acids, while chemical refining gives a better product for the recovery of sterols and tocopherols from the unsaponifiable fraction.

The deodoriser distillate from palm oil refining is usually described as palm fatty acid distillate (PFAD). It is a useful source of fatty acid and of tocopherols/tocotrienols.

2.2.5 Super-refining (chromatography)

Triacylglycerols and wax esters are generally used in the cosmetics and pharmaceutical industries as carriers of more costly materials such as pigments, perfumes, or drugs. It is therefore desirable that they should be free of colour and odour after conventional refining. This can be achieved by chromatography on a multi-kilogram scale and is applied to oils/fats and to phospholipids.

Crude phospholipid preparations are mixtures of different phospholipid classes. It is possible to separate these into fractions containing only one type of phospholipid. These are still mixtures of compounds differing in their fatty acid composition and will vary with their origin (section 1.6.2).

2.3 Processing

The commodity oils are limited in number and composition and are not always optimised for their end-use. This applies particularly to their nutritional and/or physical properties. Efforts are being made to breed plants with a more desirable fatty acid composition by conventional seed breeding procedures and also by genetic manipulation, but this approach cannot provide immediate solutions. Throughout the twentieth century, technologists designed and improved a range of techniques to solve this problem. These include fractionation, partial hydrogenation and interesterification.

2.3.1 Blending

The mixing of oils and fats to produce blends with improved nutritional or physical properties has a long history. Most spreads, for example, contain blends of two or more oils in order to achieve desirable nutritional and essential physical properties. Interesterification is usually carried out on oil blends. Oils are also blended to obtain the desired mix at minimum cost and computer programs have been developed to give the best blend. Mixtures of vegetable oils with appropriate fatty acid composition, sometimes containing fish oils, and effective antioxidants are now available as 'healthy oils' (section 9.3).

2.3.2 Fractionation

Fractionation is a procedure for adding value to an oil or fat by separating it into two (or more) fractions differing in fatty acid and triacylglycerol composition, These changes affect physical properties and so increase the oil's range of usefulness. Separation can be conducted in a solvent and while this may add to the efficiency of the separation it also adds to the cost. The most common procedure (dry fractionation), involves crystallisation of less soluble triacylglycerols from the liquid oil. The crystals will be less soluble, higher melting and more saturated and are called the 'stearin fraction'. The portion that remains liquid is more soluble, lower melting, more unsaturated and is the 'olein fraction'. In simple terms triacylglycerols of type SSS and SUS will concentrate in the stearin and those of type SUU and UUU will concentrate in the olein. However, this simple view will be modified by the chain length of the saturated acids and by the degree of unsaturation of the unsaturated acids.

Fractionation is a cheaper process than either hydrogenation or interesterification partly because there is no loss of oil and no post-treatment is required. Sometimes, both fractions have added value, but on other occasions only one fraction is of enhanced value and attempts have to be made to find a use for the less valuable fraction. Fractionation can be repeated to give other fractions but this is only commercially practicable when high-value products are obtained, such as cocoa butter replacers.

Successful and efficient separation requires the production of good quality crystals (easily separable from the liquid fraction), in the correct proportion followed by efficient separation of the solid and liquid phases. This requires that the minimum amount of olein remains in the stearin fraction. Both stages are becoming more fully understood and improved equipment has been devised. Good filtration – aiming at complete separation of solid and liquid – is important and may be carried out under reduced pressure using a Florentine filter or under pressure with a membrane filter. Membrane filter presses are usually operated at 4–8 bar. Higher pressures (up to 30 bar) may be needed for more critical separations such as the production of cocoa butter replacers.

There are three stages of the crystallisation process: super-cooling of the melt, nucleation and crystal growth. As crystallisation is an exothermic process and oil is a poor heat conductor, gentle agitation is essential to dissipate the heat without fragmenting any of the crystals. The rate of crystallisation depends on the nature of the fatty oil (partially hydrogenated fats crystallise more readily than palm oil) and on crystalliser design which affects the efficiency of heat removal during crystallisation. Other components in the oil may affect crystallisation. Diacylglycerols, such as those present in palm oil, retard nucleation. Waxes accelerate nucleation, but this results in unsatisfactory crystal morphologies leading to more difficult filtration. Fractionation is applied mainly to palm oil but also to lauric oils (coconut and palmkernel), butter oil, beef tallow, other animal fats, hardened soybean and cottonseed oil.

Palm oil is fractionated more than any other oil and processing plants handling up to 3000 tonnes/day are in operation. The olein is the more valuable product and Malaysia exports most of its palm oil in the form of palm olein. It is used for frying and for salad oils. Palm stearin is used as hardstock in the production of spreads. Palm mid-fraction is used to produce CBE fats. A single fractionation converts palm oil (IV 51–53) to palm olein (IV 56–59) and to hard stearin (IV 32–36), Each of these can be fractionated a second or a third time to give a range of products including superolein (IV 64–66), top-olein (IV 70–72), soft stearin (IV 40–42), superstearin (IV 17–21), soft palm mid-fraction (IV 42–48) and hard palm mid-fraction (IV 32–36). These materials have a wide range of food and non-food uses and extend considerably the use of palm oil. The IV (iodine value) is a measure of average unsaturation (section 5.2.4). Palmkernel oil (IV 18) is fractionated to give a stearin of IV around 7 which can be used as a cocoa butter substitute and an olein of IV around 25. These fractions are also useful after complete hydrogenation.

Anhydrous milk fat (AMF) contains many more triacylglycerol species than palm oil so its fractions are less distinct. The fractions, either alone or after being added back into AMF, find specific uses including production of spreading butter (mixing hard stearin with top olein) and of baking products such as puff pastry (AMF and stearin).

Partially hydrogenated soybean oil (IV 77, MP 35ºC) can be crystallised to produce a stearin with IV reduced to 61 and MP raised to 45ºC, and an olein with an IV raised to 81 and MP 31ºC. Both of these fractions can be recrystallised to give a mid-stearin (IV 73, MP 35ºC) and a mid-olein (IV 88, MP 18ºC).

Fractionation of animal fats (mainly tallow, but also lard – usually after randomisation – and chicken fat) gives higher melting stearins and lower melting oleins. For the tallows, both fractions retain their full beefy flavour which is considered an advantage for some purposes. The oleins are used for frying and the stearins as biscuit fats.

2.3.2.1 Winterisation

Winterisation is a process to remove material that causes cloudiness when oil is kept at sub-ambient temperatures. This may be caused by saturated triacylglycerols or by waxes. The latter are present in sunflower and corn oils and should be reduced to 10–50 ppm. This process is generally considered to be part of the refining process (section 1.6.1).

2.3.2.2 Hydrophilisation

Hydrophilisation is a process for obtaining concentrates of oleic acid from tallow or palm fatty acids. After crystallisation at about 20ºC, 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 (70–75%) along with palmitoleic, linoleic and around 10 per cent of saturated acids.

2.3.2.3 Urea fractionation

Urea normally crystallises in tetragonal form, but in the presence of certain aliphatic compounds it forms hexagonal prisms containing some of the aliphatic material. 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 fulfils certain dimensional requirements. It must not be too short or it will not be held within the channel, and it must not be too wide if it is to fit into the free space, variously estimated at between 4.0 and 6.0×10^{-10} m. Many straight-chain acids and their alkyl esters satisfy these conditions and thus readily form complexes (also called adducts or inclusion compounds) with urea.

Saturated acids form stable complexes more readily than do unsaturated acids, and oleic acid forms inclusion compounds more readily than do polyunsaturated acids. In practice, urea and mixed acids are dissolved in hot methanol or urea and methyl esters in a hot methanol-ethanol mixture. The solution is crystallised at temperatures between 4 and 20ºC. After separation the adduct and the mother liquor will each furnish acids or esters when mixed with water and extracted with ether or petroleum ether in the usual way.

This procedure is used for two purposes. It separates straight-chain acids or esters from branched-chain or cyclic compounds with the former concentrating in the adduct and the latter in the mother liquor. Urea fractionation is also used to separate acids or esters of differing unsaturation. For example, urea fractionation of fish oil fatty acids will provide a concentrate of highly unsaturated acids in the liquor with saturated and monounsaturated acids being removed as adduct.

2.3.3 Hydrogenation (see also section 7.1)

Partial hydrogenation is another important way of processing oils and fats to enhance their value. The technique, first applied to such materials by the German chemist Normann, has been in operation for over 100 years and has been subject to continuous improvement during that time. The objective is to convert a liquid oil (vegetable or fish) into a semi-solid fat which can be used as a component of a spread.

As a consequence of partial hydrogenation there is a change in melting behaviour because of the increased level of solids present. This affects spreadability, oral response and baking performance. In addition there are two other changes which have to be considered. There is an increase in oxidative stability through the complete or partial removal of the easily oxidised polyunsaturated fatty acids (sections 7.2.2 and 7.2.3) and there is a decrease in nutritional value through the destruction of essential fatty acids and the formation of acids with *trans* unsaturation (section 7.1.1).

At the molecular level, one or more of the following changes may occur: hydrogenation (saturation) of unsaturated centres, stereomutation of natural *cis* olefins to their *trans* isomers, double bond migration and conversion of polyunsaturated fatty acids to monounsaturated and saturated acids. These are the consequences of reaction between a liquid (fatty oil) and a gas (hydrogen) occurring at a solid surface (the catalyst). In partial hydrogenation, the reaction may proceed along different pathways. The concept of selectivity between these is important and is detailed in section 7.11.

Dikstra in Rajah (2002) has proposed a modification of the Horiuti-Polanyi mechanism to explain the changes that occur during partial hydrogenation of fatty acids and their esters. In the following sequence, the horizontal line shows the conversion of diene to monoene and of monoene to saturated acid/ester via the half hydrogenated states DH and MH. The steps shown vertically are the reverse processes, whereby DH goes back to D and MH goes back to M. It is during these stages that *trans* and positional isomers are formed. There are six stages altogether (see equation below) and it is important to understand the relative rates of these.

- In the conversion of D to M the first step is rate-determining and the second step is fast. The conversion of DH back to D is slow and is only important in the unusual situation that hydrogen is present in very low concentration.
- In the conversion of M to S the final stage is slow and rate-determining thus making it more likely that there will be considerable recycling of M and MH leading to formation of stereochemical and positional isomers.

$$\begin{array}{ccccccc} D & \rightarrow & DH & \rightarrow & M & \rightarrow & MH & \rightarrow & S \\ & & \downarrow & & & & \downarrow & & \\ & & D & & & & M & & \end{array}$$

The catalyst used on a commercial scale is nickel on an inert support at a 17–25 per cent level encased in hardened fat. This preserves the activity of the nickel in a form which is easily and safely handled. The reaction is generally conducted at 180–200ºC and 3 bar pressure in vessels containing up to 30 tonnes of oil. To minimise the use of a catalyst, it is desirable to use refined oil and the highest quality of hydrogen. Through improvements in the quality of a catalyst and in equipment, the requirement for catalyst has been gradually reduced. In 1960, 0.2 per cent nickel was required but by 2000, this was reduced to between 0.025 and 0.05 per cent (i.e. a 4- to 8-fold reduction). Reaction may proceed in a batchwise manner with up to 8–10 batches in a 24 hour period or in a semi-continuous fashion

The variables that have to be considered are:

- The nature of the oil being treated.
- The extent of hydrogenation which is desired.
- The selectivity to be achieved in terms of the PUFA-MUFA-saturated ratios and the ratio of *cis* to *trans* isomers.
- The quality and quantity of catalyst in terms of pore length, pore diameter, activity and amount used.
- The reaction conditions of temperature, pressure and degree of agitation.

An important factor is the competition between hydrogenation and isomerisation. This depends on the availability of hydrogen at the catalyst surface in relation to the demand. A plentiful supply of hydrogen will promote hydrogenation, and an inadequate supply of hydrogen will allow isomerisation to become more significant. The availability of hydrogen is enhanced by increased pressure and increased agitation. The demand for hydrogen is increased with higher temperatures, higher catalyst quantity, higher catalyst activity and a more highly unsaturated oil. The reaction is exothermic and appropriate cooling and stirring is required to distribute the heat.

The progress of the reaction can be followed in a number of ways. These vary in simplicity, in speed of completion and in the information they provide. They include: the volume of hydrogen used which will measure saturation but not isomerisation, iodine value measured by an accelerated technique that will provide similar information, refractive index, solid fat content measured by low-resolution ^{1}H-NMR, solid fat index measured by dilatometry, slip melting point or gas chromatography of methyl esters.

Most of the comments above refer to the normal procedure of partial hydrogenation whereby oils are converted to the plastic fats required to make spreads. An alternative ‘brush hydrogenation’ is designed to enhance shelf life of oils containing linolenic acid (soybean oil, rapeseed/canola oil) by reducing slightly the level of triene acid which is easily oxidised to compounds with very undesirable odours and flavours (section 7.2.5). Brush hydrogenation only requires a low level of hydrogenation.

2.3.4 Interesterification with chemical catalysts (see also section 8.3.4)

The production of fat spreads as an alternative to butter led to an increased demand for solid fats. For the most part, this demand has been met by the use of partially hydrogenated vegetable oils (section 2.3.3) but concern about the health effects of *trans* unsaturated acids has raised interest in an alternative way of producing fats with the required melting behaviour. This can be achieved by interesterification of blends of natural or fractionated fats. Products obtained in this way will probably contain slightly more saturated acids than their partially-hydrogenated equivalents, but they will have no *trans* acids. This section is devoted to interesterification carried out under the influence of a chemical catalyst. Similar reactions with enzymes are discussed in the following section.

Interesterification is generally effected in 10–15 tonne batches at 80–90ºC over 30–60 minutes at a cost not very different from that for partial hydrogenation. It does not require expensive equipment nor use explosive gases, but the catalyst has to be washed out and there is some loss of product leading to increased cost. To get a product with the desired properties, a soft oil is interesterified with a hard stock which may be a fractionated stearin, a lauric oil, or a fully hydrogenated seed oil. The last is a scientifically acceptable choice but has the disadvantage that the word 'hydrogenated' has to appear on the label. The average customer does not appreciate the difference between partially hydrogenated (with *trans* acids) and fully hydrogenated (without unsaturated acids).

Natural oils are mainly mixtures of triacylglycerols which differ in their physical and nutritonal properties. In vegetable oils and to a lesser extent in animal fats, the various fatty acids are linked to glycerol in groups of three in a non-random manner. If the oil, on its own or in admixture with a second oil, is interesterified, the fatty acids will be randomised and the new mixture of triacylglycerols will have different physical and nutritional properties. In a fully randomised oil, the triacylglycerol composition can be calculated from the fatty-acid composition.

The catalyst most commonly employed is an alkali metal at a level of 0.1–0.2 per cent or a sodium alkoxide at a 0.2–0.3 per cent level. The true catalyst is believed to be a diacyl glyceroxide anion.

$$Gl(Oacyl)_3 + MeO^- \rightarrow GlO^-(Oacyl)_2 + MeOacyl$$

[$Gl(OH)_3$ represents glycerol]

Since the catalyst is easily destroyed by acid, water or peroxides, the oil(s) to be interesterified must be free of these impurities. Reaction occurs between 25 and 125ºC but is usually carried out between 80 and 90ºC for 30 minutes in a batch operation.

Directed interesterification is a modification of the normal process in which the reaction is conducted at a lower temperature (25–35ºC). Under these

conditions, the less soluble triacylglycerols crystallise from the solution. This disturbs the equilibrium in the liquid phase and this has to be re-established. The result is the formation of more SSS and UUU triacylglycerols.

The following are typical applications of interesterification:

- Lard, with an unusually high level of palmitic acid in the β-position, crystallises naturally in the β form. When randomised, the content of 2–palmito glycerol esters is reduced from around 64 per cent to 24 per cent and the interesterified product crystallises in the β' form with consequent improvement in shortening properties.
- The crystal structures of margarines based on sunflower or canola oil (rapeseed) along with hydrogenated oil are stabilised in the β' form by interesterification leading to randomisation of the glycerol esters.
- Solid fats with about 60 per cent of essential fatty acids can be obtained by directed interesterification of sunflower oil and about 5 per cent of hard fat.
- Margarine made, for example, by interesterification of palm stearin and sunflower oil (1:1), contains no hydrogenated fat and therefore no *trans* acids.
- Chemical interesterification is used in the production of speciality products such as caprenin, salatrim (section 9.1) and olestra (section 9.2).

2.3.5 Interesterification with an enzymic catalyst (see section 8.3.4)

Interesterification can also be catalysed by lipases, many of which show useful properties. The 1,3-specific lipases, such as those derived from *Aspergillus niger, Mucor javanicus, M. miehei, Rhizopus arrhizus, R. delemar* and *R. niveus,* are particularly useful for interesterification. They are used to effect acyl exchange at the *sn*-1 and 3 positions while leaving acyl groups at the *sn*-2 position unchanged. Many interesting changes of this type have been effected on a bench scale, but as yet only a few have been commercialised and then only for products of high value (section 8.3.5).

Unilever developed a method for upgrading palm mid-fraction (PMF) as a cocoa butter equivalent. The PMF is too rich in palmitic acid and has too little stearic acid for this purpose, but this deficiency can be repaired by enzyme-catalysed acidolysis with stearic acid. Reaction is confined to the exchange of palmitic acid by stearic acid at the *sn*-1 and 3 positions with no movement of oleic acid from the *sn*-2 position. A similar product is produced enzymatically from high-oleic sunflower oil (rich in triolein) and stearic acid.

$$\mathrm{POP} + \mathrm{St} \rightarrow \mathrm{POSt} + \mathrm{StOSt}$$
$$\mathrm{OOO} + \mathrm{St} \rightarrow \mathrm{StOO} + \mathrm{StOSt}$$

Chemical interesterification would lead to randomisation of all the acyl chains and the products would have different melting behaviour from that required by a cocoa butter equivalent.

Betapol, a product manufactured by Loders-Croklaan, consists mainly of triacylglycerols of the type UPU. This is used as a constituent of infant formulae. Human milk fat is unusual in that it contains a significant proportion of its palmitic acid in the *sn*-2 position. While this is true also for lard (pig fat), it is not a feature of vegetable oils. Betapol is made from tripalmitin and oleic acid using the lipase from *Mucor miehei* to promote 1,3-acyl exchange. These materials are expensive and in practice fractionated palm oil, rich in tripalmitin, is reacted with 'oleic acid' from high-oleic sunflower or safflower, or from olive oil.

Bohenin (BOB) is the name given to glycerol 1,3-behenate 2-oleate which inhibits fat bloom when added to chocolate. It is produced in Japan by enzymic interesterification of triolein and behenic acid (22:0) or ester in the presence of a 1,3 stereospecific lipase (section 10.6).

Diacylglycerols are being produced and used in growing quantities because of their beneficial effects in the management of obesity. Cooking oil with at least 80 per cent of diacylglycerols (mainly the 1,3-isomer) has been marketed in Japan since 1999 and thereafter in other countries (see section 9.8.1).

There are many reports showing how, with an appropriate enzyme (*Mucor miehei* and *Candida antarctica* are frequently used), long chain PUFA such as EPA and/or DHA can be introduced into vegetable oils or synthetic glycerides to give products with enhanced nutritional value. In a similar way, C_8 and C_{10} acyl chains can be introduced into vegetable oils or fish oils with a consequent change in nutritional properties and energy values. The products are triacylglycerols with either one long and two short chains (LS_2) or two long and one short chain (L_2S). This reaction can be used to produce triacylglycerols with easily metabolisable short and medium chain acids at the *sn*-1 and 3 positions and an essential fatty acid at the crucial *sn*-2 position.

Bibliography and references

Akoh, C.C. & Min, D.B. (eds), (2002) *Food Lipids – Chemistry, Nutrition, and Biochemistry*, 2nd edn, Marcel Dekker, New York.

Dikstra, A.J. (1997) Hydrogenation revisited, *Inform,* **8**, 1150–1158.

Garti, N. & Sato, K. (eds) (2001) C*rystallisation Processes in Fats and Lipid Systems*, Marcel Dekker, New York.

Gunstone, F.D. & Padley, F.B. (eds) (1997) *Lipid Technologies and Applications*, Marcel Dekker, New York.

Hamm, W. & Hamilton, R.J. (eds) (2000), *Edible Oil Processing,* Sheffield Academic Press, Sheffield.

Hui, Y.H. (1996) (ed.) *Bailey's Oil and Fat Products,* 5th edn, volume 4 is devoted to processing technology and contains several relevant chapters, Wiley, New York.

Rajah, K.K. (ed.) (2002), *Fats in Food Technology*, Sheffield Academic Press, Sheffield.

3 Structure of fatty acids and lipids

3.1 Introduction

Since the physical, chemical and nutritional properties of oils and fats are all related to their chemical structures, it is imperative to understand the chemical nature of the various types of lipids. Almost all of these are derivatives of fatty acids and, therefore, it is appropriate to start with the acids before examining the structure of the lipids. Over 1000 natural fatty acids have been identified, but it is not necessary to know about all of these. Most workers in this field will probably have experience of only some 25–50. This chapter will cover these and a few beyond these narrow limits. Some websites containing information relevant to this chapter are listed in the bibliography.

There is no agreed definition of lipids and fatty acids but it has been suggested that 'fatty acids are compounds synthesised in nature by condensation of malonyl coenzyme A units under the influence of a fatty acid synthase complex. Lipids are fatty acids and their derivatives are substances related biosynthetically or functionally to these compounds'. As will be developed in section 4.5, this explains the frequent occurrence among natural fatty acids of those with straight chains and an even number of carbon atoms.

3.2 Fatty-acid nomenclature

Most fatty acids have a common name. In the first instance, these were used when the detailed structure was not known and were generally indicative of the origin of the acid. Examples include palmitic acid from palm oil, oleic acid from olive oil (*Olea europea*), linoleic and linolenic acid from linseed oil and ricinoleic acid from castor oil (*Ricinus communis*). These names continued in use even when the acids had been identified, because of their convenience. They are often simpler than the full systematic name. However, common names should only be used for a single structure and not for groups of isomeric compounds. Thus, oleic acid should only indicate *cis*-9-octadecenoic acid and not other 18:1 isomers. The disadvantage of common names is that, in contrast to systematic names, they contain no structural information.

Fully systematic names, based on international rules agreed among chemists and biochemists, show the number of carbon atoms and, where relevant, the number, position, type and configuration of unsaturated centres. For example, oleic acid is correctly named *cis*-9-octadecenoic acid. This name indicates a carboxylic acid (oic acid) with 18 carbon atoms (octadec) and one double bond (en) which is in the *cis* configuration and between carbon atoms 9 (and 10) counting from the COOH group.

Fatty acids can be represented structurally in several ways as shown for linoleic acid in Fig. 3.1. Where not otherwise indicated, a straight chain of carbon atoms is assumed. Symbols such as 18:2 indicate a fatty acid with 18 carbon atoms and two unsaturated centres. In the absence of other information, the unsaturated centres are taken to be *cis* olefinic but further descriptors are necessary to indicate the position of these. Organic chemists number fatty acid carbon chains from the carboxyl group (COOH = 1), but there are occasions when similarities among groups of fatty acids are better illustrated by counting from the methyl end of the chain (CH_3 = 1). This is indicated by symbols such as n-6 or ω6 indicating that the first unsaturated carbon atom is on carbon-6 counting from the methyl group. Other unsaturated centres are assumed to be methylene-interrupted. Symbols such as *c* (*Z*), *t* (*E*), *e* and *a* are sometimes used to indicate *cis* olefinic, *trans* olefinic, ethylenic and acetylenic unsaturation.

Another form of abbreviation makes use of acronyms as in the following examples:

CLA	conjugated linoleic acid
GLA	gamma-linolenic acid
AA	arachidonic acid
EPA	eicosapentaenoic acid
DHA	docosahexaenoic acid

cis-9, *cis*-12-octadecadienoic acid
18:2
9*c*, 12*c*-18:2 or 9*Z*, 12*Z*-18:2
n-6-18:2 or ω6-18:2
$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$

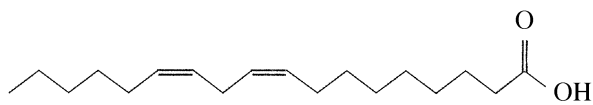


Figure 3.1 Representations of linoleic acid.

3.3 Fatty acids – main structural features

As more fatty acid structures were reported, the widening range of fatty acid structures revealed many common features. These are a consequence of common patterns of biosynthesis throughout the plant and animal kingdoms. Small variations result from changes in the synthesising enzymes which, in some cases, can now be explained in terms of their amino-acid sequence. The following generalisations are true for most common fatty acids and for many of the minor acids described below though there are exceptions which are sometimes significant.

- Natural fatty acids – both saturated and unsaturated – are straight-chain compounds with an even number of carbon atoms. Acids are known with 2–80 carbon atoms, but most commonly they lie in the range 12–22.
- Unsaturated acids are often olefinic, have *cis* configuration, and the unsaturated centres appear at certain preferred positions. The most common positions are $\Delta 9$ or $\omega 9$ as in oleic acid ($\Delta 9$–18:1) and erucic acid ($\omega 9$–22:1).
- Polyunsaturated acids generally have a methylene-interrupted arrangement of *cis*-olefinic double bonds as in linoleic acid (Fig. 3.1). This 1,4 pattern of unsaturation is characteristic of fatty acids. It is shown in Chapter 7 that this is the cause of characteristic and important patterns of reaction such as those with oxygen, hydrogen and alkali. This pattern of unsaturation differs from the 1,3 pattern observed in the carotenoids and from the 1,5 pattern in isoprenoids.
- Fatty acids rarely have functional groups beyond the carboxyl group and unsaturated centres. Nevertheless acids are known with hydroxy, epoxy, keto (oxo), oxa and halogeno groups.

Based on production figures for the major vegetable oils, it can be calculated that eight acids account for about 97 per cent of total production by nature: lauric (12:0, 4%), myristic (14:0, 2%), palmitic (16:0, 11%), stearic (18:0, 4%), oleic (18:1, 34%), linoleic (18:2, 34%), α-linolenic (18:3, 5%) and erucic (22:1, 3%). If allowance is made for all green matter (leaves, etc.) the level of α-linolenic acid would rise. The major fatty acids in animal fats and fish oils are myristic, palmitic, palmitoleic, stearic, oleic, eicosenoic, arachidonic, eicosapentaenoic, docosenoic and docosahexaenoic, but these will not have a significant effect on the figures cited above.

3.4 Saturated acids

The names and selected physical properties of some alkanoic acids and their methyl esters are given in Table 3.1. The methyl esters have lower melting and boiling points than the corresponding acids as a consequence of hydrogen bonding in the latter.

Table 3.1 Names and selected physical properties of some alkanoic acids and their methyl esters (adapted from Gunstone *et al*., 1994)

Chain	Systematic name	Common name	Acid			Methyl ester	
			Mol wt	MP (ºC)	BP (ºC)[a]	MP (ºC)	BP (ºC)[a]
4	butanoic	butyric	88.1	–5.3	164	–	103
6	hexanoic	caproic	116.2	–3.2	206	–69.6	151
8	octanoic	caprylic	144.2	16.5	240	–36.7	195
10	decanoic	capric	172.3	31.6	271	–12.8	228
12	dodecanoic	lauric	200.3	44.8	130[1]	5.1	262
14	tetradecanoic	myristic	228.4	54.4	149[1]	19.1	114[1]
16	hexadecanoic	palmitic	256.4	62.9	167[1]	30.7	136[1]
18	octadecanoic	stearic	284.5	70.1	184[1]	37.8	156[1]
20	eicosanoic	arachidic	312.5	76.1	204[1]	46.4	188[2]
22	docosanoic	behenic	340.6	80.0	–	51.8	206[2]
24	tetracosanoic	lignoceric	368.6	84.2	–	57.4	222[2]

[a] BP at 760 mm Hg or at 1 or 2 mm Hg as indicated in the superscript.

3.4.1 Short- and medium-chain acids (4:0–14:0)

Members of the C_4–C_{14} group of acids are present in two major fat sources. Milk fats contain all these acids and some vegetable oils – of which coconut and palm kernel are best known – are very rich in lauric acid with significant amounts of the 8:0, 10:0 and 14:0 acids.

The milk fats contain more of the shorter-chain acids. For example cow milk fat contains about 4 per cent (wt) butyric acid. This is equivalent to about 8.5 per cent (mol) and means that about 25 per cent of the triacylglycerols in cow milk fat contain one C_4 chain (section 1.5.1).

The 'lauric oils' are the prime source of the medium-chain acids. The C_8 and C_{10} acids, distilled from split (hydrolysed) lauric oils, are made into triacylglycerols and marketed for various purposes as medium-chain triglycerides (MCT). Attempts are being made to develop a range of cuphea oils as rich sources of one or more of these medium-chain acids. Rapeseed oil has also been genetically modified to produce a lauric-rich oil (lauric-canola) and while the product has been grown successfully in the field, it has not proved to be economic in relation to the modestly priced traditional lauric oils (sections 1.3.3, 1.3.10 and 1.3.11).

3.4.2 Palmitic and stearic acid

Palmitic acid (16:0) is the most common of all the saturated fatty acids. It is present in fish oils (10–30%), in the milk and body fats of most animals (up to 30%), and in virtually all vegetable fats at levels between 5 and 50 per cent.

Rich sources of palmitic acid include cottonseed oil (15–30%), palm oil (30–50%), Chinese vegetable tallow (~75%), lard (20–30%) and tallow from sheep and cattle (25–30%).

Stearic acid (18:0) is less common, but it is a major component in the tallows of ruminant animals (5–40%) and in a number of solid vegetable fats generally referred to as tallows or butters. These include cocoa butter (30–35%), shea butter (~45%) and Borneo tallow (~40%) (section 1.4.1). Stearic acid can also be made by complete hydrogenation of vegetable oils, rich in oleic and/or linoleic acid. Technical grade stearic acid may be rich in palmitic acid and vice versa. These saturated acids are widely used in food and non-food products (surfactants, cosmetics and personal hygiene products).

3.4.3 Long-chain acids

Saturated acids with more than 18 carbon atoms are rare. Groundnut oil contains low levels (4–7%) of such acids including arachidic (20:0), behenic (22:0) and lignoceric (24:0). Some less common vegetable oils contain these acids at higher levels. For example, rambutan tallow contains around 35 per cent of arachidic acid. Many waxes have C_{20}–C_{30} acids.

3.5 Monoene acids

Over 100 mono-unsaturated acids have been identified and are mainly olefinic compounds with *cis* configuration (the much less common *trans* acids are discussed in section 3.7). For the most part, they have 16–22 carbon atoms and are generally $\Delta 9$ or $\omega 9$ as a consequence of the presence of a very active $\Delta 9$–desaturase (section 4.5.2).

9-Hexadecenoic acid (palmitoleic or zoomaric) is a minor component of most vegetable and animal oils, but is present at more significant levels in macadamia oil (18–30%) and sea buckthorn berry oil (16–22%) (section 1.4.15).

9-Octadecenoic acid (oleic) is the most common of all monoene acids (see Fig. 3.2). It is probably present in all natural oils and achieves high levels in several, such as olive oil (60–80%), almond oil (60–70%) and low-erucic rapeseed (canola) oil (62%). Oils with high levels of oleic acid are in great demand and several of these have been developed (section 1.3.18). Animal fats frequently contain high levels of 18:1 acids, but these are not entirely oleic acid as positional and stereochemical isomers are frequently present.

Other octadecenoic acids include petroselinic (6*c*) and vaccenic acids (11*c* and 11*t*) (see Fig. 3.2). *trans*-Vaccenic acid occurs commonly at low levels in ruminant milk and depot fats, as a consequence of biohydrogenation of linoleic acid occurring in the rumen (section 7.1.13). The *cis* isomer (asclepic

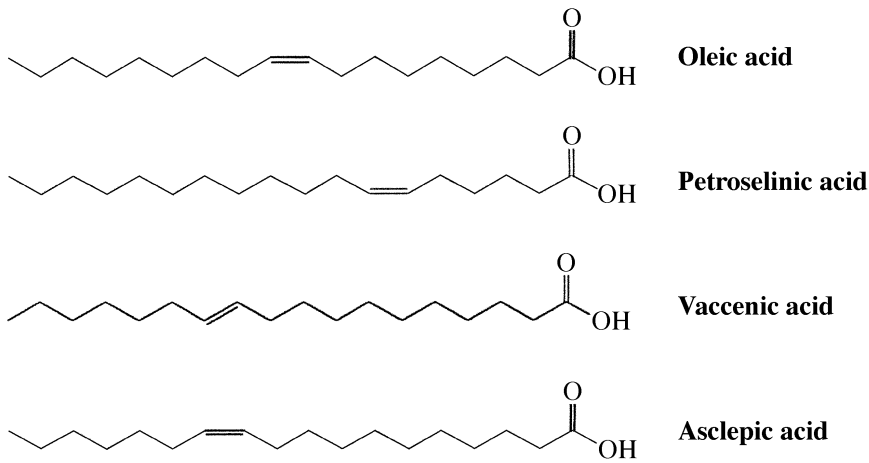


Figure 3.2 Isomeric octadecenoic acids.

acid) is a rare component in seed oils, but is likely to be present in oils rich in palmitoleic acid. Petroselinic acid is a major component in seed oils of the Umbelliferae (e.g. carrot, caraway, coriander, parsnip, parsley), Araliacea and Garryacaea families. Attempts are being made to develop a commercial coriander with around 80 per cent of petroselinic acid in its seed oil and, alternatively, to produce this acid in rapeseed oil through genetic modification (sections 1.3.11 and 1.3.18).

Eicosenoic acid (9*c* and/or 11*c*) is present in fish oils and is usually a minor component in vegetable oils with high levels of erucic acid (22:1). Meadowfoam oil (*Limnanthes alba*) is unusual in that over 90 per cent of its component acids have 20 or 22 carbon atoms in contrast to the general predominance of C_{16} and C_{18} acids. These are mainly 20:1 (5*c*, 63–67%), 22:1 (5*c*, 2–4% and 13*c*, 16–18%) and 22:2 (5*c*13*c*, 5–9%) acids.

Erucic acid (13*c*-22:1) is a major acid in the older form of rapeseed oil (section 1.3.11) and finds many technical uses, especially as its amide (section 11.3.3). It is obtained from high-erucic rapeseed oil and from crambe seed oil (*Crambe abyssinica*).

Jojoba oil (from *Simmondsia chinensis*) is unusual in that its major lipids are wax esters containing monounsaturated C_{20}, C_{22} and C_{24} acids and alcohols (section 3.13).

The most common 24:1 acid (nervonic, 15*c*) is an important component of many sphingolipids found in neurons (hence its name). Honesty (*Lunaria biennis*) seed oil contains erucic (43%) and nervonic acids (25%). Attempts are being made to grow it commercially, because of claims that it may be useful in the treatment of demyelinating disease.

3.6 Methylene-interrupted polyene acids

Methylene-interrupted polyene acids are the most common type of polyunsaturated fatty acids. They have the general formula shown below with m = 2–6, i.e. dienes through to hexaenes. They are divided into families depending on the value of n which is most commonly 1, 4, 5, or 7. The two

$$CH_3(CH_2)_n(CH{=}CHCH_2)_m(CH_2)_pCOOH$$

most important are the ω3 (n = 1) and ω6 (n = 4) families based on α-linolenic acid and linoleic acid respectively (section 4.5.4). Some members of these two families are listed in Table 3.2. Animals cannot make linoleic or linolenic acid and must obtain them from dietary (vegetable) sources. They are able to metabolise these to produce other members of each polyene family. The C_{20} and C_{22} acids are not available from plant sources. Because of the nutritional importance of these acids, studies are being made to develop plants by genetic modification that will produce such acids in their seed oils. This has been achieved in the laboratory at a low level (proof of concept), but much work remains to be done before a commercial crop can be developed.

The n-6 family: linoleic acid is the most common polyene acid and is the major acid in many seed oils such as soybean (45–60%), sunflower (20–75%), etc. (sections 1.3.15 and 1.3.16). γ-Linolenic acid has attracted interest as a dietary supplement and is available from a restricted group of seed oils (section 1.4.2). Arachidonic acid is important as the source of a wide range of metabolites, such as prostaglandins and leukotrienes, known collectively as eicosanoids (section 7.3.5). This C_{20} acid is a common component of animal phospholipids and is present in eggs and in liver. It has also been identified in mosses, ferns, fungi and algae.

Table 3.2 Methylene-interrupted polyene acids

Common name	Structure	Unsaturation[a]
Linoleic	18:2 (n-6)	9,12
γ-Linolenic	18:3 (n-6)	6,9,12
α-Linolenic	18:3 (n-3)	9,12,15
Stearidonic	18:4 (n-3)	6,9,12,15
Dihomo-γ-linolenic	20:3 (n-6)	8,11,14
Mead's acid	20:3 (n-9)	5,8,11
Arachidonic	20:4 (n-6)	5,8,11,14
Eicosapentaenoic	20:5 (n-3)	5,8,11,14,17
Docosapentaenoic	22:5 (n-3)	7,10,13,16,19
Docosahexaenoic	22:6 (n-3)	4,7,10,13,16,19

[a] All double bonds have *cis* configuration.

The n-3 family: α-linolenic acid is an important component in green tissue (grass, leaves, stems) and therefore, an important component in the diets of many foraging animals. It is present as a minor component in two major commodity oils – soybean (8%) and rapeseed (10%) – and is a more significant component of seed oils from linseed or flaxseed (60%) and perilla (60–65%). The higher members of the n-3 family – especially eicosapentaenoic acid (20:5) and docosahexaenoic acid (22:6) – are present in fish oils (section 1.5.4) and also in some micro-organisms (section 9.3) which are cultivated as sources of these important acids. Docosahexaenoic acid is an important component of phospholipids present in the brain, retina and sperm.

3.7 Other unsaturated acids

In addition to the common *cis* monoene and polyene acids described in sections 3.5 and 3.6 there are also other acids with different patterns of unsaturation. These are found mainly in plants and must result from some minor modification of the normal biosynthetic processes. While some have attracted interest for specific reasons others remain as curiosities. It remains to be determined whether, for example, the acetylenic and allenic acids can serve any useful industrial purpose. One group of such acids are those with conjugated unsaturation. These acids contain two or more unsaturated centres adjacent to each other and are no longer methylene-interrupted.

The term conjugated linoleic acid (CLA) is used to describe any 18:2 acid with conjugated unsaturation. Such acids are present in ruminant fats (meat, milk and dairy products) and are minor components of the human diet. These sources contain a range of isomers among which, the 9*c*11*t* acid (rumenic) is dominant. This acid is produced by ruminant animals during biohydrogenation of linoleic acid through isomerisation and by Δ9–desaturation of vaccenic acid (11*t*-18:1). There is evidence that individual isomers within this group inhibit the growth of cancer cells and promote the formation of protein at the expense of fat. The first of these properties is important in the management of cancer and the second in animal husbandry. Since the isolation of pure material in reasonable quantities from animal sources would be laborious, procedures have been devised to convert linoleic acid (or seed oils rich in this acid) to a mixture of the 9*c*11*t* and 10*t*12*c* isomers by treatment with alkali.

Rumen hydrogenation 9*c*12*c*-18:2 → 9*c*11*t*-18:2 → 11*t*-18:1 → 9*c*11*t*-18:2
Alkali-isomerisation 9*c*12*c*-18:2 → 9*c*11*t*-18:2 + 10*t*12*c*-18:2

The best-known acids of this type are C_{18} trienes or tetraenes with unsaturation starting on carbon 8 or 9. Some differ only in configuration. Names, structures and sources are collected in Table 3.3. Some of them contain an

Table 3.3 Natural acids with conjugated polyolefinic unsaturation

Configuration	Common name	Typical source (seed oils)
Trienes (18:3) from linoleic acid		
8*c*10*t*12*c*	jacaric	*Jacaranda mimosifolia*
8*t*10*t*12*c*	calendic	*Calendula officinalis*
8*t*10*t*12*t*	–	*C. officinalis*
9*c*11*t*13*c*	α-eleostearic[ab]	*Aleurites fordii*[d]
9*t*11*t*13*t*	β-eleostearic	*A. fordii*[d]
9*t*11*c*13*c*	punicic	*Punica granatum*
Tetraenes (18:4) from α-linolenic acid		
9*c*11*t*13*t*15*c*	α-parinaric[c]	*Impatiens balsamina*
9*t*11*t*13*t*15*t*	β-parinaric	–

[a] Also kamlolenic, 18-hydroxy-α-eleostearic (*Mallotus philippinensis*).
[b] Also licanic, 4-oxo-α-eleostearic (*Licania rigida*).
[c] Also chrysobalanic, 4-oxo α-parinaric (*Chrysobalanus icaco*).
[d] Tung oil.

additional functional group (see footnote to Table 3.3). There is evidence that the trienes are formed by bio-modification of linoleic acid. Not included in this list are acids with both olefinic and acetylenic systems which are partly or wholly conjugated. These latter are almost entirely C_{18} acids with unsaturation starting on carbon 8 or 9.

Another group of polyunsaturated fatty acids are not wholly methylene-interrupted. Many of these have an additional $\Delta 5$ (or 7) double bond and are found in lipids from sponges or from Gymnosperms. Some examples are listed in Table 3.4.

Acetylenic acids (see Fig. 3.3) occur only infrequently, and include tariric (6*a*-18:1), crepenynic (9*c*12*a*-18:2), santalbic or ximenynic (9*c*11*a*-18:2) and hydroxy acids such as helenynolic (9-OH 10*t*12*a*-18:2). Crepenynic acid is probably formed from oleic acid by a modified desaturation process (section 4.5.3). There also exists a group of highly unsaturated acids with extended

Table 3.4 Structure of some non-methylene-interrupted polyene acids (all-*cis*)

Structure	Common name
5,9,12-18:3*	pinolenic
5,9,12,15-18:4	coniferonic
5,11,14-20:3	sciadonic
5,11,14,17-20:4	juniperonic
7,11-20:2	dihomo-taxoleic
7,11,14-20:3	dihomo-pinolenic

* Also 5*t*9*c*12*c*-18:3, columbinic.

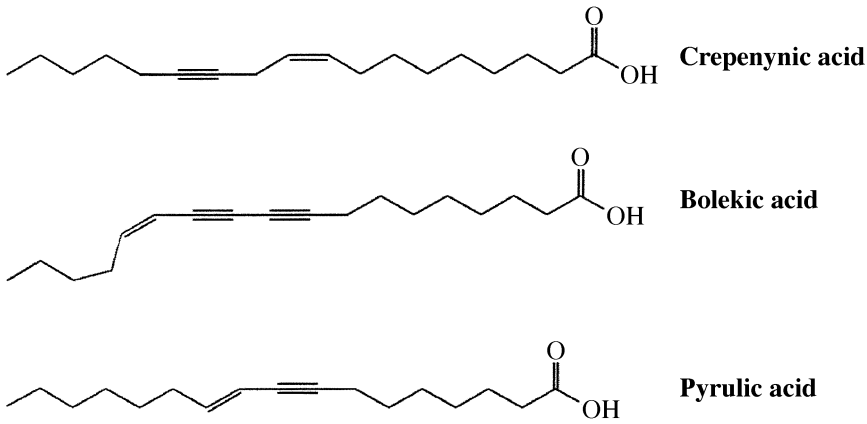


Figure 3.3 Some acetylenic acids.

$H_3C(CH_2)_{10}CH{=}C{=}CH(CH_2)_3COOH$ labellenic acid

$H_3CCH{=}CH(CH_2)_8CH{=}C{=}CH(CH_2)_3COOH$ lamenallenic acid

6a-18:1 → 5e6e-18:2

Figure 3.4 Some allenic acids and their formation from acetylenic compounds.

conjugated systems of acetylenic and olefinic unsaturation (e.g. bolekic acid 9*a*11*a*13*c*-18:3 and pyrulic acid 8*a*10*t*-17:2).

Allenic acids, containing the function -CH=C=CH-, are rare and are probably formed by biological rearrangement of appropriate acetylenic acids (see Fig. 3.4). They include the 5*e*6*e* (laballenic) and 5*e*6*e*16*c* (lamenallenic) C_{18} acids. See also stillingic acid (section 1.4.4).

Most natural olefinic acids have *cis* configuration. As indicated earlier in this section, *trans* unsaturation is often apparent among acids with conjugated unsaturation. Dietary acids with *trans* unsaturation are produced from linoleic or linolenic acid by ruminant animals or by processing (partial hydrogenation or high-temperature deodorisation) of oils containing these acids (sections 2.3.3 and 2.3.4).

3.8 Branched chain and cyclic acids

Typical acids with branched chains or containing carbocyclic systems include iso acids and anteiso acids present in woolwax and other animal fats, acids

such as tuberculostearic acid (10-methylstearic acid, present in the lipids of the tubercle bacillus) with mid-chain branching, phytanic and pristanic acids with several branched methyl groups present at low levels in fish oils resulting from metabolism of phytol (see Fig. 3.5).

Typical examples of acids with a carbocyclic system include:

- Cyclopropane acids present in bacteria.
- Malvalic and sterculic acids which contain cyclopropene units (in selected seed oils of the Malvaceae) and present at low levels in cottonseed oil (section 1.3.5) (Fig. 3.6).
- Cyclopentene acids (C_6–C_{20}) from seed oils of the Flacourticeae (Fig. 3.7).

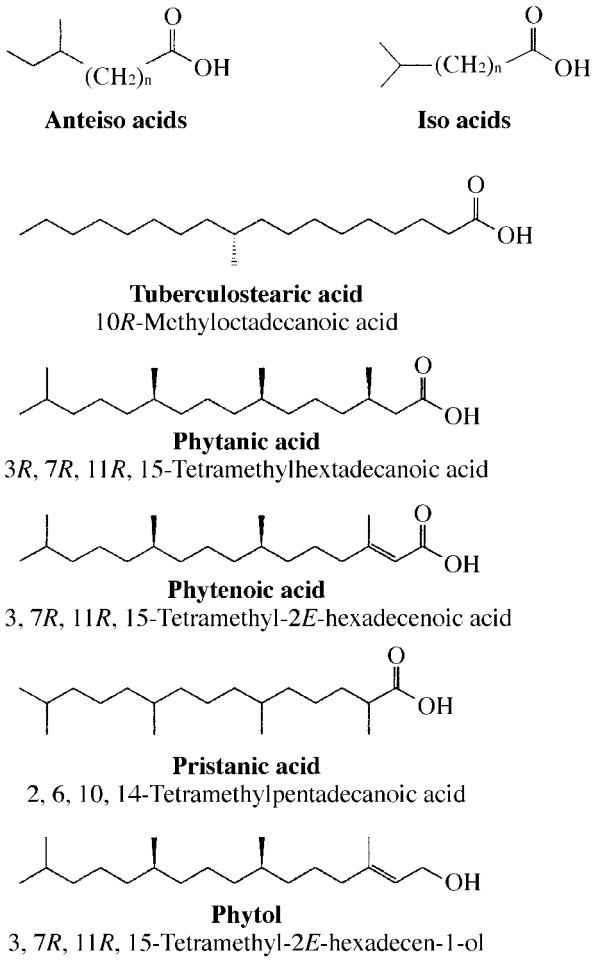


Figure 3.5 Acids with one or more methyl branches.

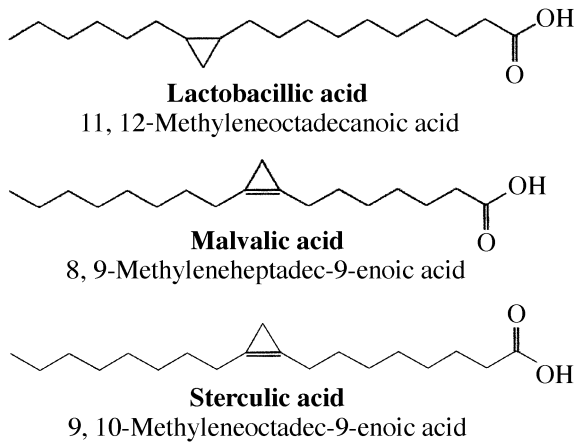


Figure 3.6 Cyclopropane and cyclopropene acids.

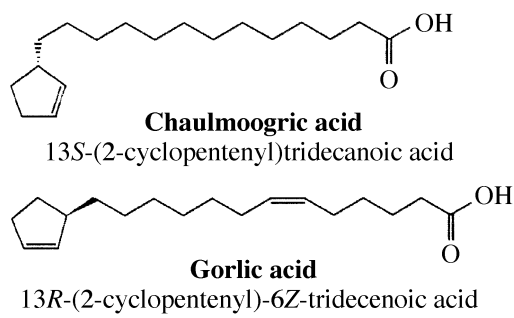


Figure 3.7 Cyclopentene acids.

3.9 Oxygenated acids

Fatty acids with functionality beyond unsaturation and a carboxyl group are rare but examples of fatty acids with an oxygenated function (hydroxy, epoxy, furanoid, oxa, oxo, methoxy) include some of importance.

3.9.1 Hydroxy acids

The hydroxyl group in a natural hydroxy acid may occur in the α (2) position (sphingolipids, section 3.1.7), in the $\omega 1$ or $\omega 2$ positions as in many fatty acid metabolites, in mid-chain positions resulting from enzymic oxidation (section 7.3.4), or in some other mid-chain position. The last group are best known because of the easy accessibility of ricinoleic acid in castor oil (section 1.1.1).

Table 3.5 Mid-chain hydroxy acids without conjugated unsaturation

Chain length and unsaturation	Position of hydroxyl group	Common name	Source (seed oils)
18:1 9*c*	12*R*	ricinoleic	*Ricinus communis*
18:1 12*c*	9*S*	isoricinoleic	*Strophanthus* and *Wightia spp*
18:2 9*c* 15*c*	12*R*	densipolic	*Lesquerella densipila*
20:1 11*c*	14*R*	lesquerolic	*L. densipila*
20:2 11*c*17*c*	14*R*	auricolic	*L. auriculata*

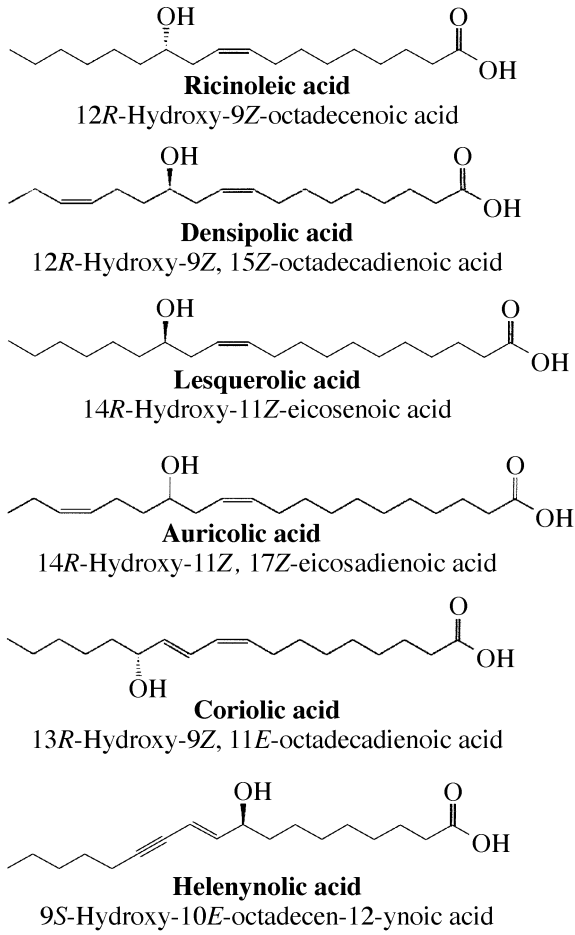


Figure 3.8 Hydroxy acids.

This, and other acids of this type are listed in Table 3.5 (see also Fig. 3.8). Many of the acids with conjugated acetylenic/olefinic unsaturation (section 3.7) also contain a hydroxyl group adjacent to the unsaturated unit (e.g. helenylic acid, 9-OH 10*t*12a-18:2).

3.9.2 Epoxy, furanoid and oxa acids

Epoxy acids occur as metabolites of polyunsaturated fatty acids (section 7.3.5) but have also been identified as component acids of some seed oils. These acids are not common but they are present at high levels in seeds from some plants. For example, vernolic acid (*cis*-12*S*,13*R*-epoxyoleic) forms up to 80 per cent of the acids of several vernonia oils and attempts are being made to develop *Vernonia galamensis* with around 75 per cent of vernolic acid as a commercial crop. Other epoxy acids which have been identified include coronaric (9*R*,10*S*-epoxy12*c*-18:1) and alchornoic (14*S*,15*R*-epoxy 11*c*-20:1). Epoxidised oils are used as plasticisers/stabilisers for PVC (section 7.4.1) (see Fig. 3.9).

Naturally occurring furanoid acids have been identified in some fish oils. During prolonged periods of fasting, the levels of these may become quite high. They have also been identified in some seed oils at very low concentrations (see Fig. 3.10).

Oxa acids (also referred to as divinyl ethers) with an oxygen atom inserted into the carbon chain between two olefinic centres, are among the products of

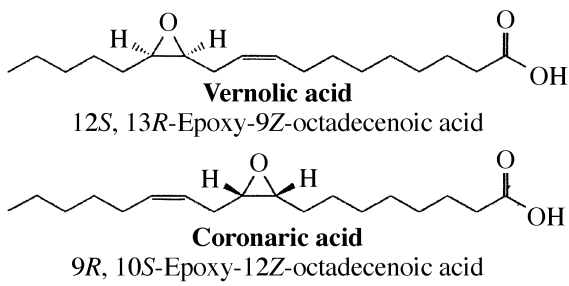


Figure 3.9 Epoxy acids.

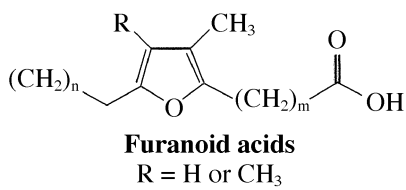


Figure 3.10 Typical furanoid acid.

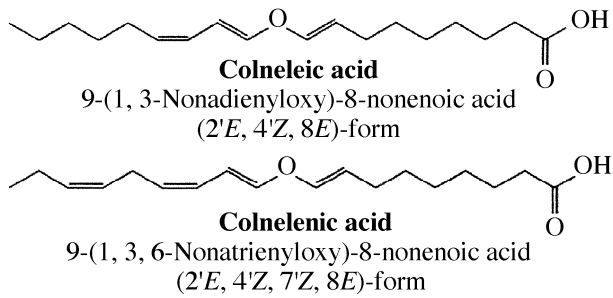


Figure 3.11 Oxa acids (divinyl ethers).

lipoxygenase-catalysed oxidation. The first members of this class to be identified were colneleic and colnelenic acids present in potato lipids and formed from linoleic and linolenic acid respectively. Other compounds of this type have been identified in brown and red algae and in parts of tomato and garlic plants. This group of fatty acids has 18 or 20 carbon atoms, an ether oxygen in the carbon chain and 3–6 double bonds (Fig. 3.11).

3.10 Halogenated fatty acids

Over 200 halogen-containing fatty acids have now been reported. Most commonly these contain chlorine or bromine, but acids with fluorine and with iodine are also known. Many are of marine origin, related to the fact that the seas contain significant levels of chlorides and bromides. The halogen may be mid-chain or it may be at, or close to, the terminal carbon.

As an example, there are some fluorine-containing acids, such as 16-fluoropalmitic acid and 18-flouro-oleic acid, with the fluorine attached to the terminal carbon atom. These acids are present in the seed oil of *Dichapetalum toxicarum*, so called because of its toxic nature. When sheep eat the seeds of this plant they die quickly. This has been explained by the fact that the metabolism (repeated β-oxidation) of these fluoro acids finally produces the very toxic fluoro-acetic acid.

3.11 Introduction to lipid structure

The previous sections of this chapter have been devoted to the structure of the natural fatty acids. These occur in the free state to a limited extent, for the most part they are combined with mono or poly-hydric alcohols as esters or with amines as amides (see Fig. 3.12).

$$RCOOH + R'OH \rightarrow RCOOR' \qquad \text{esters from alcohols}$$

$$RCOOH + R'NH_2 \rightarrow RCONHR' \qquad \text{amides from amines}$$

Figure 3.12 Esters and amides from alcohols and amines respectively.

It is convenient to divide the lipids into three major categories each of which can be further divided:

1. Simple lipids: esters of fatty acids mainly with glycerol but also with other hydroxy compounds such as sugars, sterols and long-chain alcohols.
2. Complex glycerolipids: including phospholipids, glycolipids and ether lipids.
3. Sphingolipids: amides of sphingoid bases including ceramides, cerebrosides, gangliosides and sphingomyelins.

Since glycerol (1,2,3-propanetriol) is an important component of most lipids, it is necessary to appreciate the full structural nature of this apparently simple molecule. Glycerol contains a prochiral carbon atom linked to H, OH, and two CH_2OH groups. When these last two groups are made to differ by attachment to different groups then the molecule becomes chiral and can exist in two enantiomeric forms. To designate the stereochemistry of glycerol derivatives, the three carbon atoms are steospecifically numbered (*sn*). The glycerol molecule is represented as a Fischer projection with the secondary hydroxyl group to the left of the central (prochiral) carbon atom and the three carbon atoms are designated *sn*-1, *sn*-2 and *sn*-3 from the top downwards (Fig. 3.13).

A glyceryl lipid will be chiral when the substituents in the *sn*-1 and *sn*-3 positions differ and there will be two enantiomers with different optical rotations (though the difference is often very small). More important is the fact that the enantiomers react differently in enzymic processes. The terms phospholipids (lipids containing phosphoric acid), glycolipids (lipids containing one or more sugar units), and sulfolipids (lipids containing a sulfonic acid unit) are loosely used in the way indicated.

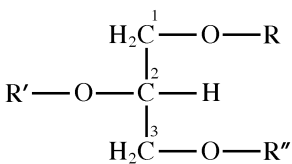


Figure 3.13 Stereospecific numbering (*sn*-1, 2 and 3). The various R groups represent different acyl chains.

3.12 Acylglycerols

When glycerol is combined with a single fatty acid it may form two isomeric monoacylglycerols, two isomeric diacylglycerols and one triacylglycerol. If the acyl chains differ then there are further possibilities of isomerism in the diacylglycerols and triacylglycerols. Oils and fats are predominantly triacylglycerols. Monoacylglycerols and diacylglycerols may be present as minor components, either as intermediates in the biosynthetic pathway or as products of partial lipolysis.

3.12.1 Monoacylglycerols (Fig. 3.14)

Monoacylglycerols (formerly called monoglycerides) exist in two forms depending on whether the primary (α) or secondary hydroxyl (β) is acylated. The unsymmetrical molecule is chiral and there are two symmetrical enantiomers with the acyl group in the *sn*-1 or *sn*-3 position. Pure α and β isomers quickly change to a 90:10 (α:β) mixture of the two compounds. This rearrangement is promoted by acid, alkali, heating and alcoholic solvents. Monoacylglycerols and their derivatives are used extensively as food emulsifiers (section 10.10) and are easily made from oils and fats by glycerolysis (section 8.3.3). 2-Monoacylglycerols are formed in the intestine during digestion of fat and are absorbed and transported before being reconverted to triacylglycerols for transport through the blood as lipoproteins (section 9.2).

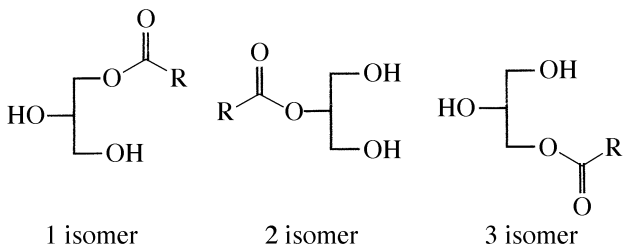


Figure 3.14 Monoacylglycerols: *sn*-1, 2 and 3 stereoisomers.

3.12.2 Diacylglycerols (Fig. 3.15)

The diacylglycerols (diglycerides) exist in symmetrical (*sn*-1,3) and unsymmetrical forms (*sn*-1,2 and 2,3) with the 1,3-isomer being the most stable. 1,2-Diacyl-*sn*-glycerols are important intermediates in the biosynthesis and metabolism of triacylglycerols and phospholipids (sections 4.6.1 and

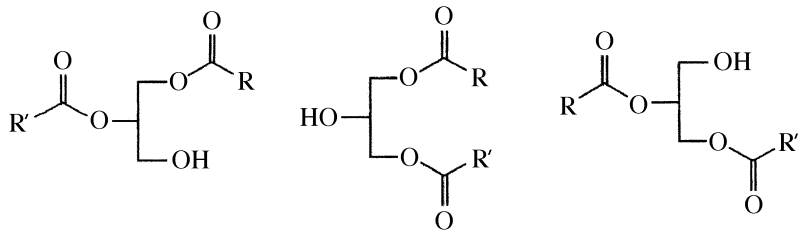


Figure 3.15 Diacylglycerols: *sn*-1,2, 1,3 and 2,3 isomers.

4.6.2). 1,3-Diacylglycerols have been recommended as dietary materials (section 9.8.1).

3.12.3 Triacylglycerols

Triacylglycerols (triglycerides) are fully acylated derivatives of glycerol and are the most common form of lipids. It is unusual for natural triacylglycerols to have only one kind of fatty acid unless a single acid exceeds around 70 per cent. For example, triolein is present in olive and other high-oleic oils. More commonly, two or three different fatty acids are present. The number of different triacylglycerol molecules present in a fat rises rapidly with the number of fatty acids present. With only two fatty acids, such as palmitic and oleic, a fat can contain six different triacylglycerols though this number reduces to four if regioisomers are ignored. These may be represented as PPP, POP (and PPO), POO (and OPO) and OOO. POP and POO each exist as enantiomeric pairs. The situation with more fatty acids is set out in Table 3.6. The triacylglycerol composition of some natural oils has been discussed in Chapter 1. Hydrolysis of these molecules under enzymic and non-enzymic conditions is discussed in section 8.2.

In the unusual case where hydroxy acids are present, there is the possibility of molecules with more than three ester groups. These are sometimes described as estolides. This is not apparent in castor oil which is rich in triricinolein, but an example is to be found in stillingia oil (section 1.4.4).

Table 3.6 Relation between the number of fatty acids and the maximum number of triacylglycerols that can be formed from them

Number of acids	5	10	20	n
Number of triacylglycerols				
All isomers distinguished	125	1000	8000	n^3
Excluding enantiomeric pairs	75	550	4200	$(n^3 + n^2)/2$
No isomers distinguished	35	220	1540	$(n^3 + 3n^2 + 2n)/6$

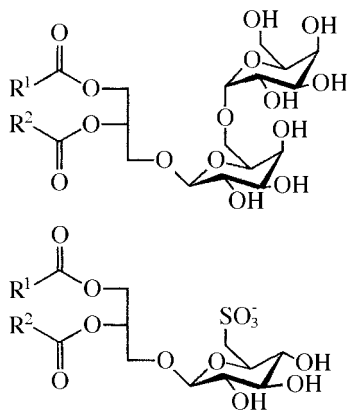
3.13 Wax esters

Though most commonly esterified with glycerol, fatty acids can also be associated with other hydroxy compounds such as sugars, sterols (section 1.6.3), and long-chain alcohols (wax esters, section 1.6.1). Many so-called waxes contain long-chain hydrocarbons (such as squalene), acids, alcohols and diols, aldehydes, ketones, etc. along with the wax esters. The latter are monoesters of long-chain acids with long-chain alcohols and generally contain around 40 carbon atoms. For the most part, both moieties are saturated or monounsaturated and the esters are often solid. They are produced by animals (beeswax and wool wax or lanolin) and by plants (candelilla, carnauba, jojoba). Leaf surfaces are covered by a layer of wax to reduce passive loss of moisture. The seed oil of *Simmindsia chinensis* (jojoba oil) is unusual in that it is not a triacylglycerol, but a mixture of wax esters made up of 18:1, 20:1 and 22:1 acids combined with 18:1, 20:1, 22:1 and 24:1 alcohols. The wax is thus a mixture of C_{38}–C_{44} esters with a double bond in each part of the molecule.

3.14 Glycosyldiacylglycerols (Fig. 3.16)

These compounds are also described as glycoglycerolipids. They are 1,2-diacylglycerols with one or more sugar units attached to the *sn*-3 position. The most common, present in plant membranes especially in chloroplasts, contain one or two units of galactose. Similar compounds have also been recognised in animal tissues.

Sulfoquinovosyldiacylglycerol, referred to as 'plant sulfolipid', is found exclusively in chloroplasts. It is a glucosyldiacylglycerol in which the sugar CH_2OH has been replaced by CH_2SO_3H.



Digalactosyldiacylglycerols

Sulfoquinovosyldiacylglycerol

Figure 3.16 Some glycosyldiacylglycerols.

3.15 Phospholipids

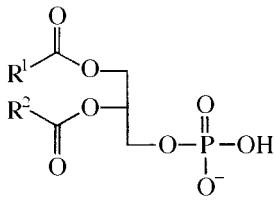
Many lipids are derivatives of phosphoric acid, which is a tribasic acid with the structure H_3PO_4 or $O{=}P(OH)_3$. Lipids that also contain glycerol are strictly glycerophospholipids to distinguish them from those sphingolipids also containing phosphoric acid. Despite this, the glycerophospholipids are generally referred to simply as phospholipids. These are derivatives of 3-glycerophosphoric acid, which in its diacylated form gives rise to the phosphatidic acids (Fig. 3.17). These occur naturally only at very low levels but they are important as intermediates in the biosynthesis and metabolism of other phospholipids (section 4.6.2).

The most common phospholipids are phosphatidic acids in which the phosphoric acid unit has reacted with another hydroxy compound. These are formulated in Tables 3.7 and 3.8 and Fig. 3.17. The phosphatidylcholines are the most abundant lipid in animal membranes and are often a major lipid in plant systems. The phosphatidylethanolamines are generally the second most abundant phospholipid in animal membranes. These phospholipids contain four different ester bonds and while it is difficult to distinguish between them using chemical reagents, there are phospholipases which promote reaction specifically at each ester bond (Table 3.7). The names of phospholipids should be cited in the plural because they are classes of compounds and not molecular individuals (apart from some which have been synthesised). This is because they contain two fatty acids selected from a pool. Most phospholipids have a saturated acyl chain in the *sn*-1 position and an unsaturated acyl chain in the *sn*-2 position. Some information on the component acids of one set of phospholipids is given in Table 3.8 and shows how the various classes of phospholipids from a single source differ in their fatty acid composition. Lysophospholipids have only one acyl group, usually at the *sn*-1 position, and one free hydroxyl group making the molecule more polar and a powerful surfactant (Fig. 3.17).

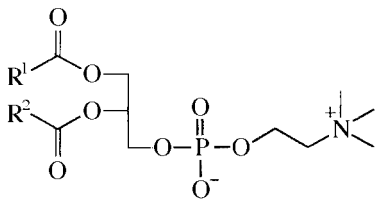
Table 3.7 The major glycerophospholipids

Name of class	Abbreviation	X[a]
Phosphatidylcholines	PC	$CH_2CH_2NMe_3$
Phosphatidylethanolamines[b]	PE	$CH_2CH_2NH_3$
Phosphatidylserines	PS	$CH_2CH_2(NH_3)COO$
Phosphatidylinositols[c]	PI	$C_6H_{11}O_5$
Phosphatidylglycerols[d]	PG	$CH_2CH(OH)CH_2OH$

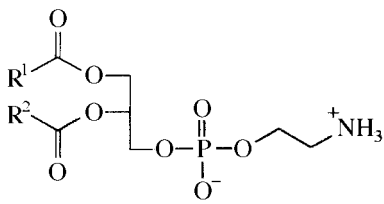
[a] Based on the structure of the phosphatidic acids. The charges on these molecules are shown in Fig. 3.17. The nitrogen atom is frequently quaternised.
[b] Also occur as derivatives of phosphonic acid (H_3PO_3).
[c] A derivative of *myo*-inositol.
[d] Also cardiolipin (PGP) containing two phosphatidic acid moieties linked through a third glycerol unit and present in heart muscle.



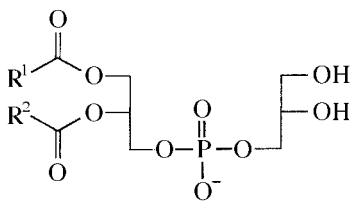
Phosphatidic acids



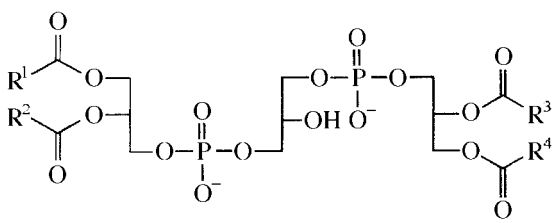
Phosphatidylcholines



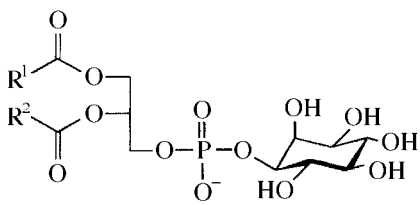
Phosphatidylethanolamines



Phosphatidylglycerols

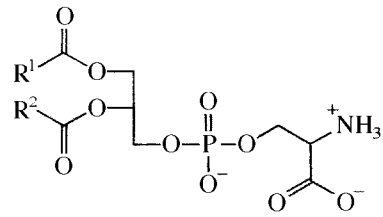


Diphosphatidylglycerols (cardiolipin)

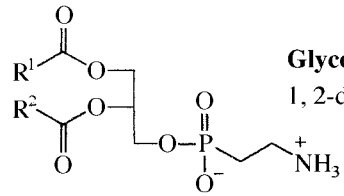


Phosphatidylinositols

Figure 3.17 Structures of the major phospholipids (continued on next page).

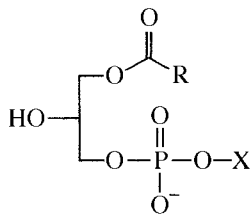


Phosphatidylserines



Glycerophosphonolipid

1, 2-diacyl-sn-glycero-3-(2'-aminoethyl)phosphonate



2-Lysophospholipids

LPC: X = choline

LPE: X = ethanolamine

Figure 3.17 (*continued*)

Table 3.8 Fatty acid composition (wt%) of phospholipids in the human red blood cell

Acid	Total PL	SPM	PC	PE	PS
16:0	20	24	31	13	3
18:0	17	6	12	11	37
18:1	13		19	19	8
18:2	9		23	7	3
20:3	1		2	1	3
22:0	2	9	2	1	3
20:4	13	1	7	24	24
24:0	5	23			
22:4	3			7	4
24:1	5	24			
22:5	2			4	3
22:6	4		2	8	10

PL = phospholipids, SPM = sphingomyelins, PC = phosphatidylcholines,
PE = phosphatidylethanolamines, PS = phosphatidylserines.
Adapted from Vance & Vance (2003).

3.16 Ether lipids

Some lipids contain ether (alkoxy) rather than ester (acyloxy) groups. These are almost always in the *sn*-1 position and are found as alternatives to triacylglycerols (especially in fish oils) or to phospholipids (particularly PE). The alkoxy groups are of two types. Some are saturated or have unsaturation only in conventional positions, while others contain a novel structural feature which diffentiates them from the first group. They have (*trans*) unsaturation between C1 and C2 and are therefore vinyl ethers with the unusual properties associated with such compounds. It has been suggested that these two groups of PE compounds be designated plasmanylethanolamines and plasmenylethanolamines respectively (see Figs 3.18 and 3.19).

Chemical hydrolysis of the first group furnish fatty acids, phosphoric acid if based on phospholipids and a glycerol ether since cleavage of ether bonds requires harsher reaction conditions than hydrolysis of esters. The glycerol

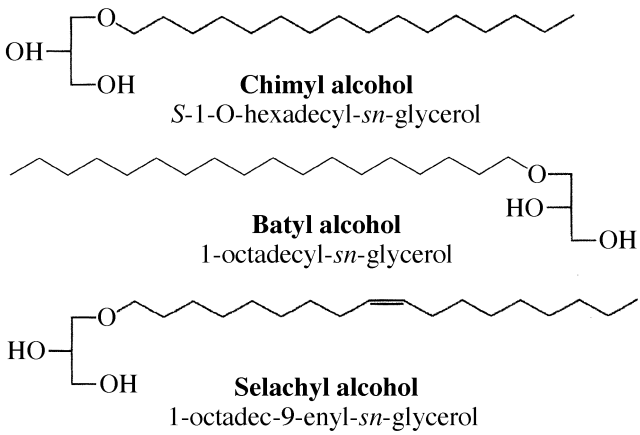


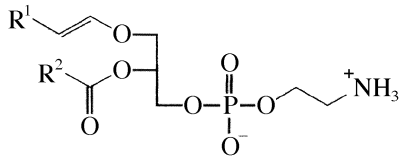
Figure 3.18 Glycerol ethers resulting from hydrolysis of ether lipids.

Table 3.9 Enzymic hydrolysis of phosphatidylcholines[a]

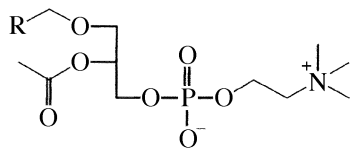
Phospholipase	Lipolysis products	Source
A1	fatty acids (sn-1), lyso PC	snake venom
A2	fatty acids (sn-2), lyso PC	snake venom
B	fatty acids (sn-1 and 2), GPC	–
C	DAG, phosphorycholine	bacteria (e.g. *Clostridia*)
D	PA, choline	most plant tissues

PC = phosphatidylcholines, GPC = glycerophosphorylcholine, DAG = diacylglycerols, PA = phosphatidic acids.

[a] These enzymes are also effective with phosphatidylethanolamines.



Typical plasmalogen
1-alk-1'-enyl-2-acyl-3-phosphoethanolamine-*sn*-glycerol



Platelet activating factor
1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine

Figure 3.19 Typical plasmalogen and PAF.

ethers are compounds like chimyl (16:0), batyl (18:0) and selachyl (18:1) alcohols (Fig. 3.18). In contrast, the vinyl ethers, though not cleaved under alkaline conditions, are hydrolysed under acidic conditions and instead of a glycerol ether, they yield glycerol and a long-chain aldehyde.

$ROCH{=}CH(CH_2)_nR' \rightarrow ROH + HOCH{=}CH(CH_2)_nR'$

The product is the aldehyde $OHCCH_2(CH_2)_nR'$ in its enolic form.

Phosphatidylethanolamines with ether groups occur in animal tissues and were originally called plasmalogens to reflect the production of aldehydes on acid-catalysed hydrolysis. A special group of ether-containing phosphatidylcholines show biological properties and are bound to receptors in blood platelets. These are described as platelet-activating factor (PAF) and are 1-O-alkyl-2-acetyl-*sn*-glycerol-3-phosphocholines.

3.17 Sphingolipids

The final group of lipids to be discussed are amides, rather than esters, with the amine itself being a long-chain compound. The long-chain bases (sphingoids) are usually C_{18} or C_{20} amines with two (sphingosines) or three (phytosphingosines) hydroxyl groups. These molecular fragments with three or four functional groups on adjacent carbon atoms, have some resemblance to glycerol. In the natural derivatives the amine is acylated with a long-chain fatty acid and the primary hydroxyl group is associated with a sugar moiety or a phosphorylcholine unit. The names of some sphingolipid types are given in Table 3.10 and Fig. 3.20.

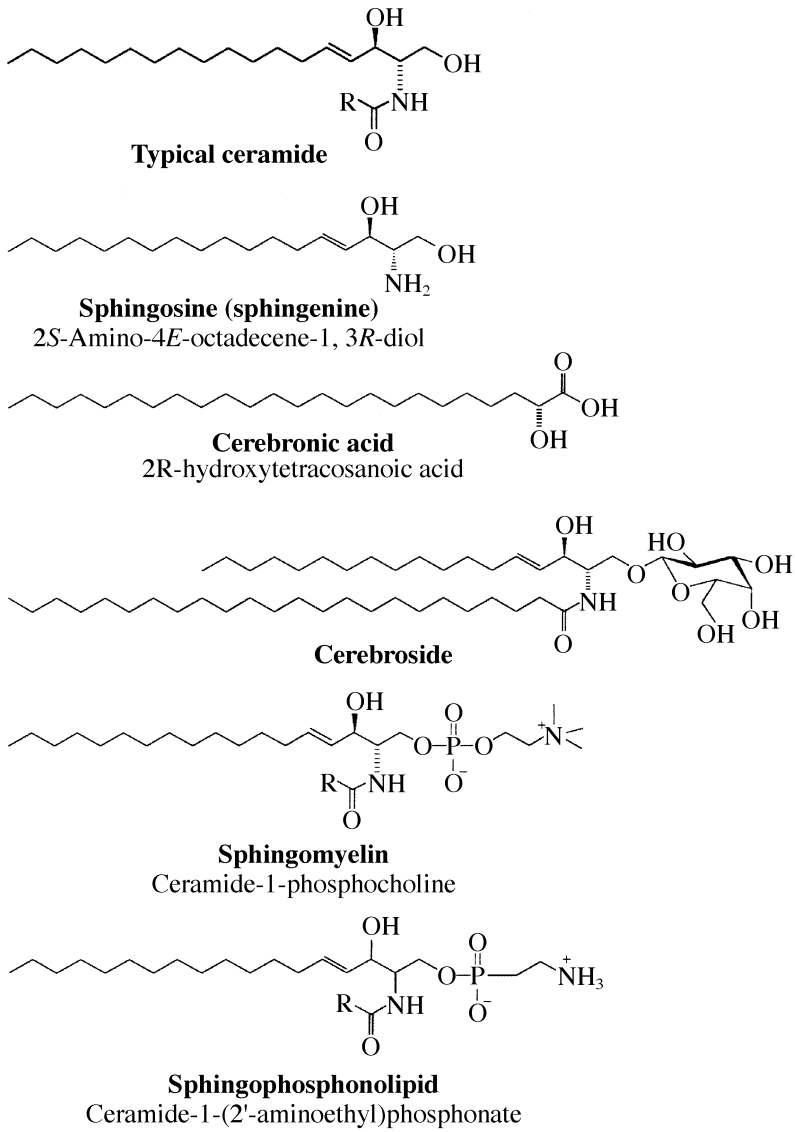


Figure 3.20 Structure of some sphingolipids.

Despite their differences, phosphoglycerides and sphingolipids resemble one another in that they both contain two lipophilic chains and a polar head group, and are conveniently located in lipid bilayers. Sulfate esters of glycoceramides are significant components of brain lipids.

Table 3.10 Structure and occurrence of some sphingolipids based on the general formula: $RCH{=}CHCH(OH)CHNHR^1CH_2OR^2$

Name	R^1	R^2	Occurrence
Sphingenine	H	H	–
Ceramides	COR^1	H	skin
Cerebrosides	COR^1	glu or gal	brain, spleen, ganglia
Gangliosides	COR^1	glu-gal-sialic acid	myelin sheath
Sphingomyelin	COR^1	$PO_3CH_2CH_2NMe_3$	animal membranes

There are several diseases (lipidoses) associated with names such as Tay-Sachs, Fabry, Gaucher, Farber, Niemann-Pick and Crabbe, in which sphingolipids accumulate in certain organs because of some defect in their metabolism. Sphingolipids frequently contain α-hydroxy acids and in one unusual ceramide present in the epidermis there is an ω-hydroxy acid esterified with linoleic acid. This illustrates how nature adapts existing molecules to produce compounds with appropriate functionality.

Acknowledgement

Structures in Figs 3.2, 3.3, 3.5, 3.6, 3.7 and 3.8 have been copied with permission of Gunstone & Herslof (2002) and the Oily Press.

Bibliography and references

Gunstone, F.D. (forthcoming) Vegetable Oils in Bailey 6, (ed F. Shahidi).
Gunstone, F.D. & Herslof, B.G. (2000) *Lipid Glossary 2*, The Oily Press, Bridgwater.
Gunstone, F.D., Harwood. J.L. & Padley, F.B. (1994) *The Lipid Handbook*, 2nd edn, Chapman and Hall, London.
Huang, Yung-Sheng & Ziboh, V.A. (2001) *γ-Linolenic Acid: Recent Advances in Biotechnology and Clinical Applications*. AOCS Press, Champaign, Illinois.
Sebedio, J-L, Christie, W.W. & Adlof, R. (2003) *Advances in Conjugated Linoleic Acid Research,* Volume 2, AOCS Press, Champaign, Illinois.
Ucciani, E. (1995) *Nouveau Dictionnaire des Huiles Vegetales,* Lavoisier TEC & DOC, Paris.
Vance, D.E. & Vance, J. (eds) (2003) *Biochemistry of Lipids, Lipoproteins, and Membranes*, Elsevier, Amsterdam.
<www.aocs.org/member/division/analysis.fanames> Common (non-systenatic) names for fatty acids
<www.bagkf.de/sofa>
<www.cyberlipid.org>
<www.lipid.co.uk>
<www.natri.tamuk.edu>

4 Chemical and biological synthesis of fatty acids and lipids

4.1 Fatty acid synthesis

4.1.1 Introduction

Where do research workers or technologists go when they want a fatty acid or glycerol ester that is pure, or at least of defined composition? Material available from suppliers of fatty acids and lipids is generally expensive and may be available only in gram or milligram quantities. These materials have probably been synthesised in the laboratory or have been isolated from appropriate natural sources using standard isolation procedures (section 4.7). Of those available, many of the saturated acids and of the more common unsaturated acids are of natural origin. For some purposes, materials rich in the desired component may suffice. For example, high-oleic sunflower seed oil (section 1.3.16) may be used as a source of oleic acid or of triolein and may be adequate when large quantities are needed and when price is important.

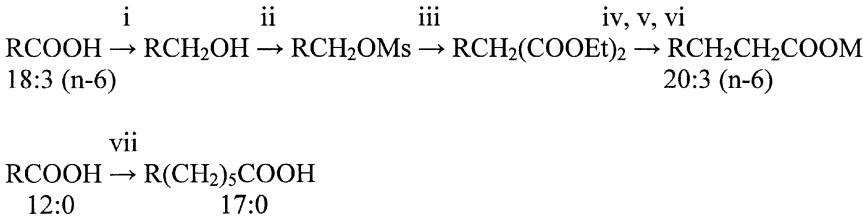
For fatty acids the major isolation procedures include crystallisation, urea fractionation, molecular distillation, chromatography and enzymic enhancement based on appropriate selectivity (section 4.7), but isolation from a natural source is not always appropriate. It cannot be applied:

- for acids for which there is no convenient natural source from which they could be isolated,
- for acids that do not occur naturally, such as those with unsaturation in unusual positions and *trans* isomers,
- for acids required in isotopically labelled form.

4.1.2 Partial synthesis by chain extension

Organic chemists have developed procedures by which a molecule such as a fatty acid can be efficiently chain-extended by:

- one carbon atom usually provided by cyanide or carbon dioxide,
- two carbon atoms by chain extension with ethyl malonate,
- five or six carbon atoms provided by cyclopentanone or cyclohexanone respectively.



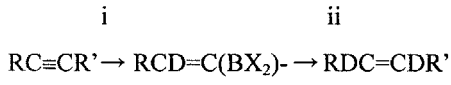
Scheme 4.1 Conversion of γ-linolenic acid to its bis-homologue (20:3) and of lauric acid to heptadecanoic acid (17:0). (i) $LiAlH_4$; (ii) CH_3SO_2Cl (MsCl), NEt_3; (iii) $NaCH(COOEt)_2$; (iv) KOH; H_2SO_4; (v) heat at 165°; (vi) MeOH, H_2SO_4; (vii) chain-extension with cyclopentanone (S. Hunig & W. Eckhardt, *Chem. Ber*. 1962, **95**, 2493–2497) Kettenverlangerung der Δ10-undecensaure um 5 and 6 C-atome.

These useful procedures can be applied to acids easily isolated from natural sources. For example dihomo-γ-linolenic acid (20:3 n-6) can be obtained by malonation of γ-linolenic acid (18:3 n-6) that has been isolated from evening primrose or borage oil. Heptadecanoic acid (17:0) can be made by chain extension of lauric (12:0) or palmitic acid (16:0). The former route is preferable because it is easier to separate the product from unreacted C_{12} starting material (see Scheme 4.1).

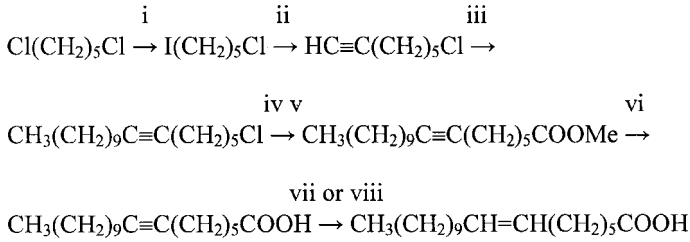
4.1.3 Synthesis via acetylenic intermediates

The synthetic versatility of acetylenic compounds, developed during the 1960s, was quickly applied to unsaturated fatty acids. Over the following 40 years, the procedures have been improved and widely used to prepare polyunsaturated-fatty acids with up to seven olefinic centres. This route has been particularly useful in the preparation of acids with the 1,4-pattern of unsaturation characteristic of fatty acids. The usefulness of this approach depends on the ease with which 1-alkynes (RC≡CH) can be alkylated with appropriate saturated or unsaturated compounds to give disubstituted alkynes (RC≡CR′) and then reduced in controlled fashion to *cis* or *trans* olefinic compounds (RCH=CHR′).

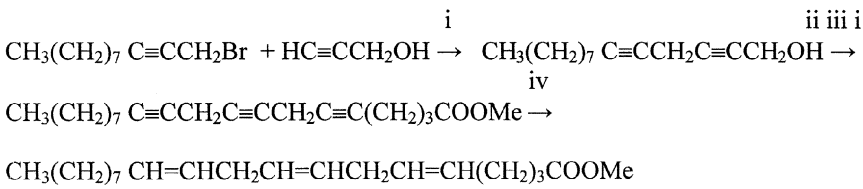
Partial reduction of alkynes has been achieved with Lindlar's catalyst (palladium partially poisoned with lead) or with P-2 nickel (prepared immediately before use by reduction of nickel(II) acetate with sodium borohydride). These catalysts give the *cis* olefin as the major reaction product. Selectivity between the *cis* and *trans* isomers is improved by addition of quinoline to the reaction with Lindlar's catalyst or of ethylene diamine to the reaction with P-2 Ni. A *trans* isomer is obtained by reduction with lithium or sodium in liquid ammonia. Alkynes can also be reduced to *cis* alkenes by hydroboration with a sterically hindered borane such as di-isoamylborane or dicyclohexylborane. Reaction with a deutero borane and CH_3COOD is a convenient route to alkenes containing the group –CD=CD– (Scheme 4.2).



Scheme 4.2 Deuteration of an alkyne. (i) BX_2D (X=C_5H_{11} or C_6H_{11}, see text); (ii) CH_3COOD.



Scheme 4.3 Synthesis of 7-octadecenoic acid. (i) NaI, $COMe_2$; (ii) NaC≡CH, NH_3; (iii) $NaNH_2$, NH_3, $CH_3(CH_2)_9Br$; (iv) KCN, DMSO; (v) MeOH, H^+; (vi) KOH, H_3O^+; (vii) H_2/Pd to give the *cis* isomer, (viii) Li/NH_3 to give the *trans* isomer.



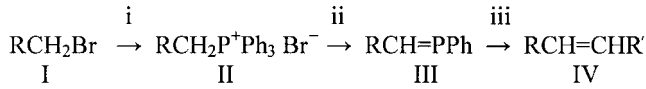
Scheme 4.4 Synthesis of 14,15-dihydroarachidonic acid (5,8,11-eicosatrienoic acid, 20:3 n-9) as the methyl ester (adapted from Jeffery *et al*., 1992). (i) CuI, *n*-Bu_4NCl, Na_2CO_3, DMF, room temperature; (ii) CBr_4, PPh_3, CH_2Cl_2, 0ºC; (iii) $HC{\equiv}C(CH_2)_3COOMe$; (iv) $Ni(OAc)_2.4H_2O$, $NaBH_4$, EtOH, $H_2N(CH_2)_2NH_2$, H_2.

The acetylenic route is illustrated by reaction sequences in Schemes 4.3 and 4.4 leading to octadec-7-enoic acid and to 14,15-dihydroarachidonic acid (5,8,11-eicosatrienoic acid). The latter is based on the coupling of a propargylic halide or tosylate with 1-alkynes in the presence of copper(II) iodide and tetra-*n*-butylammonium chloride. This reaction is highly regio- and chemo-selective and avoids the use of a Grignard reagent.

However, the acetylenic route has some disadvantages. Polyacetylenic compounds are unstable and the desired all-*cis* acid may be contaminated with other compounds including acids with *trans* unsaturation. Accordingly, alternative routes have been developed, particularly those based on the Wittig reaction described in the following section.

4.1.4 Synthesis by the Wittig reaction

At its simplest, the Wittig reaction is a method for making olefinic compounds (IV). An alkyl halide (I), converted to its phosphonium salt (II) and then to an



Scheme 4.5 The Wittig reaction. (i) PhP_3; (ii) base; (iii) R′CHO.

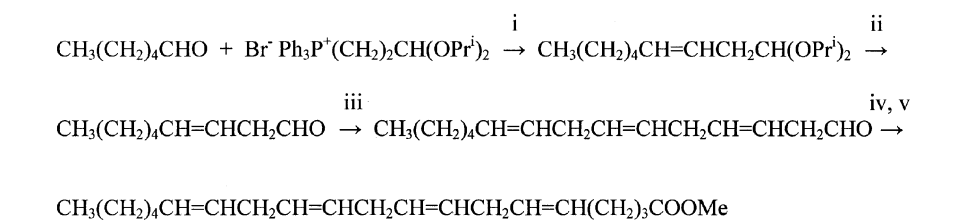
ylid (III), is condensed with an aldehyde. The carbon atoms in the olefinic product (IV) are confined to those originally present in the alkyl halide and the aldehyde (Scheme 4.5).

The reaction conditions can be adjusted to give mainly the *cis* or *trans* olefin and continual developments have increased the yield and configurational purity of the product. Unsaturated centres may already be present in the alkyl halide or the aldehyde, and the reactants may also contain other functional groups. Additionally, the coupling reaction may be carried out more than once. Appropriate C_3 synthons, used to produce the 1,4-diene unit in polyunsaturated fatty acids, are acetals such as $X^- Ph_3P^+(CH_2)_2CH(OR)_2$, made from propenal ($CH_2$=CHCHO). The synthesis of arachidonic acid (20:4), detailed in Scheme 4.6, is based on C_6 and C_5 compounds and a C_3 synthon used three times. In a similar way, eicosapentaenoic acid (5,8,11,14,17-20:5) was synthesised from $OHC(CH_2)_3COOMe$ (C_5), $CH_3CH_2CH_2Br$ (C_3) and two C_6 synthons (I and II). The two bromo compounds are reacted as ylids.

I $Br(CH_2)_2CH$=$CHCH_2CH(OEt)_2$
II $Bu^tMe_2SiO(CH_2)_2CH$=$CHCH_2CHO$

4.1.5 Other synthetic procedures

1,4-Dienes and enynes can be prepared by allylation of vinyl or alkynyl metallic compounds as illustrated in Scheme 4.7. This reaction occurs in tetrahydrofuran-hexamethylphosphoramide solution at temperatures between −78ºC and +20ºC.



Scheme 4.6 Synthesis of methyl arachidonate by an iterative Wittig process (Viala & Santelli, 1998). (i) $NaN(SiMe_3)_2$, THF-HMPA, −100ºC; (ii) TsOH, THF, heat; (iii) repeat the C_3 homologation twice; (iv) phosphonium salt from $Br(CH_2)_4COOH$, $NaN(SiMe_3)_2$, THF-HMPA, −100ºC; (v) CH_2N_2.

$$R^1CH{=}CHCH_2Br + LiCu[C{\equiv}CR^2]_2 \longrightarrow R^1CH{=}CHCH_2C{\equiv}CR^2$$

Scheme 4.7 Preparation of 1,4-enynes by allylation of a metallic 1-alkyne.

4.1.6 Synthesis of isotopically labelled acids

Adlof (1999) has prepared a very extensive review of this topic. Fatty acids may be labelled with stable isotopes (commonly ^2H [D] or ^{13}C) or with radioactive isotopes (^3H, ^{11}C, or ^{14}C). This distinction is important because the stable and radioactive materials are used in different ways and are detected by different procedures.

Lipids made with isotopically-labelled fatty acids are used *in vivo* and *in vitro* to study reaction mechanisms, biosynthesis, absorption and metabolism but only those with stable isotopes can be used in human dietary studies. Isotopically-labelled compounds are analysed by mass spectrometry (all labelled compounds), nuclear magnetic resonance spectrometry (^2H and ^{13}C), scintillation procedures (^3H and ^{11}C) or positron emission (^{14}C).

Acids made by the methods already described for non-isotopic acids can be modified using isotopically-labelled forms of reagents such as sodium borohydride, hydrazine, lithium aluminium hydride, water, hydrogen with suitable catalysts, di-isoamylborane, carbon dioxide and cyanides. Examples include:

- Partial reduction of acetylenic compounds with tetradeuterohydrazine (N_2D_4) or with D_2 and Lindlar's catalyst

  $RC{\equiv}CR' \rightarrow RC^2H{=}C^2HR'$ [i.e. RCD=CDR′]

- Conversion of alcohol (as mesylate or tosylate) or alkyl halide to fatty acid with one additional carbon atom by reaction with labelled (^{13}C or ^{14}C) cyanide or carbon dioxide.

  RX → R*COOMe (where *C is a labelled carbon atom)

- The Barton reaction (section 8.10) provides a convenient route to fatty acids labelled at C-1. In this reaction, any fatty acid can be converted to its *nor*-bromide (i.e. a molecule with one less carbon atom) which can then be reacted with labelled cyanide or carbonate to reform the acid with a labelled *COOH group. The Barton reaction involves conversion of RCOCl to RCl or RBr by reaction with 2-mercaptopyridine *N*-oxide in $BrCCl_3$ in the presence of 4-dimethyaminopyridine.

4.2 Acylglycerol synthesis

4.2.1 Introduction

The acylation of glycerol or some equivalent molecule has a long history and improved procedures are continually being developed. A balance has to be struck between the complexity, purity, quantity required and cost/value of the desired product. For the purest products, the acylating agent must be prepared from a pure fatty acid obtained as described in section 4.1.

Because of the possibilities of isomerism in glycerol esters discussed in section 3.12, attention must be directed to the regiospecificity and stereospecificity of the desired product. Most of the early procedures furnished racemic products and the methods had to be modified to produce enantiomeric esters.

In the following discussion attention is directed to the following:

- Selection of the C_3 synthon that will furnish the three glycerol carbon atoms.
- Minimising the danger of acyl migration from one oxygen atom to an adjacent one.
- Selection of acylating agent and reaction conditions.
- Introduction of appropriate protecting (blocking) groups and their safe removal at a late stage in the reaction scheme.
- Methods of analysis to confirm the structure and purity of the synthetic product (see section 5.4.2).

4.2.2 C_3 synthons

Glycerol esters with only one type of acyl group can be made by acylation of glycerol itself, using appropriate acylating conditions (section 4.2.3). When more than one acyl group is to be introduced then a derivative of glycerol with appropriate protecting group(s) (section 4.2.5) should be employed. Alternatively, glycerol is replaced by a C_3 compound with functional groups of differing reactivity, such as those listed in Table 4.1.

The preparation of enantiomeric glycerol esters is possible through the use of enantiomeric C_3 synthons such as the *R* and *S* forms of 1,2-glycerol acetonide prepared from natural enantiomeric materials such as D-mannitol, L-serine, or L-arabinose.

4.2.3 Acylation procedures

Acylation is the reaction in which a free hydroxyl (or amino) group reacts with a fatty acid to form an ester (amide) thus:

$$RCOOH + R'OH \rightarrow RCOOR'$$

This formulation is too simple. Free carboxylic acids are not generally

Table 4.1 Glycerol and other synthons commonly employed in the synthesis of glycerol esters

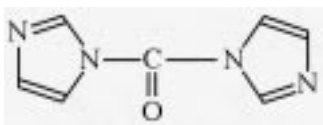
Common name	Systematic name
Glycerol	1,2,3-trihydroxypropane
1,2-Glycerolacetonide	1,2-O-isopropylideneglycerol[a]
–	1,3-O-benzylidene glycerol
Benzyl and substituted benzyl ethers of glycerol[b]	
Dihydroxyacetone	1,3-dihydroxypropan-2-one
Allyl alcohol	propenol
Glycidol	2,3-epoxypropanal
–	3-chloropropane-1,2-diol

[a] available in *R*, *S* and racemic forms. [b] 4-methyl and 2-iodo-benzylchloride.

sufficiently active to react with an alcohol. Either a catalyst or a more active acylating agent must be employed. The latter may have to be prepared prior to the acylation step or, more conveniently, be produced *in situ* from appropriate reagents. The following systems have been reported:

- Reaction of the C_3 synthon at room temperature for 1–3 days or at 100ºC for 4 hours with a slight excess of acyl chloride (RCOCl) and an equivalent amount of pyridine or other tertiary base to remove the hydrogen chloride produced along with the ester. Acid chlorides are pre-formed by reaction of carboxylic acid with thionyl chloride ($SOCl_2$) for saturated acids or with oxalyl chloride [$(COCl)_2$] for unsaturated acids.
- As above, but replacing the acid chloride with a mixture of anhydride [$(RCO)_2O$] and triflouromethanesulfonic acid (CF_3SO_3H) which together form the reactive mixed anhydride $RCOOSO_2CF_3$.
- Direct reaction of carboxylic acid and hydroxy compound in the presence of 4-toluenesulfonic acid, trifluoroacetic anhydride or sulfonated polystyrene resin as catalysts.
- Acylation with free acid also occurs in the presence of 1,1-dicyclohexylcabodiimide (DCC, this reagent is reported to be potentially carcinogenic and should be handled with care) and 4-dimethylaminopyridine (DMAP) in di- or tetra-chloromethane solution over 2 to 24 hours. Alternatively, 1,1′-carbonyldiimidazole can be used at ambient temperature.

  DCC $C_5H_{11}N{=}C{=}NC_5H_{11}$ [C_5H_{11} = cyclohexyl]



1,1′-carbonyldiimidazole

- Reaction of caesium salts with alkyl bromides.

- Reaction of methyl esters with triacetin (transesterification).
- Reaction of carboxylic acids with epichlohydrin.
- Reaction of vinyl esters with hydroxy compounds.
- Reactions promoted by enzymes (sections 4.2.8 and 8.3).

4.2.4 Acyl migration

When an acyl group has a free hydroxyl group on an adjacent carbon atom, then the acyl group can migrate to the free hydroxyl group. Reaction occurs through an intermediate ortho ester with a five-membered ring that can open to produce the original ester or an isomeric ester in which the acyl group has migrated. The product will be an equilibrium mixture of two isomers. For example, 1-acylglycerol changes to a 90:10 mixture with the 2-acylglycerol. More seriously, pure 2-acylglycerols furnish the same 90:10 mixture of the 1- and 2-acylglycerols. Pure diacylglycerols (1,2- or 1,3-) will also give an equilibrium mixture of the two isomers. Migration does not occur when the free hydroxyl group is blocked but care must be taken when the blocking group is removed and the hydroxyl group is uncovered (Scheme 4.8).

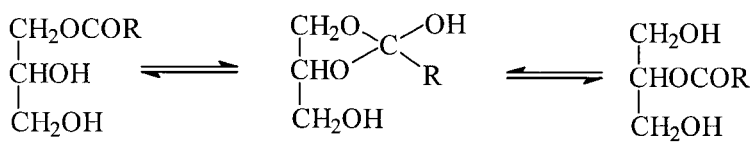
Acyl migration is promoted under acidic or alkaline conditions, in polar solvents, and by heating, so all these conditions should be avoided when preparing glycerol esters. This is reflected in the conditions selected for removing protecting (blocking) groups (section 4.2.5).

4.2.5 Protecting (blocking) groups

Protecting groups are used to block reaction at a particular site and are removed after they have served that purpose. It is important that the attachment and removal of these are achieved without any other change in the compound being produced. Some examples of protecting groups are listed in Table 4.2.

4.2.6 Synthesis of racemic acylglycerols

Following the general points made in the previous sections (4.2.2–4.2.5) it is now possible to examine some examples of the synthesis of glycerol esters. Mono- and di-acylglycerols may be end-products or they may be intermediates in the production of triacylglycerols. Examples include the preparation of 1-monoacylglycerols (Scheme 4.9), 2-monoacylglycerols (Scheme 4.10), 1,3-diacylglycerols (Schemes 4.11 and 4.12), 1,2-diacylglycerols (Schemes 4.13) and triacylglycerols with two or three different acyl groups (Scheme 4.14).

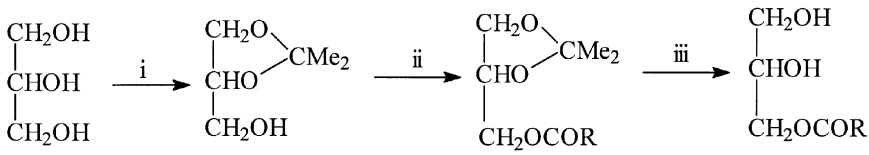


Scheme 4.8 Interconversion of 1- and 2-monoacylglycerols.

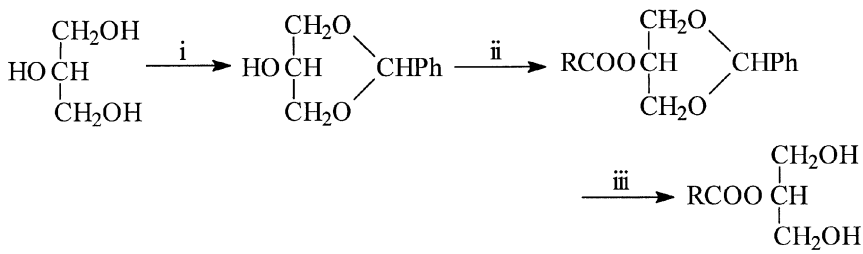
Table 4.2 Protecting groups used in the formation of acylglycerols (for further examples and details see Sonnet, 1999)

Protecting group	Reagent for formation	Reagent for removal
isopropylidine	acetone, TSA[a]	aqueous acid (or) $B(OMe)_3$ and HBO_3 (or) $B(OEt)_3$ followed by H_2O (or) Me_2BBr_2 -50^oC
benzylidine	benzaldehyde, TSA[a]	H_2, Pd/C
benzyl	benzyl chloride	PriBr or BF_3 or H_2-Pd/C
trityl (Ph_3C)	trityl chloride	$(CF_3CO)_2O$
tetrahydropyranyl	dihydropyran	$(CF_3CO)_2O$

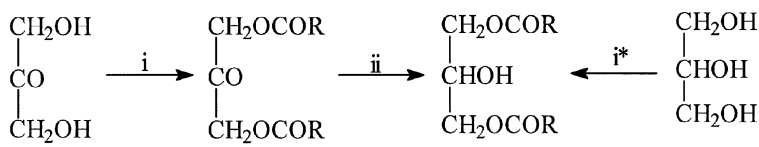
[a] 4-toluenesulfonic acid.



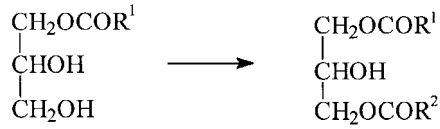
Scheme 4.9 The preparation of 1-monoacylglycerols. (i) $COMe_2$, C_6H_6, 4-$MeC_6H_4SO_3H$; (ii) RCOOH, 4-$MeC_6H_4SO_3H$, (iii) H_3BO_3, $B(OMe)_3$; H_2O.



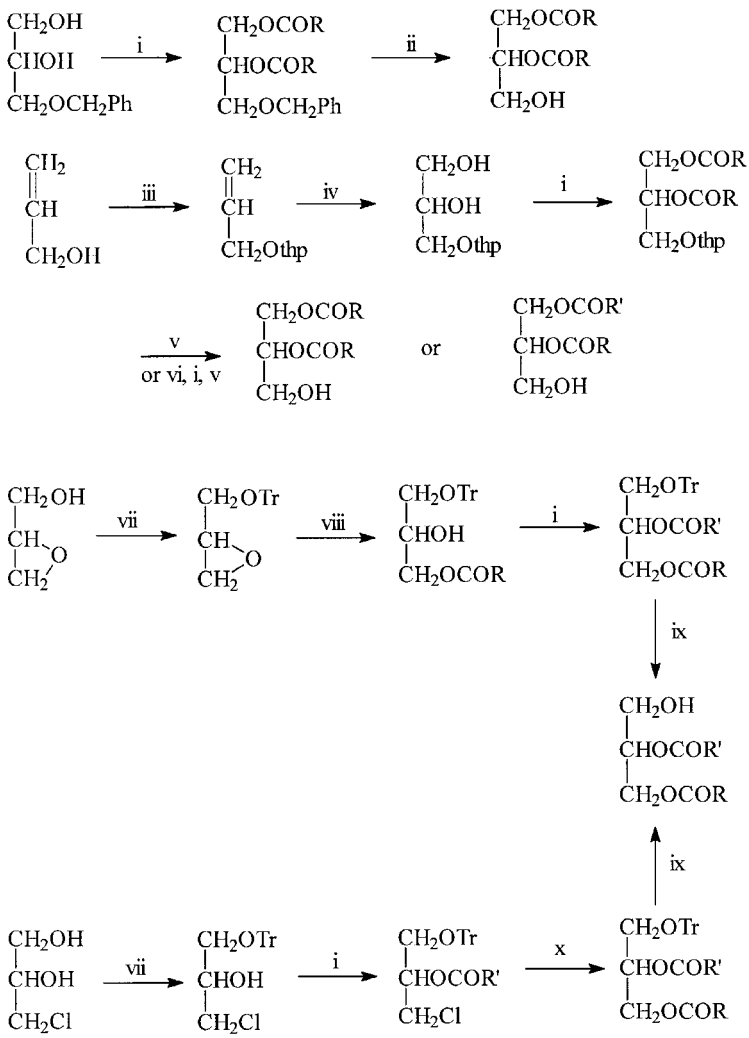
Scheme 4.10 The preparation of 2-monoacylglycerols. (i) PhCHO, 4-$MeC_6H_6SO_3H$; (ii) RCOCl, pyridine; (iii) H_3BO_3, $B(OMe)_3$; H_2O.



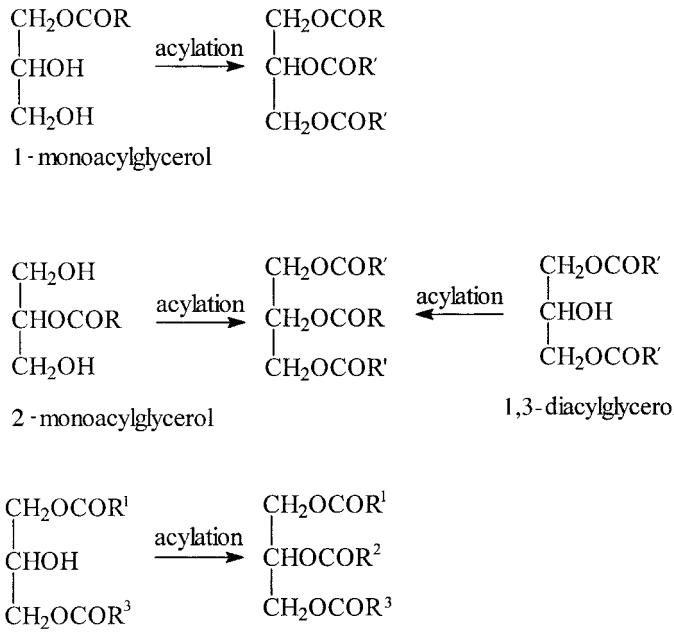
Scheme 4.11 The preparation of 1,3-diacylglycerols with identical acyl groups from dihydroxyacetone or from glycerol. (i) RCOCl, pyridine; (ii) $NaBH_4$. * Primary hydroxyl groups are more reactive than secondary alcohol groups but the product will not be pure.



Scheme 4.12 The synthesis of 1,3-diacylglycerols with different acyl groups. The monoacylglycerol is reacted with R^2COCl and pyridine. Primary hydroxyl groups are more reactive than secondary hydroxyl groups but the product will not be pure.



Scheme 4.13 Four routes to 1,2-diacylglycerols. (i) acyl halide, pyridine; (ii) Ni, H_2; (iii) dihydropyran, H^+; (iv) $KMnO_4$; (v) HCl or $B(OH)_3$; (vi) pancreatic lipase; (vii) Ph_3Cl; (viii) RCOOH; (ix) $(CF_3CO)_2O$, MeOH; (x) RCOONa: thp = tetrahydropyranyl, Tr = triphenylmethyl.



Scheme 4.14 The preparation of triacylglycerols.

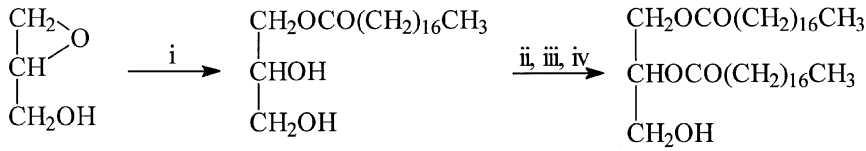
Triacylglycerols containing only one type of acyl group are made by acylation of glycerol with free acid, acid chloride or acid anhydride. For example, reaction of glycerol and free acid with toluene-4-sulfonic acid (TSA) as a catalyst, tetrahydrfuran as a solvent and a molecular sieve as desiccant, gives a good yield of triacylglycerol after reaction at 110–120ºC for about six hours.

4.2.7 Synthesis of enantiomeric acylglycerols

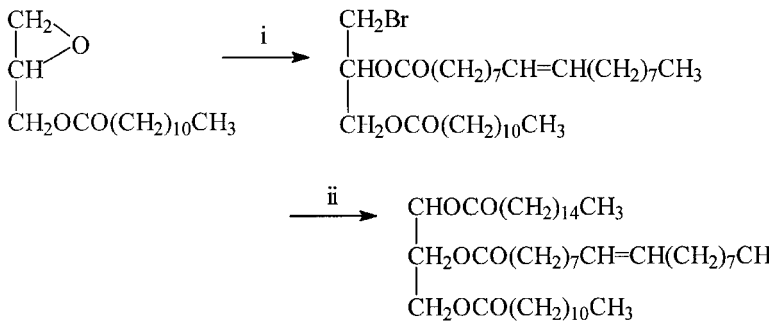
Using enantiomeric C_3 synthons, the procedures described in the previous section can be adapted to give enantiomeric glycerol esters. Two examples are cited using *S*-glycidol (Scheme 4.15) and *R*-glycidol (Scheme 4.16). The 1,2-diacyl-*sn*-glycerols shown in Scheme 4.16 can be used to prepare enantiomeric triacylglycerols or phospholipids.

4.2.8 Structured lipids

This term is used to describe triacylglycerols with a particular specification, usually prepared in large quantities. For example, MLM triacylglycerols are glycerol esters with medium-chain acids in the 1 and 3 positions and long-



Scheme 4.15 The synthesis of 1-monoacyl and 1,2 diacyl-*sn*-lglycerols from *S*-glycidol. (i) stearic acid, Ti(OPri)4; (ii) Me_2Bu^tSiCl, imidazole (to block the *sn*-3 position); (iii) stearoyl chloride, pyridine; (iv) NBS, DMSO, H_2O, THF (to remove blocking group). The 1,2-diacylglycerol can be used to prepare enantiomeric triacylglycerols or phospholipids.



Scheme 4.16 The synthesis of enantiomeric triacylglycerols from *R*-glycidol. (i) oleic anhydride, LiBr, THF or C_6H_6; (ii) palmitic acid as salt, THF, HMPT, 45–50°C. Starting with palmitate instead of laurate and with appropriate adjustment of reagents it is possible to prepare the enantiomer.

chain acids (frequently DHA) in the 2 position. Such compounds are often made by modification of natural lipid mixtures using the specificity of enzymes in several types of esterification (section 8.3).

4.3 Phospholipid synthesis

This topic will not be covered in detail, but the major routes are outlined. Further information is available in reviews by Bittman (1993, 1999).

- Preparation from 1,2-diacylglycerols (section 4.2.6) involves conversion of the diacylglycerol (ROH) to a more reactive iodo compound (RI) and thence to a PC or PE.
- Conversion of pure PC or PE classes of phospholipids from natural sources to the required enantiomeric GPC or GPE by complete deacylation followed by controlled stepwise re-acylation with the required fatty acids (Doig & Diks, 2003a,b).
- Conversion of natural PC or PE to other phospholipid classes by exchange of the choline or ethanolamine moieties with other groups using phospholipase D (transphosphatidylation) (Doig & Diks, 2003a,b).

4.4 Sphingolipid synthesis

The structures of the sphingolipids based on sphingosine and phytosphingosine have been described in section 3.17. These bases have two and three chiral centres respectively, and for the synthesis of enantiomeric products it is necessary to develop appropriate chirality at each of these centres. This has been achieved by basing the synthetic pathways on natural products already having the desired chirality. Examples include compounds such as D-glucosamine, D-mannose, D-galactose, D-xylose, L-serine and L- or D-tartaric acid. Details are given in the review by Jung & Schmidt (1999).

4.5 Fatty-acid biosynthesis

4.5.1 Introduction

An account of the biosynthesis of glycerol esters requires a preliminary discussion of fatty-acid biosynthesis. The so-called 'acetate-malonate' biosynthetic pathway leads to three different kinds of natural products depending on the detailed route followed. Fatty acids result from a reductive pathway but acetate and malonate are also precursors for isoprenoids (terpenes and sterols) produced via mevalonic acid (C_6) and for a wide range of phenolic compounds resulting from cyclisation of polyacetate. This illustrates the fact that nature is economical in the range of both substrates and reactions employed in biosynthesis.

The major biosynthetic pathways to fatty acids involve three stages:

- *De novo* synthesis of palmitic (or other alkanoic) acid from acetate (C_2, a product of carbohydrate metabolism) by reaction with malonate (C_3), itself formed from acetate.
- Further chain-elongation of saturated or unsaturated acids by one or more two-carbon units.
- Desaturation: particularly of stearic acid, first to oleic acid and then to linoleic and linolenic acids.
- Further sequences of desaturation and elongation producing discrete families of polyunsaturated fatty acids.

Whether in plants or animals these changes take place in different parts of the cell, under the influence of specific enzymes or enzyme complexes, and require the acids to be in appropriate substrate form.

While animals can produce their own supplies of fats, they also get these fats as part of their dietary intake. Much of this will be metabolised through oxidation to produce energy, stored in part as ATP, but some will be stored as lipid, perhaps after modification. Phospholipid bilayers are fundamental com-

ponents of all living matter and these must contain selected fatty acids. It will be explained later that humans (and many other animals) cannot make all the fatty acids they require and some must be obtained from dietary plant sources (section 4.5.4).

4.5.2 De novo *synthesis of saturated acids*

In plant systems, *de novo* synthesis occurs in the plastid and results mainly in the conversion of acetate to palmitate. All sixteen carbon atoms in palmitic acid are derived from acetate – half from the methyl carbon and half from the acyl carbon. Two of the carbon atoms (C_{15} and C_{16}) come directly from acetate while the other fourteen come from acetate via the more reactive malonate. Production of malonate requires the incorporation of an additional carbon atom into the acetyl group. This is supplied as bicarbonate and subsequently lost as carbon dioxide. The acyl groups are attached as thioesters to co-enzyme A (CoASH) during part of the cycle and to acyl carrier protein (ACPSH) during another part. The abbreviated symbols used for these co-enzymes emphasise the thiol groups (SH) to which the acyl chains are attached.

In the *de novo* pathway, acetate and malonate react through a series of steps converting acetate first to butanoate (C_4), then to hexanoate (C_6), and sequentially thereafter, two carbon atoms at a time, to palmitate (C_{16}). At this stage a thioesterase liberates the acyl chain from ACP. The thioesterase is not completely chain-length specific and acids of other chain lengths may be produced. This is obviously true in the lauric oils where the major saturated acid (lauric, 12:0) is accompanied by lower levels of caprylic (8:0), capric (10:0), myristic (14:0) and palmitic acid (16:0).

The four-step cycle includes condensation of acetate and malonate to give ketobutanoate with subsequent reduction to butanoate in three further steps: reduction to the 3*R* hydroxy acid, dehydration to the 2*t* acid and reduction again. Reduction is effected by NADPH (as a source of hydride ion) and a proton. The process is then repeated to add further two-carbon units until a thioesterase liberates the free acid. This sequence requires fatty acid synthase which contains all the enzymes needed for the four steps and shown in Scheme 4.17.

In the *de novo* process, acetate provides the $\omega 1$ and $\omega 2$ carbon atoms and by combination with malonate, which is equivalent to further acetate units, leads to the common fatty acids with an even number of carbon atoms. Sometimes acetate and/or malonate is replaced by another molecular unit and thereby produce some of the less common acids. In the following examples starter acetate is replaced by another acid such as:

- propionate (C_3) which leads to the formation of odd-chain acids and especially 17:0.

Conversion of acetyl-CoA to malonyl-CoA with acetyl-CoA carboxylase

$$CH_3COSCoA + HCO_3^- + ATP \rightarrow HO_2CCH_2COSCoA + P_i^- + ADP$$

Conversion of acetyl-ACP to butanoyl-ACP (four-step cycle)

condensation with β-ketoacyl-ACP synthase

$$CH_3COSACP + HO_2CCH_2COSACP \rightarrow CH_3COCH_2COSACP + CO_2 + ACPSH$$

reduction with β-ketoacyl-ACP reductase

$$CH_3COCH_2COSACP + NADPH + H^+ \rightarrow CH_3CHOHCH_2COSACP + NADP^+$$

dehydration with β-hydroxyacyl-ACP dehydrase

$$CH_3CHOHCH_2COSACP \rightarrow CH_3CH{=}CHCOSACP + H_2O$$

reduction with enoyl-ACP reductase

$$CH_3CH{=}CHCOSACP + NADPH + H^+ \rightarrow CH_3CH_2CH_2COSACP + NADP^+$$

Scheme 4.17 Conversion of acetyl-CoA to malonyl-CoA with a biotin enzyme (acetyl-CoA carboxylase) and conversion of acetyl-ACP to butanoyl-ACP in a four-step cycle.

- branched acids with four and five carbon atoms such as $(CH_3)_2CHCOOH$ and $CH_3CH_2CH(CH_3)COOH$. These lead to the series of iso (even-chain and mainly C_{18}) and anteiso (odd-chain and mainly C_{17}). The short-chain starters are products of protein metabolism being formed from valine and isoleucine respectively.
- cyclopent-2-enyl carboxylic acid is the starter acid for the production of the cyclopentenyl acids (section 3.8).

If malonate (from acetate) is replaced by methylmalonate (from propionate) then branched methyl groups appear in the chain (section 3.8).

4.5.3 Desaturation to monoene and polyene acids and elongation in plant systems

The first desaturation of a saturated acyl chain occurs in the plastid. The most common is the conversion of stearate to oleate and involves the removal of pro-(*R*) hydrogen atoms from C-9 and C-10 to give a *cis* olefinic bond under the influence of a Δ9 desaturase. The system is oxygen-dependent and involves the reduced form of ferredoxin. Other saturated acids can be desaturated similarly so there is a group of Δ9 monoene acids such as myristoleic (9*c*-14:1), palmitoleic (9*c*-16:1), oleic (9*c*-18:1) and gadoleic (9*c*-20:1).

Elongation by two carbon atoms occurs commonly in fatty-acid biosynthesis. It is a variant of *de novo* chain-lengthening and occurs with acetyl or malonyl CoA or ACP derivatives. The substrate is any pre-formed saturated or unsaturated acid. For example, erucic (22:1) in high-erucic acid rapeseed oil and nervonic

acid (24:1) in honesty seed oil are formed from oleic acid by two and three elongations respectively. These belong to a family of n-9 monoene acids.

18:1 (9)	→	20:1 (11)	→	22:1 (13)	→	24:1 (15)
oleic		cetoleic		erucic		nervonic

Further desaturation in the cytoplasm converts oleate (in the form of a phosphatidylcholine) to linoleate (Δ12 desaturase) and converts linoleate (as its monogalactosyldiacylglycerol derivative) to linolenate (Δ15 desaturase). The additional double bonds have *cis* configuration and are in a methylene-interrupted relation to each other. This 1,4 diene unit is characteristic of polyunsaturated fatty acids and is to be distinguished from the 1,3 (conjugated) systems in carotenoids and the 1,5 system in terpenes.

It is of interest that enzymes converting oleate to the acetylenic acid crepenynic (9c12a-18:2) and to the epoxy acid vernolic (12,13-epoxyoleic) are very similar to Δ12 deaturase which converts oleate to linoleate. A small change in the amino acid sequence of the desaturating enzyme is sufficient to lead to these different fatty acids from the same substrate.

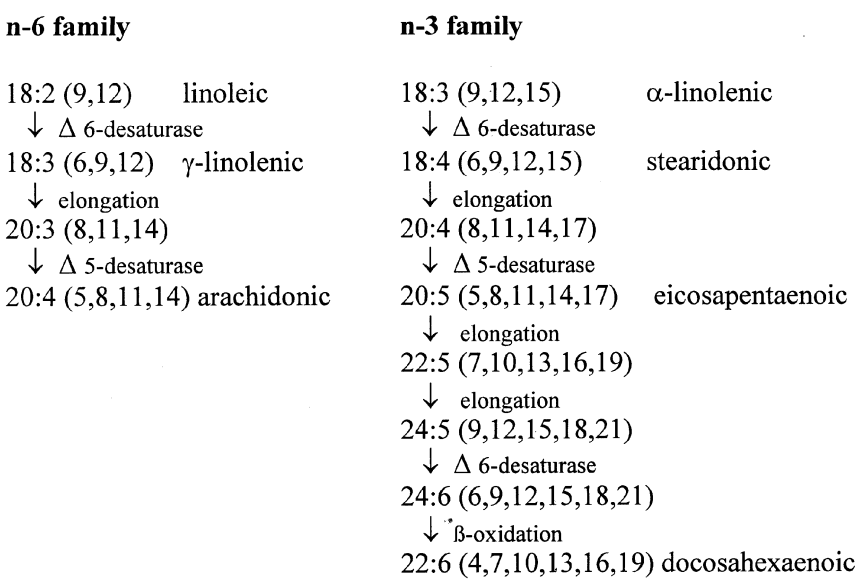
Fatty acid desaturases are proteins with di-iron centres and differ from one another in several ways:

- They use different electron donors – animals use NADH, plants use reduced ferredoxin.
- The intermediate electron carrier is generally cytochrome b_5 but need not be.
- The nature of the fatty acid substrate varies between CoA esters in mammals, stearoyl-ACP in plants, and phosphoglycerides or glycosylglycerides in cyanobacteria, plants and some yeasts.
- The position in which a double bond will be inserted may be related to the COOH group, the methyl group, or to an existing olefinic centre.

Though common in animal systems, the Δ6 desaturase is less apparent in the plant world. However it operates in the biosynthesis of γ-linolenic acid (6,9,12-18:3) from linoleate and of stearidonic acid (6,9,12,15-18:4) from α-linolenate. The C_{20} and C_{22} polyenes that characterise animal systems and particularly fish lipids either do not exist in plant systems or are exceedingly rare. The production of important acids such as arachidonic (5,8,11,14-20:4), eicosapentaenoic (5,8,11,14,17-20:5) and docosahexaenoic (4,7,10,13,16,19-22:6) in plant systems is a challenge for plant geneticists. Research has got as far as proof of concept but much remains to be done before an economically viable system is produced.

4.5.4 Desaturation and elongation in animal systems

Families of polyene acids are produced by a combination of elongation and desaturation processes starting with palmitoleic acid (n-7 family), oleic (n-9



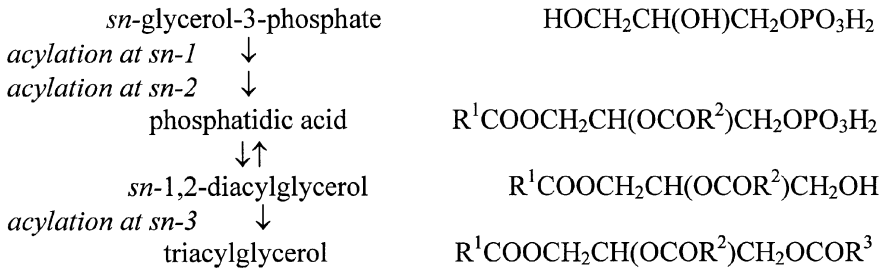
Scheme 4.18 The n-6 and n-3 families of polyunsaturated fatty acids. The most significant acids in these sequences are linoleic and arachidonic in the n-6 family and α-linolenic, eicosapentaenoic, and docosahexaenoic acid in the n-3 family. The two C_{20} acids are precursors of an important group of eicosanoids including the prostaglandins and leukotrienes (section 7.3.5). The numbers in parenthesis indicate the positions of the double bonds all of which have the *cis* configuration.

family), linoleic (n-6 family), and linolenic acid (n-3 family). The acids in each family share a common structural feature, i.e. the position of the double bond closest to the methyl end of the molecule. These changes are particularly important in animal systems and lead to the long-chain polyunsaturated fatty acids that are of considerable nutritional significance (section 9.3). The changes occurring in mammalian systems are set out in Scheme 4.18. The same enzymes are used in each family and there is competition for access to these. The ratio of n-6 to n-3 acids required in the diet for optimum health is a matter of present debate (section 9.3).

4.6 Lipid biosynthesis

4.6.1 Formation of triacylglycerols

The glycerol carbon atoms present in glycerolipids are derived from several C_3 compounds including glycerol (1,2,3-trihydroxypropane), glyceraldehyde (2,3-dihydroxypropanal) and dihydroxyacetone (1,3-dihydroxypropan-2-one), all of which are products of carbohydrate metabolism.



Scheme 4.19 Conversion of *sn*-glycerol-3-phosphate to triacylglycerol via phosphatidic acid and *sn*-1,2-diacylglycerol with selective acylation at the *sn*-1, 2, and 3 positions.

In animals, plants, and yeasts, triacylglycerols are made by the Kennedy pathway from phosphatidic acids or 1,2-diacylglycerols which are themselves interconvertible. Acylation of both of these is carried out by fatty acids as their CoA thiol esters. The three esterification reactions are enzyme-controlled with marked selectivity leading to non-random distribution of fatty acids in plant lipids. Saturated acids are preferentially bound at the *sn*-1 position and unsaturated acids at the *sn*-2 position. The acyl transferase for the *sn*-3 position is less selective and fatty acids in this position are largely controlled by the acyl-CoA pool. The phospatidic acid/1,2-diacylglycerol duo is also an intermediate in the pathway to the phospholipids (PC, PE, PI, PG and PS) and the mono- and di-galactosyldiacylglycerols (section 4.6.2).

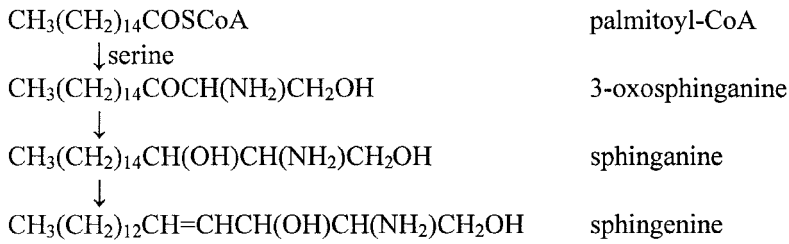
4.6.2 Phospholipids

Phospholipids are produced biosynthetically from the interchangeable duo phosphatidic acids and diacylglycerols (section 4.6.1 and Scheme 4.19) as indicated in Table 4.3.

Table 4.3 Biological sources of phospholipids

Product	Reacting materials
PI	PA CMP inositol
PG	PA CMP G-3P
PC	DAG CMP-phosphorylcholine
PE	DAG CMP-phosphorylethanolamine
MGDG and DGDG	DAG UDP-galactose

PI = phosphatidylinositols; PG = phosphatidylglycerols; PC = phosphatidylcholines;
PE = phosphatidylethanolamines; MGDG = monogalactosyldiacylglycerols;
DGDG = digalactosyldiacylglycerols. PA = phosphatidic acids; DAG = diacylglycerols;
G-3P = glycerol 3-phosphate, CMP = cytidine monophosphate; UDP = uridine diphosphate.
Phosphidylethanolamines are a source of phosphatidylserines by base exchange and provide an alternative route to phosphatidylcholines by stepwise methylation with *S*-adenosylmethionine.



Scheme 4.20 Biosynthesis of sphingenine from palmitoyl-CoA and serine.

4.6.3 Sphingolipids

Sphingenine is made from palmitoyl-CoA and serine (Scheme 4.20). It can then be converted to a cerebroside or to sphingomyelin with the materials indicated:

- Cerebroside via psychosine (UDP-galactose and then acyl-CoA) or ceramide (acyl-CoA and then UDP-galactose).
- Sphingomyelin via sphingenine phosphorylcholine (CDP-choline and then acyl-CoA) or ceramide (acyl-CoA and then CDP-choline).

4.7 Isolation procedures

4.7.1 Crystallisation

Crystallisation from selected solvents 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.

4.7.2 Distillation under reduced pressure

Distillation of unsaturated methyl or ethyl esters (for boiling points see Table 3.1) is not generally advised because they are exposed to high temperatures. Under these conditions, double bond isomerisation (both positional and configurational) or cyclisation may occur, though oxidation can be minimised by working under vacuum. Exposure to heat is reduced by short-path or molecular distillation. Reduced pressure distillation, which depends on boiling

point, 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 cuts rich in C_{14}, C_{16}, C_{18}, C_{20} and C_{22} acids.

4.7.3 Chromatography including silver ion systems

Fatty acids, usually as methyl esters, can be separated by several chromatographic techniques. These processes are used mainly for analytical purposes and will be described in Chapter 5. Silver ions react reversibly with olefinic centres so that saturated, monoene and various polyene esters can be effectively separated. This is mainly operated on a very small scale but useful separation on a mg scale has been described by Christie (section 5.3.2).

4.7.4 Urea fractionation

Urea fractionation is a useful method of separating fatty acids or their methyl esters, mainly on the basis of total unsaturation. Double-bond position and chain length have only a minor effect, but there is a significant difference in the behaviour of *cis* and *trans* isomers. When urea crystallises from methanol or ethanol in the presence of long-chain aliphatic compounds it forms hexagonal crystals containing a channel in which fatty acids or their esters can be trapped if they are the appropriate dimensions. Saturated acids form stable complexes more readily than unsaturated acids and oleic acid forms an inclusion compound more readily than polyene acids. In a typical case, urea and mixed acids are crystallised from methanol at 0–4ºC. The crystals are separated from the mother liquor and the fatty acids are recovered from each fraction. Polyunsaturated fatty acids generally remain in the mother liquor. The procedure is simple, does not damage the highly unsaturated acids, can be repeated one or more times to enhance the enrichment of a particular acid and can be effected on a gram, kilogram or tonne scale. Products from urea fractionation can be further upgraded by chromatography or by enzymic enhancement.

As an example, the n-3 polyunsaturated fatty acids in fish oil fatty acids are easily concentrated by urea fractionation. Herring oil with 12 per cent of n-3 acids (18:4. 20:5 and 22:6) gave a fraction with 69 per cent of these three acids combined. With menhaden acids, the level was raised from 22 to 91 per cent.

4.7.5 Enzymic enhancement

Glycerol and alkyl esters are related to free acids by hydrolysis and esterification, procedures which generally have to be catalysed. There are several enzyme preparations that can be used for this purpose and these display a

range of specificities which can be exploited to promote the reaction of one type of acid or ester against another.

Enzymes are catalysts, not reagents, and under appropriate experimental conditions can catalyse reversible reactions in either direction. Thus lipases, normally associated with lipid hydrolysis, can also effect esterification, transesterification and acidolyis (section 8.3).

Enzymic reactions have several potential advantages over the corresponding non-enzymic reaction. Reaction generally occurs under milder conditions of temperature and pH and there is reduced danger of undesirable side-reactions. Of greater importance, however, is their specificity. Enzymes may distinguish between:

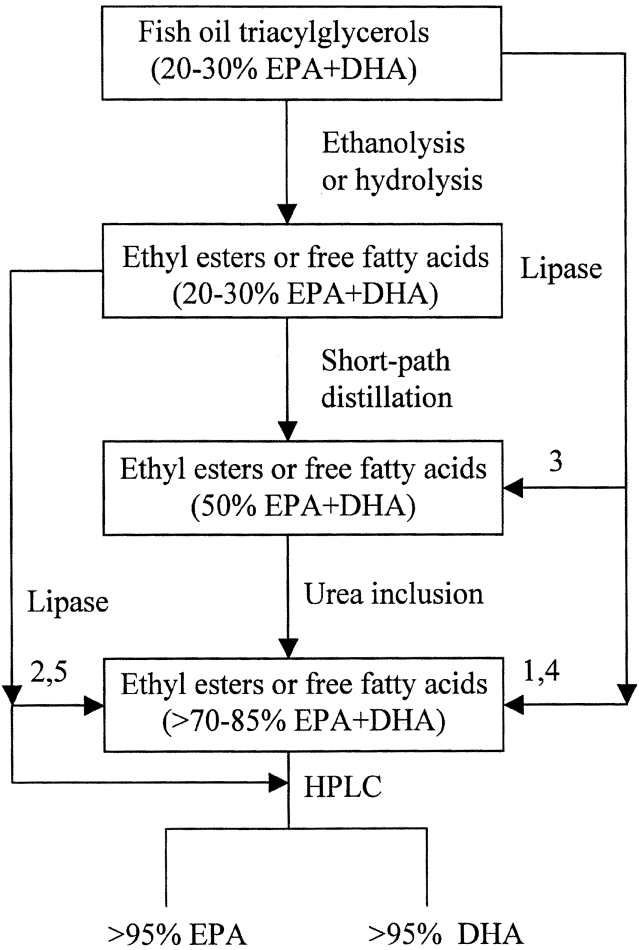
- acids according to their chain length or double bond position,
- the nature of the acylating molecule (acid or ester),
- the nature of the alcohol as between primary and secondary hydroxyl groups.

Of particular value in this context are those enzymes (for example the lipases from *Candida cylindricea, C. rugosa, C. antarctica, Rhizopus miehei, R. delemar, Pseudomonas fluorescens* and *Geotrichum candidum*) which distinguish between unsaturated acids according to double bond position. Especially important in this connection are DHA with a double bond at $\Delta 4$, EPA and AA each with a double bond at $\Delta 5$ and GLA with a double bond at $\Delta 6$. Several enzymes discriminate against these so that they tend to remain unchanged during hydrolysis or esterification. These possibilities are illustrated in Scheme 4.21 showing how it is possible to prepare EPA and DHA each of at least 95 per cent purity.

4.7.6 Chemical methods

Chemical methods of isolating fatty acids according to the number of double bonds depend 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. The reaction of olefinic acids with silver ions might be considered to fall into this category though the complex is never isolated. Olefinic compounds react with mercuric acetate to give adducts which 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.

Appropriate olefinic acids react with $KHCO_3$, I_2 and KI to give iodolactones. Acids with a $\Delta 4$ double bond (such as DHA) form an iodo γ-lactone and acids with a $\Delta 5$ double bond (such as AA or EPA) form an iodo



Scheme 4.21 Possible routes to EPA and DHA from fish oil triacylglycerols by enzymatic enhancement. Adapted from Bornscheuer *et al*. (2003, 167) with permission.

1. *Pseudomonas fluorescens* lipase, hydrolysis of tuna oil, 80% DHA+EPA in FFA.
2. *Rhizomucor miehei* lipase, ethyl esterification of FFA of tuna oil, 74% DHA, 3% EPA in the free acids.
3. *Pseudomonas* sp.lipase, ethanolyis of fish oil, 50% EPA+DHA in acylglycerols.
4. Two-step process: *Pseudomonas* sp. lipase, ethanolysis, 46% EPA+DHA in acylglycerols; urea-fractionation, 85% EPA+DHA.
5. Novozyme 435 lipase, esterification of PUFA from cod liver oil with glycerol, above 70% EPA+DHA in triacylglycerols.

δ-lactone. This scheme is formulated in Figure 7.25. There are several interesting features about these reactions:

- The iodolactones are neutral molecules and are therefore easily separated from unreacted acids. This provides the basis of a method for separating and isolating $\Delta 4$ and $\Delta 5$ acids from acids which do not have unsaturation at these positions.
- The original unsaturated acids can be recovered from their iodolactones by reaction with trimethylsilyl iodide (or Me_3SiCl and NaI).
- The iodolactonisation reaction is dependent on solvent, reaction temperature, and on the ratio of iodine to iodide. For example, with ethanol as a solvent and a reaction temperature of 25ºC, DHA gives a maximum yield of iodo-γ-lactone in 10 minutes but EPA requires 90 minutes to produce the iodo-δ-lactone.

Bibliography and references

Adlof, R.O. (1999) Isotopically labelled fatty acids. In: *Lipid Synthesis and Manufacture* (ed F. D. Gunstone), Sheffield Academic Press, Sheffield, pp. 46–93.

Bittman, R. (1993) Chemical preparation of glycerolipids: a review of recent syntheses. In: *Phospholipid Handbook* (ed G. Ceve), Marcel Dekker, New York, pp. 131–232.

Bittman, R. (1999) Chemical synthesis of glycerophospholipids and their analogs. In: *Lipid Synthesis and Manufacture* (ed F.D. Gunstone), Sheffield Academic Press, Sheffield, pp. 185-207.

Bornscheuer, U.T., Adamczak, M. & Soumanou, M.M. (2003) Lipase-catalyzed synthesis of modified lipids. In: *Lipids for Functional Foods and Nutraceuticals* (ed F.D. Gunstone), The Oily Press, Bridgwater, pp. 149–182.

Camps, F. & Guerrerro. A. (1999) Synthesis of long-chain compounds with conjugated unsaturation. In: *Lipid Synthesis and Manufacture* (ed F.D. Gunstone), Sheffield Academic Press, Sheffield, pp. 94–126.

Christophe, A.B. & De Vriese, S. (2000) *Fat Digestion and Absorption*, AOCS Press, Champaign, Illinois.

Crombie, L. (1996) Synthesis in the isotopic labelling of plant fatty acids. In: *Synthesis in Lipid Chemistry* (ed J.H.P. Tyman), Royal Society of Chemistry, Cambridge, England, pp. 34–56.

Desai, T. Gigg, J. Gigg, R., and Martin-Zamora, E. (1996) Synthesis of phosphatidylinositol phosphates. In: *Synthesis in Lipid Chemistry* (ed J.H.P. Tyman), Royal Society of Chemistry, Cambridge, England, pp. 67–92.

Doig, S.D. & Diks R.M.M. (2003a) Toolbox for exchanging constituent fatty acids in lecithins. *European Journal of Lipid Science and Technology*, **105**, 359–367.

Doig, S.D. & Diks R.M.M. (2003b) Toolbox for modification of the lecithin headgroup. *European Journal of Lipid Science and Technology*, **105**, 368–376.

Gunstone, F.D. (1999) The availability of polyunsaturated fatty acids. In: *Lipid Synthesis and Manufacture* (ed F. D. Gunstone), Sheffield Academic Press, Sheffield, pp. 39–45.

Harwood, J.L. (1999) Lipid biosynthesis. In: *Lipid Synthesis and Manufacture* (ed F.D. Gunstone), Sheffield Academic Press, Sheffield, pp. 422–466.

Jeffrey, T., Gueugnot, S. & Linstrumella, G. (1992) An efficient route to skipped diynes triynes, (Z,Z) dienes and (Z,Z,Z) trienes, *Tetrahedron Letters*, **33**, 5757–5760.

Jung, K.-H. & Schmidt, R.R. (1999) Sphingolipids. In: *Lipid Synthesis and Manufacture* (ed F.D. Gunstone), Sheffield Academic Press, Sheffield, pp. 208–249.

Kuo, T.-M. & Gardner, H.W. (2002) *Lipid Biotechnology*, Marcel Dekker, New York.

Lie Ken Jie, M.S.F. (1993) The synthesis of unusual and rare fatty acids. *Progress in Lipid Research,* **32**, 151–194.

Lie Ken Jie, M.S.F. & Chueng, S.W.H. (1999) Synthesis of special acids: A review. In: *Recent Developments in the Synthesis of Fatty Acid Derivatives* (eds G. Knothe & J. T. P. Derksen), AOCS Press, Champaign Illinois, pp. 20–43.

Parkin, K.L. (2002) Biosynthesis of fatty acids and storage lipids in oil-bearing seed and fruit tissue of plants. In: *Food Lipids – Chemistry, Nutrition, and Biotechnology* 2nd edn, (ed C.C. Akoh & D.B. Min), Marcel Dekker, New York, pp. 909–966.

Schmidt, R.R. (1996) Sphingosines, phosphosphingolipids, and glycosphingolipids. In: *Synthesis in Lipid Chemistry* (ed J.H.P. Tyman), Royal Society of Chemistry, Cambridge, pp. 93–118.

Sonnet, P.E. (1999) Synthesis of triacylglycerols. In: *Lipid Synthesis and Manufacture* (ed F.D. Gunstone), Sheffield Academic Press, Sheffield, pp. 162–184.

Taylor, R.J.K. (1996) Pyrylium-based routes to polyunsaturated natural products. In: *Synthesis in Lipid Chemistry* (ed J.H.P. Tyman), Royal Society of Chemistry, Cambridge, pp. 15–33.

Tyman, J.H.P. (1996) Recent developments in the synthesis of pheromones. In: *Synthesis in Lipid Chemistry* (ed J.H.P. Tyman), Royal Society of Chemistry, Cambridge, pp. 213–226.

Vance, D.E. & Vance, J. (ed) (2003) *Biochemistry of Lipids, Lipoproteins and Membranes,* 4th edn, Elsevier, Amsterdam.

Vatele, J-M (1999) Polyene acids. In: *Lipid Synthesis and Manufacture* (ed F.D. Gunstone), Sheffield Academic Press, Sheffield, pp. 1–38.

Viala, J. & Santelli, H. (1998) Efficient stereoselective synthesis of methyl arachidonate via C_3 homologation, *J. Org. Chem*., **53**, 6121–6123.

Weselake, R.J. (2002) Biochemistry and biotechnology of triacylglycerol accumulation in plants. In: *Lipid Biotechnology* (ed Tsung Min Kuo & H.W. Gardner), Marcel Dekker, New York, pp. 27–56.

5 Analytical procedures

5.1 Introduction

Quantitative analysis is an important part of lipid science, and developments in this field are often dependent on new and improved analytical procedures. Traditional procedures were essentially chemical in nature. They involved chemical reagents and solvents, were generally labour-intensive and many required gram quantities of material. Some of these still have a place, but increasingly they have been replaced by procedures based on physics rather than chemistry – in particular chromatography and spectroscopy. These are generally quicker, less labour-intensive, more accurate and require less material. Equipment is more sophisticated and more expensive: spectrometers and chromatography systems have largely replaced burettes and pipettes.

This chapter will outline the more commonly used analytical procedures. However, this is not a book devoted only to analysis – other texts concentrate on this topic and the fullest account at the present time is Christie's recent book (2003) and the website that he manages.

5.2 Classical analytical procedures

5.2.1 Introduction

Analytical procedures for oils and fats are driven partly by the desire to identify and quantify materials being examined in the research laboratory and partly by commercial demands. Goods are bought and sold according to a specification and there is a need to check that the specification is being met. If goods are to be traded internationally, procedures of analysis must be robust and widely recognised.

Organisations such as those listed below provide similar but not identical directions. In addition there are other national oil/fat organisations that develop analytical procedures. Full details of these tests will not be found in this book but their nature and purpose will be described.

AOAC The Association of Official Analytical Chemists
AOCS The American Oil Chemists' Society

BSI The British Standards Institution
ISO The International Organisation for Standardisation
IUPAC The International Union of Pure and Applied Chemists

Before any test is carried out it is necessary to obtain a representative sample of material and perhaps to transport and store this before any measurement is made. There are standard procedures for all these stages. Attention must be given to the storage temperature, the nature of the container, the inhibition of enzyme activity and the possible addition of antioxidants. Unless care is taken in all these matters, even the most careful analysis will be valueless.

5.2.2 Extraction

Different ways of quantitatively extracting lipid from a sample are available and depend on the nature of the matrix in which the lipid exists. For oilseeds, the oil is generally extracted from crushed seed by the Soxhlet procedure using hexane or a suitable hydrocarbon fraction such as that boiling between 40 and 60ºC. This method provides a sample of oil which can also be used for further tests. Non-destructive methods suitable for routine assessment of many samples are based on NMR (section 5.3.5) or NIR (section 5.3.8). Extraction of oils and fats on an industrial scale is described in section 2.1.

More complex methods are required for biological sources such as a liver or blood, often associated with a high proportion of water. In foodstuffs, lipid is accompanied by protein and/or carbohydrate and these sources may also require special procedures (McLean & Drake, 2002).

Biological samples are extracted with chloroform-methanol according to the well-established methods of Folch *et al*. (1957) or Bligh & Dyer (1959). In the Folch extraction, ground or homogenised tissue is shaken with a 2:1 mixture of chloroform and methanol, and the organic extract is subsequently partitioned with aqueous potassium chloride solution. The combined layers should have a volume ratio of 8:4:3 (chloroform/methanol/water). Extracted lipid is in the (lower) chloroform layer. The Bligh and Dyer method was developed for fish muscle and other wet tissue assumed to contain about 88 g of water in every 100 g of tissue. The tissue (100 g) is homogenised with chloroform (100 ml) and methanol (200 ml) and, after filtering, residual tissue is homogenised a second time with chloroform (100 ml). The two organic extracts are combined and shaken with aqueous potassium chloride (0.88%, 100 ml). After settling the lipid partitions into the lower layer.

Fat in food has been defined in Europe as total lipids including phospholipids and in the United States as fatty acids from monoacylglycerols, diacylglycerols, triacylglycerols, free acids, phospholipids and sterol esters. These assessments have generally been made by extraction with an appropriate

solvent assuming that all lipid is extracted and that the extract is only lipid. There may be problems when lipid is associated with protein or with carbohydrate, therefore modified methods are needed. An alternative method is to hydrolyse the total sample with acid or alkali after adding tri-undecanoin (glycerol ester of 11:0) as internal standard. The resulting fatty acids are extracted, converted to methyl esters, and examined by gas chromatography. The results are converted to triacylglycerol equivalents and expressed as fat.

5.2.3 Melting behaviour

Fats are not pure organic compounds with sharp melting points but mixtures of many individual triacylglycerols, each of which may be solid or liquid. Many fats are plastic solids that deform under pressure as they are mixtures of solid and liquid components. The proportion of these two phases changes with temperature and it is necessary to know the solid/liquid ratio at appropriate temperatures. This is important in assessing the quality of spreading fats and confectionary fats (sections 10.2 and 10.6). The temperature at which solid first appears on cooling is also important in frying oils and in salad oils (sections 10.4 and 10.5).

The 'titre' denotes the solidification point (ºC) of the fatty acids derived from a fat while the slip melting point is the temperature at which a column of fat (10±2 mm), contained in an open capillary tube and immersed in water to a depth of 30 mm, starts to rise.

Of greater value is the measurement of solid fat content by low-resolution ^{1}H NMR spectroscopy (section 5.3.5). Measurements made at a range of temperatures give a plot of solid content against temperature. The slope of this line and the temperature at which there is no solid phase provide useful information about the melting and rheological behaviour of the sample under investigation.

5.2.4 Unsaturation

Oils and fats contain saturated and unsaturated acids, and many of their properties depend on the ratio of these two acid types. Traditionally, average unsaturation has been measured as the iodine value based on reaction with iodine monochloride (Wijs' reagent) or other mixed halogen under controlled conditions. It is still cited in most specifications relating to oils and fats. However, it has a number of disadvantages and limitations. The measurement is time-consuming, labour-intensive and uses undesirable reagents and solvents. For this reason it is now often calculated from the fatty acid composition determined by gas chromatography using the theoretical iodine values of individual components. The theoretical iodine values of methyl stearate, oleate, linoleate and linolenate are 0, 85.6, 173.2 and 260.3, respectively, based on the function: 25380 $\times$ (number of double bonds) $\div$

$$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$$

Scheme 5.1 Linoleic acid with two allylic groups and one bis-allylic group.

molecular weight. However the agreement between the observed and the calculated value is not good. Reasons for this include the fact that no allowance is made for unsaponifiable material, which generally contains olefinic compounds and that the GC trace may contain several minor peaks which are ignored. The iodine values of polyunsaturated fatty acids may also be low through incomplete halogenation. An important limitation is that the iodine value does not distinguish between *cis* and *trans* isomers and this information is important when following partial catalytic hydrogenation.

Knothe (2002) has drawn attention to the fact that average unsaturation distinguishes between saturated and unsaturated acids, but does not reflect the important difference in reactivity between monounsaturated and polyunsaturated acids. He has suggested new indices measuring the allylic position equivalent (APE) related to mono and polyunsaturated acids and the bis-allylic position equivalent (BAPE) related to polyunsaturated acids only (Scheme 5.1). These can be determined by gas chromatography or from ^1H and ^{13}C NMR signals characteristic of each of these acid types (sections 6.2.4 and 6.2.5).

5.2.5 Acidity, saponification and unsaponifiable material

The level of free acid is an item in most specifications for crude and refined oils. It is measured by titration with standard sodium hydroxide solution. The amount of alkali required to hydrolyse (saponify) a fat is a measure of the average chain length of the acyl chains, though this value is affected by unsaponifiable material also present in the oil. This parameter may be reported as 'saponification value' (SV) or 'saponification equivalent' (SE). These are inversely related by the expression SE = 56100/SV. With increasing chain-length, saponification equivalent rises, but saponification value falls. Typical saponification values for some common oils include coconut 248–265, palmkernel oil 230–254, cocoa butter 192–200, palm oil 190–209, cottonseed 189–198, soybean 189–195, sunflower 188–194, corn 187–195, groundnut 187–196, olive 184–196 and rape 182–193.

When a natural fat or oil is hydrolysed, it gives fatty acids (soluble in aqueous alkali), glycerol (soluble in water) and other material (insoluble in aqueous alkali). The latter can be extracted with an appropriate organic solvent and is described as unsaponifiable or non-saponifiable material. It includes sterols, tocopherols, hydrocarbons, long-chain alcohols, etc. There is a growing interest in these compounds and methods of analysing this fraction in more detail are available (section 5.4.4). Unsaponifiable material is

normally less than two per cent of the total oil though sometimes it will be higher. Wax esters, for example, are hydrolysed to long-chain acids and alcohols with the latter being part of the unsaponifiable fraction. Spreads with added phytosterol esters (section 9.8.2) will also have elevated levels of unsaponifiable material.

5.2.6 Measurement of oxidative deterioration and of oxidative stability

In common with other olefinic compounds, oils and fats react with oxygen. The process is complex (section 7.2) and usually undesirable. Two major questions are asked of the analyst in this connection: how far has this sample already been oxidised and how long will this (food) sample last before it is unacceptable, i.e. what is its shelf-life? The first requires a measurement of present status, while the second requires a predictive measurement. The most common oxidative process (autoxidation) occurs with an induction period, during which deterioration is not severe and it is useful for food producers to know the length of this period. Several stages of oxidation can be recognised and tests are available for each stage:

- Primary products of oxidation are allylic hydroperoxides and are measured as peroxide value or as conjugated dienes.
- Secondary products are mainly aldehydes and are measured by the anisidine value.
- Tertiary oxidation products include short-chain acids measured by the Rancimat or oil stability index (OSI) and malondialdehyde measured by the TBA test.

Although oxidative deterioration is most important for goods stored at ambient or refrigerator temperatures, the changes can be accelerated at elevated temperatures. Unfortunately reaction at higher temperature is not always a good predictor of reactions occurring at lower temperatures. The most common method of assessing oxidative status is by measurement of hydroperoxides. These molecules react with acidified potassium iodide to liberate iodine that can be determined volumetrically by reaction with sodium thiosulfate. The value represents mmol of oxygen per 2 kg of fat and this means that in an oil with a peroxide value of 2, around 0.1% of the olefininic molecules have been oxidised. Freshly refined material should have a peroxide value below 1. A fat is considered to be rancid at a peroxide value of 10. Refining destroys hydroperoxides, but it does not regenerate the fat in its original form. Hydroperoxides are converted to aldehydes during refining. Volatile aldehydes are removed during subsequent refining but aldehydes attached to the glycerol moiety remain and can be detected by the anisidine value. Refining an oil that has already been oxidised will therefore reduce the peroxide value but increase the anisidine value. These two measurements may

be combined in a totox value representing the sum of twice the peroxide value plus the anisidine value. The anisidine value is based on the measurement of the intensity of the chromophore at 350 nm produced by reaction of anisidine (4-methoxyaniline, $ArNH_2$) with carbonyl compounds which are mainly 2-enals ($R'CH{=}CHCHO$). This value varies depending on the enals actually present and is therefore only strictly comparable across results for a single oil. An anisidine value of 1 corresponds with around 0.1% of oxidised material.

$$ArNH_2 + OCHCH{=}CHR' \rightarrow ArN{=}CHCH{=}CHR'$$

Early stages of autoxidation can also be detected by measurement of uv absorption at 234 nm resulting from conjugated dienes formed during oxidation of polyunsaturated fatty acids. The method is not suitable for heated fat, for fat that already contains conjugated dienes, and for fats with a high content of oleic acid and consequent low levels of linoleic acid.

The thiobarbituric acid test (TBA) depends on the reaction of this compound with malondialdehde ($OHCCH_2CHO$) formed during oxidative breakdown of polyunsaturated acids.

In the rancimat and omnium oxidative stability measurements, a stream of air is drawn through heated oil (100–140ºC) into a vessel containing deionized water. Short-chain acids – mainly formic – increase the conductivity of the water and the induction period is indicated by the time that elapses before there is a rapid increase in conductivity. These measurements may be of limited value for predicting the stability of a range of oils, but for repeated samples of the same oil they give useful comparative values. They have largely replaced older active oxygen methods (AOM).

In the older accelerated tests (Schaal, active oxygen) the oil or fat is held at a temperature up to 100ºC and the time taken to reach an arbitrary peroxide value is measured. This is taken as an indication of the induction period and hence shelf life under normal storage conditions.

In biological experiments, the presence of short-chain hydrocarbons in breath may be measured. Ethane comes from n-3 acids and pentane from n-6 acids.

Headspace analysis may be carried out in various ways using gas chromatography to separate and identify short chain compounds – mainly aldehydes – formed by decomposition of hydroperoxides. Compounds such as 4-heptanal, and the 2,6- and 3,6-nonadienals are considered to be the most significant flavour notes, but many of the volatile materials have little sensory effect.

5.3 Present-day analytical techniques

5.3.1 Chromatography and spectroscopy

Most lipid analytical systems are now based on the powerful separating ability of a chromatographic procedure. Those of greatest interest are thin layer chromatography (TLC), high performance liquid chromatography (HPLC), and gas chromatography (GC). These will be described in the following sections and their use in specific lipid analyses will be reported in section 5.4.

Chromatography was first developed as a means of separating coloured compounds – hence its name – but with more sophisticated methods of following the separation, this technique can now be applied to the separation of a wide range of mixtures. Christie (2003) identifies several modes of chromatography in which a solute mixture is separated into its components by partition between a stationary and a mobile phase.

- Adsorption using mainly silica as stationary phase and a mobile phase consisting of either a single solvent (isocratic elution) or a mixture of two solvents of differing polarity (gradient elution).
- Normal-phase liquid-partition.
- Liquid-liquid or gas-liquid partition. In such systems the more polar phase is stationary (usually a liquid held on to a solid or a surface) and the less polar phase (liquid or gas) is mobile. In reversed phase chromatography, the less polar phase is stationary.
- Complexation chromatography with silver ions. Silver salts are incorporated into the stationary phase and silver ions react reversibly with pi electrons in unsaturated solutes. The strengths of these interactions vary with several structural features in the solute molecules and even small and subtle differences are sufficient to achieve useful separations. In some cases, the silver ions are bound to silica-based ion-exchange materials.
- Gel-permeation or size exclusion chromatography. Separation is based mainly on the size and shape of the solute molecules. Larger molecules elute before smaller ones, which are more easily trapped in the pores of the adsorbent.
- Chiral-phase chromatography. By incorporating a chiral molecule into the stationary phase it is possible to separate enantiomeric compounds. Alternatively, the enantiomers can be converted to diastereisomers with sufficiently different physical properties to be separated by more conventional chromatographic techniques.

All these separations result from differing molecular interactions between the several components of the solute and the mobile and stationary phases. With lipids, these interactions generally arise from (weak) polar forces.

Important spectroscopic procedures include ultraviolet and infrared spectroscopy, mass spectrometry (see Christie website), and nuclear magnetic

resonance spectroscopy (see Christie website). They are described further in sections 5.3.5, 5.3.6 and 5.3.8 and their use is illustrated in section 5.4.

5.3.2 Thin-layer chromatography (TLC) and related chromatographic systems

In thin-layer chromatography a layer of adsorbent – usually silica – is held on a flat glass, metal or plastic surface. The mixture to be separated is placed as a spot or a streak, 1.5–2.0 cm from one edge of the plate. The plate is then placed upright in a closed glass jar or tank containing the developing solvent up to a level below the spot. The solvent rises up the plate through capillary attraction and carries the various components of the mixture to different heights thus effecting a separation. The solvent is often hexane alone or hexane with increasing proportions of diethyl ether, but other solvents and solvent mixtures can be used. If the spot is placed near a corner of the plate it is possible to develop it in one direction and then turn the plate through 90º and develop again in a second solvent (2D-TLC). When separation is complete the plate can be treated with material that will render the lipids visible. Typical examples are of such reagents are iodine vapour and 2′7′-dichlorofluorescein. Separation on a silica layer will depend on the polarity of the solutes. For example, 1- and 2-monoacylglycerols, 1,2- and 1,3-diacylglycerols and triacylglycerols can all be separated as can the various classes of phospholipids. Also, esters of the common fatty acids can be separated from those with an oxygenated function. These separations can be carried out on an analytical or semi-preparative scale.

Other separations are possible if an appropriate chemical is incorporated into the silica layer. Older examples include borates and arsonates to separate polyhydroxy compounds according to the number of hydroxyl groups, their position in the acyl chain and their stereochemistry. Better known in this connection is the use of silver nitrate. Argentation or silver ion chromatography is widely used to separate *cis* and *trans* isomers and esters according to the number of double bonds. Examples are given in sections 5.4.1 and 5.4.2.

Solid phase extraction (SPE) frequently involves the use of small commercial chromatography columns made of impermeable plastic material, which is generally pre-packed with about 0.5 g of a variety of adsorbents. Silica columns are most widely used by lipid analysts though those chemically bonded with octadecylsilyl groups (ODS) have many non-lipid applications. Columns packed with silica are used for lipid class separation as an alternative to TLC. They are also useful in the study of clinical samples to separate cholesterol (that might interfere with gas chromatography) from methyl ester. Columns with bonded phenylsulfonic groups can be converted to their silver ion forms (section 5.3.2).

Christie (2003) has described a useful procedure in which short ion-exchange solid-phase extaction columns are charged with $AgNO_3$-CH_3CN-H_2O. Esters (0.25 mg) with 0–6 double bonds are eluted with solvents ranging from dichloromethane to acetone-acetonitrile (60:40). The quantities are small but sufficient for further examination by GC and/or MS.

5.3.3 High-performance liquid chromatography (HPLC)

HPLC involves chromatography with solvent mixtures as mobile phase and microparticles (3–10 micron) of silica- or alkyl- (most often C_{18}) bonded silica as stationary phase. In normal (straight) phase HPLC the stationary phase is polar (silica) and the mobile phase is non-polar. Reversed-phase HPLC refers to (non-polar) columns with alkyl-bonded silica and polar solvent mixtures. HPLC can also be used in the silver ion mode where the silver ions are bonded to the stationary phase. The most widely-used columns are 250 mm long with a 4–5 mm internal diameter and can be used in an analytical or semi-preparative mode. With careful use they have quite a long life.

Appropriate injection systems and high-pressure pumps are also required. Detection systems depend on UV absorption, differential refractometry, fluorescence, and evaporative light scattering. Each system has its limitations in respect of solute, solvent, sensitivity, linearity of response and must be selected carefully for each type of analysis. HPLC can be used for separations that can be achieved by TLC and *vice versa.* Examples of the use of HPLC are found in sections 5.4.1, 5.4.2 and 5.4.3.

Size exclusion chromatography (SEC) or gel permeation chromatography (GPC) effect separation by molecular weight and are useful for the separation of lipid polymers which are eluted before triacylglycerols and before free acids.

5.3.4 Gas chromatography (GC)

Gas chromatography is the analytical procedure most widely used by lipid analysts. It is employed mainly to separate and quantify component acids in the form of their methyl esters. This efficient separation procedure is based on partition chromatography where the stationary phase is usually coated on the inner wall of a fused silica capillary tube 10–100 metres in length and is liquid, at least at the temperature at which it operates. This phase may be non-polar, weakly polar, or highly polar. The gas phase is usually nitrogen, helium or hydrogen in order of increasing resolving power. The column is heated to a range of temperatures, limited only by the thermal stability of the stationary phase and of the analyte. Elution is slower at lower temperatures, but separation is improved and it may be necessary to make a compromise between time of elution and efficiency of separation. Separation is monitored with a flame ionisation detector that is remarkable for its robust nature and for

its linear response over a wide range of concentration. If the analyte contains volatile components that co-elute with the solvent or non-volatile components that are not eluted, then it is desirable to add an internal standard so that absolute rather than relative values can be measured. The separation is carried out at constant temperature (isothermal) or according to a prearranged programme during which the temperature is gradually raised. Lipids can be examined in many forms and the column can be connected to a mass spectrometer (section 5.3.6).

The column allows partitioning of the separate constituents in the analyte between the stationary phase as a thin film on the inner surface of the capillary column and the mobile phase (gas). The components of the analyte travel down the column and are eluted after different times (retention time) depending on the proportion of time spent in the stationary and mobile phases. The efficiency of a chromatographic system depends on the nature and flow rate of the carrier gas, column dimensions, liquid phase thickness and column temperature. These parameters should be optimised within practical constraints such as the time that can be given to each analysis.

5.3.5 NMR spectroscopy

Low-resolution ^{1}H NMR spectroscopy is used to determine the ratio of solid and liquid phases in a plastic fat. This is important in chocolate manufacture and in the understanding of melting behaviour in spreads (sections 10.2 and 10.6). Low resolution NMR has almost completely replaced the older method of dilatometry to measure solid fat content. The percentage of solids is given by the expression 100 (hydrogen nuclei in the solid phase) ÷ (all the hydrogen nuclei in the sample). These two types of hydrogen environment can be distinguished by observation of the relaxation signal. The signal for hydrogen atoms in solids decays quickly – less than one per cent remains after 70 μsec – while that from liquids decays very slowly requiring about 10 000 sec. There are practical reasons why measurements cannot be made at the instant of the pulse and are usually made after 10 μsec (SS + SL) and after 70 μsec (SL only). Because some of the SS signal will have already decayed after 10 μsec the observed value has to be corrected by a factor determined by calibration of the system using samples of solid plastic (35–70%) in liquid paraffin.

These measurements require only about six seconds and are used routinely for the study of spreads and confectionery fats. However, fats needing polymorphic stabilisation such as cocoa butter have to be equilibrated before measurements are made and a tempering routine requiring up to about 40 hours has been described.

By further adaptation the NMR system can be modified to distinguish between oil and moisture and it is possible to measure the oil and moisture content of around 1000 samples of seeds per day.

The use of high resolution NMR spectroscopy is described in sections 5.4.1–5.4.3. ^{1}H, ^{13}C and ^{31}P all find application and the technique is also discussed in sections 6.2.4 and 6.2.5.

5.3.6 Near-infrared and Fourier transform infrared spectroscopy

The near-infrared region of the spectrum, composed of overtones and combinations of the fundamental bands, was considered unimportant until developments in computing made it possible to exploit this information. Near-infrared reflectance spectroscopy (NIRS), based on commercial instruments, is now used extensively in agriculture and beyond. It is used, for example, to determine the content of moisture, protein and fat in a batch of seeds. Its use has been extended to the determination of fatty-acid composition and this may be carried out on a single seed. This is of great benefit in breeding programmes. The procedure is rapid, non-destructive and involves neither sample-grinding nor chemical modification. Calibration equations based on a large number of samples are required, but instruments from different laboratories can be integrated in a network with calibration equations developed on a master instrument and then used in all the satellite instruments in the network.

Fourier transform infrared (FTIR) spectroscopy has advanced dramatically in recent years and is now being used as an alternative way of measuring several important properties for lipid analysts. An FTIR spectrometer can record the entire infrared spectrum in one second and this can be added to many other scans through a fast Fourier transform algorithm to produce a conventional infrared absorption spectrum. Such spectra based on interferometry have several advantages over spectra from more conventional dispersive instruments. There is a marked improvement in signal to noise ratio, higher energy throughput, superior resolution and greater wavelength accuracy through the use of an internal laser. Undiluted edible oils are particularly suited to FTIR analysis as they are liquid, easy to handle and have relatively simple spectra. Preliminary calibration is necessary to convert spectral information into useful data and once this is available the system may be used to measure parameters such as *cis-trans* ratios, iodine value, saponification number, free acid content, peroxide value, and anisidine value. Details are available on the website <www.agrenv.mcgill.ca.foodsci.irg.irhomepg.htm>

5.3.7 Mass spectrometry

When a molecule (M) is bombarded with electrons of sufficient energy in the vapour phase at low pressure, the molecule becomes ionised to give a molecular ion (M^{+}). At greater electron energies, the ionised molecules fragment, usually in several ways, to give one uncharged particle (A) and one

positively charged particle (B^+). The mass spectrometer separates the charged particles (M^+ and B^+) according to their mass/charge ratio (*m/z* where *z* is usually 1). With high-resolution instruments, this value can be measured with such accuracy as to indicate the molecular formula of each ion.

$$M \underset{-2e}{\overset{+e}{\rightarrow}} M^+ \rightarrow A + B^+$$

Modifications of the spectroscopic system either inhibit fragmentation (soft techniques) so that that the molecular ion dominates or promote fragmentation. In the former case, the results can be used quantitatively to determine the relative amounts of all the molecular ions produced from the mixture. Enhanced fragmentation assists in structural identification. Sometimes it is desirable to bleed an inorganic ion into the system to promote ionisation.

The traditional form of mass spectrometry operates through electron impact (EI). Modifications include:

- APCI (atmospheric pressure chemical ionisation) which is a mild ionisation technique.
- MS-MS (tandem mass spectrometry). Molecular ions are separated and then fragmented to form daughter ions. These are separated and monitored in another part of the instrument.
- FAB (fast atom bombardment). Complex molecules not considered sufficiently volatile for study by the normal procedures are bombarded in an inert matrix with heavy atoms such as argon. Secondary ions are released from the matrix for MS examination.
- MALDI-TOF (matrix-assisted laser desorption/ionisation linked to a time-of-flight mass spectrometer). This involves direct injection of the lipid sample in an appropriate matrix.
- Electrospray ionisation is a mild but sensitive ionisation process used with compounds of high molecular weight. It gives information on molecular weight primarily, but in combination with tandem mass spectrometry a full structural determination may be possible. There are many applications to phospholipids and glycolipids. This technique is the basis of the new studies of lipidomics which require a large number of analyses without any chromatography.

The identifying ability of the mass spectrometer has been coupled with the separating ability of GC and HPLC to provide powerful techniques to analyse complex lipid mixtures both quantitatively and qualitatively. This requires the selection of derivatives that give good separation in the GC system and good fragmentation in the MS system (section 5.4.2). However, increasingly samples are being analysed without the chromatographic step using computers to analyse the complex spectra obtained.

5.3.8 Enzymatic procedures

One challenge to lipid analysts is to distinguish between the fatty acids in the *sn*-1, 2 and 3 positions in triacylglycerols or between the *sn*-1 and 2 positions in phospholipids. This has been achieved through the use of lipases or phospholipases which effect deacylation stereospecifically. Because these reactions are frequently accompanied by acyl migration, the experimental instructions must be followed meticulously if meaningful results are to be obtained. Some examples are detailed in section 5.4.2.

5.4 Lipid analysis

Lipid samples isolated from a plant or animal system and available in mg to kg quantities can be examined at various levels of complexity:

- General properties of the sample such as iodine value, level of free acid, state of oxidative deterioration, solid/liquid ratio, etc. (section 5.2).
- Recognition of the proportion of different lipid classes such as acylglycerols, phospholipids and sphingolipids.
- Component acid analysis of the total lipid or of individual lipid classes.
- Further information on triacylglycerols such as separating fatty acids in the *sn*-1 and 3 positions from those in the *sn*-2 positions, separating triacylglycerols with different double bond numbers or measuring levels of molecular species.
- This last can be examined at various degrees of refinement. For example, there are six isomeric triacylglycerols, each containing one mol of palmitic, oleic and linoleic acid. The analyst has to decide how far these are to be distinguished.

It is necessary to appreciate the potential complexity of the problem. If a wax ester contains five acids and five alcohols, then these can be combined to give 25 possible esters. The situation with individual phospholipid classes is similar. Phosphatidylcholines with five component acids can exist in 25 different forms, so that a full analysis would involve the recognition of all these in both qualitative and quantitative terms. With triacylglycerols, the situation is even more complex. With five different acids there could be 125 (5^3) triacylglycerols, though some could be present at zero or very low levels. These figures become even larger: 10 acids could exist in 1000 triacylglycerols. Many of the triacylglycerols are isomeric and the researcher and analyst have to consider what level of information is required. There may also be a conceptual problem. If all 125 compounds were identified quantitatively would it be possible to grasp the significance of the information? The analyst would probably group the components or focus only on the major individuals.

The chromatographic and spectroscopic procedures used to conduct these analyses have been outlined (section 5.3) and the following sections provide a brief account of several types of lipid analysis.

5.4.1 Fatty acids

Component acid analysis is the most widely practised analytical technique in lipid science. In its most common form, mixed triacylglycerols are converted to methyl esters and these are separated on an appropriate GC column. Modern chromatographic systems will provide a trace, retention times and an area percentage based on total eluted material (see Fig. 5.1). Careful attention to a number of parameters is necessary to get accurate results. Unless specially calibrated, the system will not identify the esters present in each peak. This has to be done by the analyst. For the most frequently-occurring esters this can be achieved by comparison with standards derived from oils of known composition or with mixtures available from lipid suppliers in qualitative or quantitative mixtures.

When closely related methyl esters are not separated (sometimes called critical pairs) then the GC conditions should be modified. The column should be replaced by one of differing polarity or the chromatogram can be run at a lower temperature. In the latter case, separation is improved but elution will

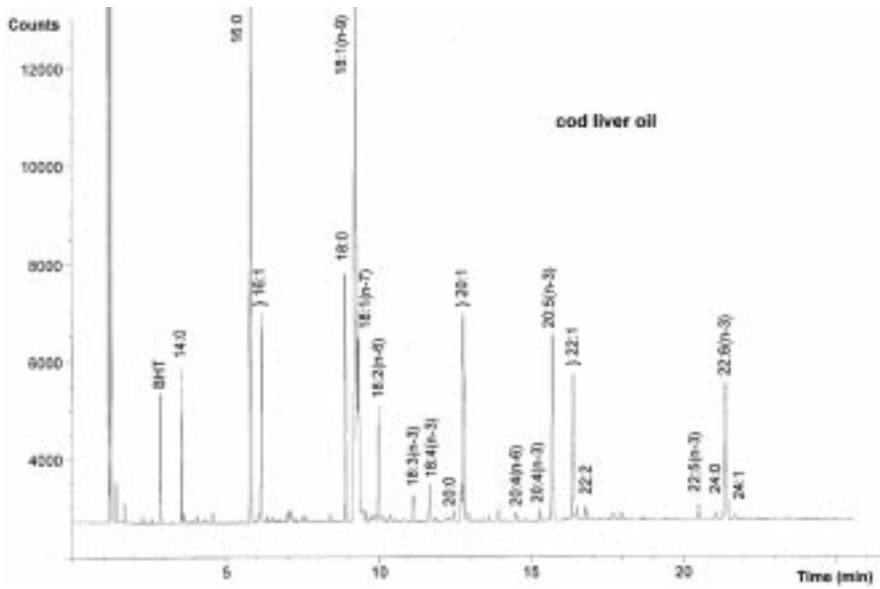


Figure 5.1 GC separation of the methyl esters derived from cod liver oil by gas chromatography using a CP-Wax 52CB with hydrogen as mobile phase. The vertical axis is Counts and the horizontal axis is Time (min). Copied with permission of the author and the publisher from Christie (2003).

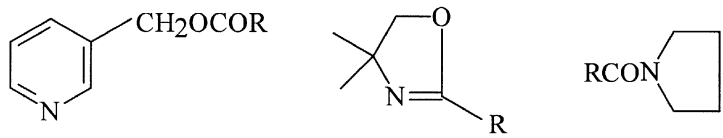


Figure 5.2 Picolinate, dimethyloxazoline and pyrrolidide from the carboxylic acid RCOOH.

take longer. Sometimes replacing methyl esters with isopropyl esters will give better resolution. Ethyl, butyl and 2-methoxyethyl esters have also been used. For short-chain acids, the methyl esters may be too volatile, and butyl or decyl esters can be used in their place.

For GC-MS, the derivatives must be chosen to give good separations on the GC column and to give mass spectroscope information. The most favoured derivatives are N-containing compounds such as:

- picolinyl esters made by acylation of picolinyl alcohol either with fatty acid and 1,1′-carbonyldiimidazole or with acid chloride (Figs 5.2 and 5.3),
- pyrrolidides made by interaction of pyrrolidine and free acid or methyl ester,
- 4.4-dimethyloxazolines (DMOX) formed by reaction of 2-amino-2-methyl-1-propanol with free acid, ester or acid chloride (Figs 5.2 and 5.3).

Fatty acids are identified by comparison of GC retention time with standard materials, but GC-MS is the surest method of structural identification (section 5.3.6). Useful separations can also be made using silver ion chromatography in TLC or HPLC systems. By these means, *cis* and *trans* isomers are easily separated. The Δ6c, Δ9c and Δ11c-18:1 isomers can also be separated by silver ion chromatography.

By a combination of silver ion TLC and GC it is possible to separate *cis* and *trans* isomers, first as groups and then as individual isomers. Because there is incomplete separation of *cis* and *trans* isomers by GC alone, results obtained without the preliminary silver ion separation underestimate the content of *trans* isomers by an average margin of 35 per cent. Detailed analyses of this type show that while dairy fats and partially hydrogenated vegetable fats both contain a range of *trans* 18:1 acids, they vary markedly in the relative proportions of the different isomers.

More sophisticated methods of fatty-acid analysis can also be carried out by regiospecific and stereospecific analysis which provide information about the distribution of acyl chains at the various glycerol hydroxyl groups (section 5.3.2).

Interesting developments in GC are apparent in its application to biotechnology. Plant breeders may need to analyse hundreds or thousands of samples for fatty-acid composition and this can only be achieved by careful management and by automation of standard chromatographic procedure.

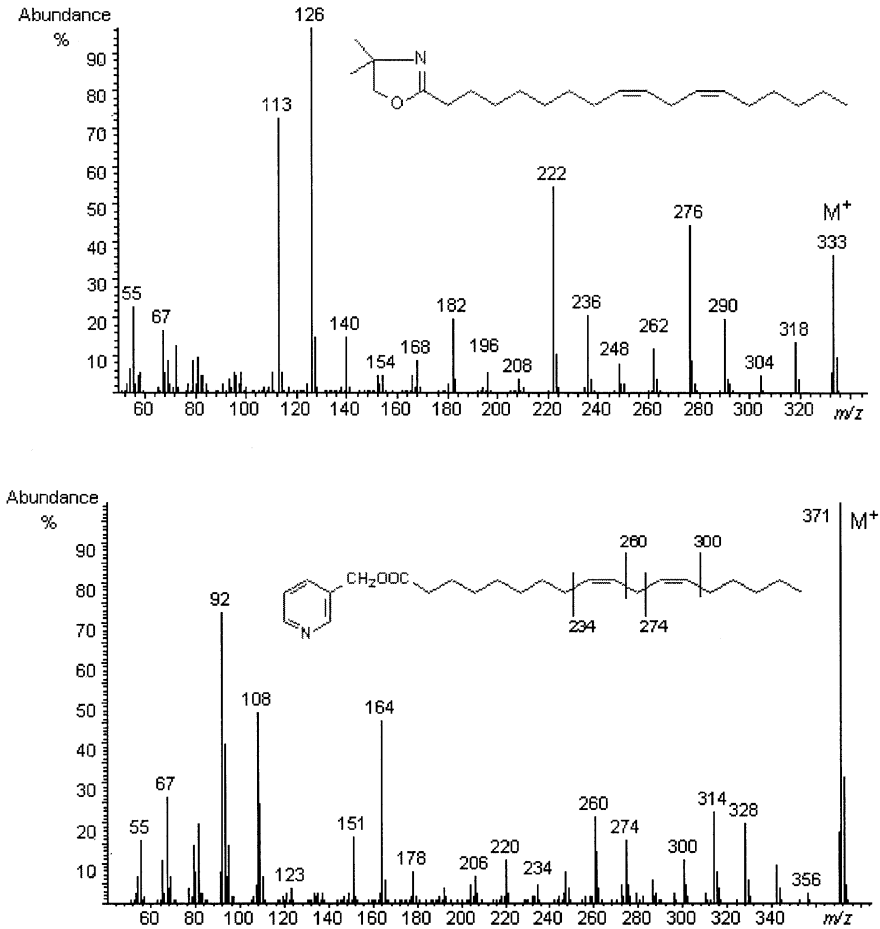


Figure 5.3 Mass spectra of linoleic acid as DMOX derivative and as picolinate. Downloaded with permission from <www.lipid.co.uk>.

Individual seeds or small samples must be extracted, converted to methyl esters, chromatographed and the results monitored. In one project, all samples are bar-coded and are typically presented in 25-well breeder trays adapted so that seeds cannot mix. The seeds are crushed with a 25-pin pestle tool and a robotic system then carries out the passage from crushed seed to production of an ester solution placed in an autosampler vial. Each GC oven has two injectors, two columns, and two detectors.

Using high-resolution ^{1}H NMR spectroscopy it is possible to determine the level of DHA in an oil sample and to measure total n-3 acids. In both cases ethylene glycol dimethyl ether can be used as an internal standard. DHA and

other acids with Δ4 unsaturation have characteristic signals at 2.38 ppm for the four hydrogen atoms on C_2 and C_3 while acids without Δ4 unsaturation have a signal at 2.28 ppm for the two C_2 hydrogen atoms. The proportion of n-3 acids can be determined from the CH_3 signal. This is at 0.81–0.89 ppm for all n-3 acids and at 0.90–0.98 ppm for all other acids/esters.

5.4.2 Acylglycerols

The following description applies mainly to triacylglycerols, but the methods can usually be adapted to examine monoacylglycerols, diacylglycerols and phospholipids. In several of the procedures to be described, the original sample is separated into a number of fractions for independent examination. In producing a final composition it will be necessary to know the proportion of each fraction. When these are too small to be weighed appropriate information can be obtained by adding an internal standard to each fraction. Subsequent GC analysis of the methyl esters will then allow the relative weight of each fraction to be determined.

The first step in analysing triacylglycerol mixtures is to determine the fatty acid composition by GC as described in the previous section. If for no other reason, this information may be used at the end of a triacylglycerol analysis to check that the data are reliable. If, for example, a mixture has been separated into several fractions, then the fatty-acid composition of these, suitably weighted, should add up to that of the original mixture. If they do not then the analysis is faulty.

Procedures for distinguishing the acyl groups in the two α positions from those in the β position provide a regiospecific analysis. Those that distinguish all three positions provide a stereospecific analysis. Both give insight into the nature of the mixed triacylglycerols beyond that provided by GC of the total esters, but neither gives complete information on molecular species. Some authors have used their data to calculate levels of individual molecular species based on assumptions concerning the association of acids in these differing positions. But these assumptions are not always justified.

In regiospecific analysis, GC gives information on the distribution of fatty acids between the primary OH groups of glycerol (*sn*-1 and 3) and the secondary OH group at the *sn*-2 position. This limited distinction can be made with a 1,3-specific enzyme such as pancreatic lipase. The mixed triacylglycerols in a tris buffer are shaken with calcium chloride, bile salt and pancreatic lipase for 2–4 minutes at 40ºC. The reaction is stopped by addition of ethanol and 6M HCl, and total organic products are extracted with diethyl ether. The product is separated by TLC and the recovered 2-monoacylglycerols are converted to methyl esters for examination by GC. This gives the composition of the fatty acids in the *sn*-2 position. These values can be subtracted from the composition of the original triacylglycerols to give the

$$GlOCOR + EtMgBr \longrightarrow GlOH + RC(Et)_2OH$$

$$TAG \longrightarrow DAG\ (1,2\text{-},\ 2,3\text{-}\ \text{and}\ 1,3) \longrightarrow MAG\ (1\text{-},\ 2\text{-},\ \text{and}\ 3\text{-})$$

Scheme 5.2 Reaction of triacylglycerol with EtMgBr to give tertiary alcohols and deacylated triacylglycerols as detailed in the second line.

combined composition of the 1 and 3 positions. These may be the same but it must not be assumed that they will be.

Alternative procedures for conducting regiospecific analysis of triacylglycerols are based on non-specific partial deacylation of glycerol esters with the Grignard reagent EtMgBr in a reaction of less than one minute. This is combined with chromatographic methods for separating the partially deacylated products (monoacylglycerols and diacylglycerols) from each other and from the tertiary alcohol also formed. The experimental conditions must be selected to keep acyl-migration to a minimum. The separated glycerol esters are subject to further detailed chromatographic analysis in various ways (Scheme 5.2).

In one procedure, two monoacylglycerol fractions (*sn*-1/3 and *sn*-2) are isolated and each is converted to methyl esters for GC examination. The results compare favourably with those obtained by pancreatic lipase.

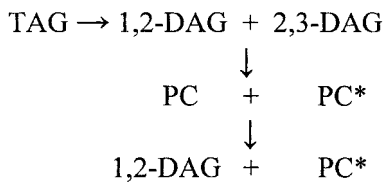
The acyl (C-1) signals in a ^{13}C NMR spectrum are also useful in that their chemical shifts depend on the position of the double bond closest to the carboxyl group and on whether the acyl chain is in the *sn*-1/3 positions or the *sn*-2 position. This makes it possible, for example, to compare the distribution of $\Delta 4$ acids (virtually only docosahexaenoic acid) and $\Delta 5$ acids (eicosapentaenoic acid and/or arachidonic acid) between the 1/3 and 2 positions The appropriate chemical shifts are detailed in section 6.2.5.

In stereospecific analysis, the reaction with EtMgBr has been extended to determine the fatty acid composition at all three of the glycerol positions. This has been achieved in two ways based on the conversion of a mono- or diacylglycerol fraction to a urethane by reaction with an isocyanate.

$$\text{Mono or diacylglycerol (ROH)} + R'NCO \rightarrow \text{urethane (ROCONHR}')$$

Takagi and his colleagues (Christie, 2003) react the mixed α-monoacylglycerols with 3,5-dinitrophenylisocyanate, producing two enantiomeric sets of bisurethanes which are then separated on a chiral column. Each set is finally converted to methyl esters for GC analysis.

Christie (2003) avoids the need for a chiral column by reacting an enantiomeric isocyanate [S-(+)-1-(1-naphthyl)ethyl isocyanate] with the mixed 1,2- and 2,3-diacylglycerols. In this way the enantiomeric diacylglycerols are converted to two groups of diastereoisomeric urethanes which can be separated by silca-HPLC. Each group can then be converted to methyl esters for normal GC analysis.



Scheme 5.3 Stereospecific analysis of triacylglycerols.
TAG = triacylglycerols, DAG = diacylglycerols, PC = natural phosphatidylcholines based on 1,2-diacylglycerols, PC* = unnatural phosphatidylcholines based on 2,3-diacylglycerols.

Older methods of stereospecific analysis exploit the selectivity of an appropriate phospholipase. As an example, one method involves reaction with EtMgBr for 25 seconds, isolation of the mixed 1,2- and 2,3-diacylglycerols, and reaction of this mixture with choline chloride to produce phosphatidylcholines. Those based on 1,2-diacylglycerol are identical to natural phosphatidylcholines and are hydrolysed to the diacylglycerols in the presence of phospholipase C in two minutes. In contrast, 2,3-diacylglycerols give a set of enantiomeric phosphatidylcholines which are not natural substrates for phospholipase and react much more slowly, requiring a reaction time of four hours. After a short reaction, it is possible to separate diacylglycerols (from the natural phosphatidylcholines) from unreacted unnatural phosphatidylcholines. It is also necessary to carry out a lipolysis of the original triacylglycerols with pancreatic lipase. Appropriate fractions are converted to methyl esters for GC examination and the composition of the fatty acids attached to each of the glycerol hydroxyl groups can be calculated by suitable arithmetical manipulation (Scheme 5.3).

As an alternative, the mixture of natural and unnatural phosphatidylcholines described above can be deacylated in the presence of phospholipase A2. This will give a lyso-phosphatidylcholine and fatty acid from the 2-position from the natural phosphatidylcholine only.

Because of the reliability of the flame ionisation detector it would be good to analyse triacylglycerol mixtures by gas chromatography, but this technique is not widely used for this purpose. Apart from the milk fats and the lauric oils with their short- and medium-chain acids the low volatility of the triacylglycerols require that the columns be run at high temperatures, usually close to their experimental limits. GC is therefore not widely used to analyse triacylglycerols except in a few limited cases. It has been used to analyse palm oil and cocoa butter and its alternatives in terms of their carbon numbers. Palm oil for example, can be characterised by the content of its glycerol esters having 48, 50, 52, and 54 carbon atoms. These numbers are the sum of the carbon atoms in the three acyl chains and do not include the three glycerol carbon atoms. The C_{48} fraction contains three C_{16} chains, the C_{50} fraction contains two C_{16} and one C_{18} chain and so on. The C_{18} chain will be mainly

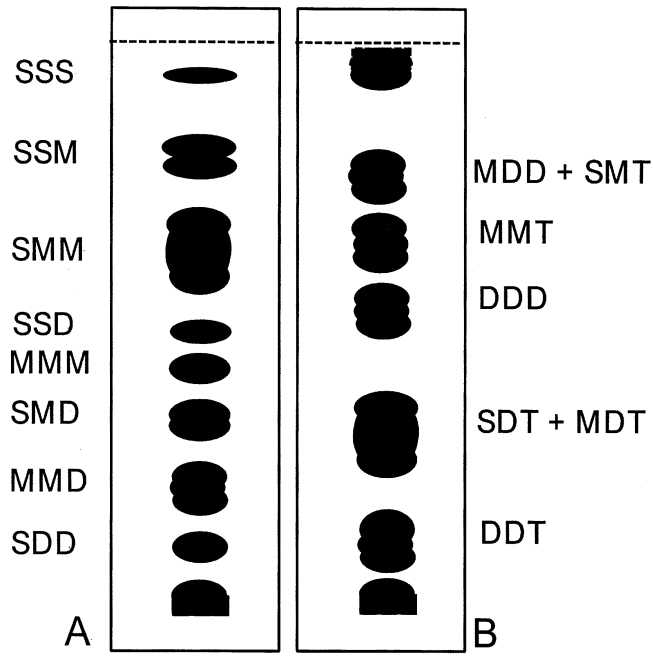


Figure 5.4 Separation of soybean triacylglycerols by silver ion chromatography using chloroform-methanol at a 99:1 ratio for plate A and 99:6 for plate B. S, M, D and T relate to saturated, monoenoic, dienoic and trienoic acyl chains, respectively. The order does nor reflect positional distribution. Copied with permission of the author and the publisher from Christie (2003).

oleate but may be stearate or linoleate so these fractions refer to groups of triacylglycerols and not to individual compounds. These techniques were developed with packed columns. When short capillary columns are used, the groups are still apparent but each is further divided so that the C_{52} peak, for example, becomes four peaks corresponding to POSt, POO/ PLSt, PLO and PLL, where P = palmitic, St = stearic, O = oleic and L= linoleic. Each of these three letter symbols includes all the possible isomers with the three acyl chains indicated.

Silver ion chromatography with thin layer chromatography or HPLC can be used effectively to separate triacylglycerols according to their degree of unsaturation. With improved procedures bands or peaks, containing for example, three double bonds, are further separated into triacylglycerols, such as MMM, DMS and TSS where S, M, D and T represent saturated, monoene, diene, and triene acyl chains. Since interaction between silver ions and double bonds is not linearly related to the number of unsaturated centres, it is not always easy to interpret a silver ion chromatogram and it may be necessary to investigate the fractions further (Figs 5.4 and 5.5).

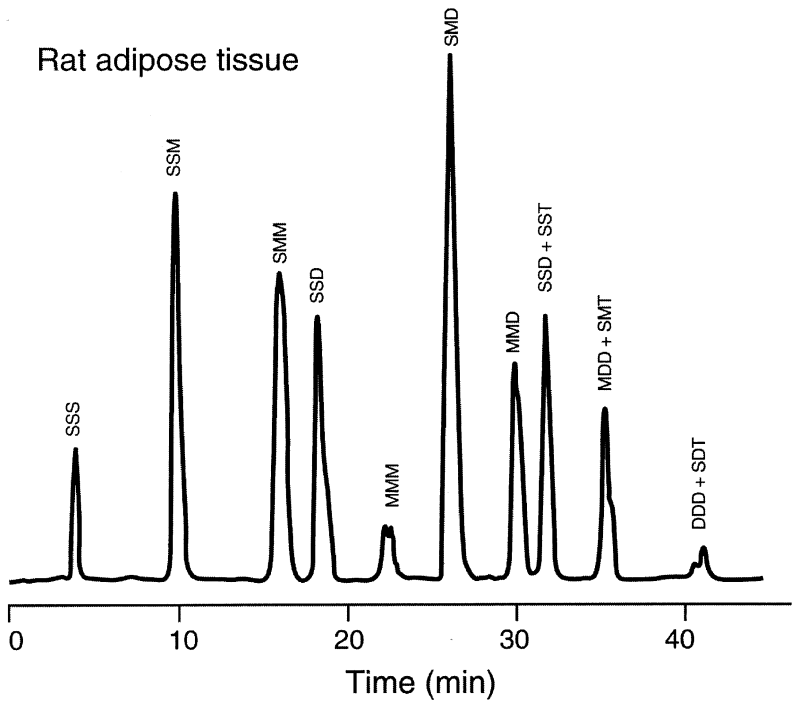


Figure 5.5 Separation of triacylglycerols from rat adipose tissue by silver ion HPLC. S, M, D and T relate to saturated, monoenoic, dienoic and trienoic acyl chains respectively. The order does not reflect positional distribution. Copied with permission of the author and the publisher from Christie (2003).

RP HPLC provides one of the best methods of analysing triacylglycerol mixtures, but here again, recognition of peaks requires experience and/or further examination of fractions. This is because separation depends on the partition number (PN), related to both chain length, carbon number (CN) and the number of double bonds (db). The simple expression PN = CN – zdb (z = 2) is only of limited value because the claim that z = 2 is only approximate. For example, based on the acids 12:0 to 18:0 and 16:1, 18:1, 18:2, and 18:3 there are over 20 triacylglycerols with a partition number of 44 which can be separated from each other. The following examples, taken from the complete group, are eluted in the order given: OLL (54:5), OOLn (54:5), LaOO (48:2), PPLn (50:3) MStLn (50:3) MMP (44:0), where L = linoleic, La = lauric, Ln = linolenic, M = myristic, O = oleic and P = palmitic. An example of RP HPLC is given in Fig. 5.6.

Mass spectrometry can be used in conjunction with GC or HPLC to identify the triacylglycerol component present in a separated peak, but it is also possible to obtain valuable analytical information from mass spectrometry alone using tandem mass spectrometry (MS-MS). This provides information

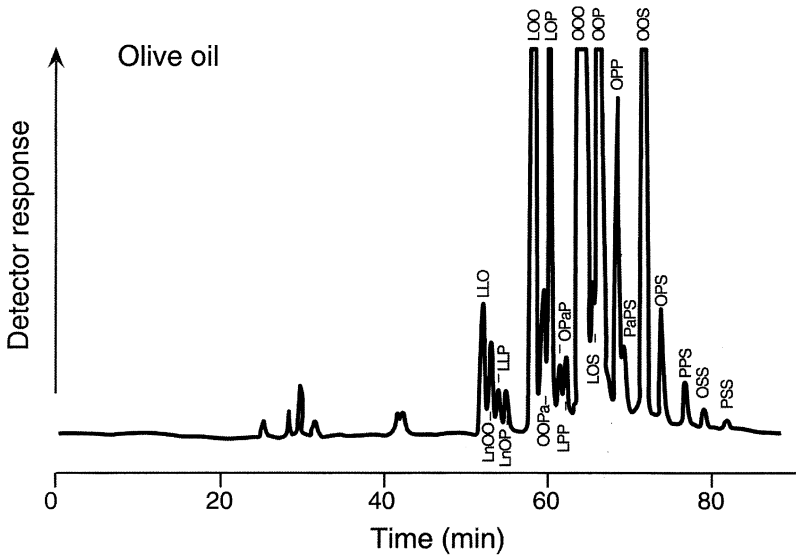


Figure 5.6 Separation of olive oil triacylglycerols by reversed phase HPLC, L, Ln, O, P, Pa and S stand for the acyl chains of linoleic, linolenic, oleic, palmitic, palmitoleic and stearic acid respectively. Copied with permission of the author and the publisher from Christie (2003).

on the size of individual molecular species, the nature of the attached acyl groups, and distinguishes acyl groups at the *sn*-2 position from those in the *sn*-1/3 positions. One example is concerned with the triacylglycerols in human milk fat (Currie & Kallio, 1993). Ammonia negative ion chemical ionisation MS gave 29 molecular ions $[M\text{-}H]^-$ of molecular weight 666.7–886.7, with 38–54 carbon atoms, and 0–4 double bonds. In addition, 28 fatty acid molecular weights were recognised. The composition of each $[M\text{-}H]^-$ cluster was determined from a study of the $[RCOO]^-$ daughter ions and the distribution of acyl groups between the *sn* 1/3 and *sn*-2 positions from a study of the $[M\text{-}H\text{-}RCOOH\text{-}100]^-$ fragments. The most abundant glycerol ester is the symmetrical 1,3-dioleate 2-palmitate, making up about 10 per cent of the total.

Increasingly, triacylglycerols are being studied through RP HPLC followed by atmospheric pressure chemical ionisation (APCI). The spectrum from each separated glycerol ester contains four significant peaks: the molecular ion peak $[M+H]^+$ and three diacylglycerol peaks corresponding to the loss of one acyl group. For example, peaks at 879, 622 [M-256] 600 [M-278] and 574 [M-304] indicate a triacylglycerol containing 16:0, 18:3 and 20:4. Identification is not too difficult but procedures for good quantitation need to be improved.

In the MALDI-TOF spectrum each triacylglycerol appears as a sodiated molecular ion $(M + 23)^+$ which can be identified from the fatty acids present in the oil. A distinction is not made between isomers, and response factors

have to be measured to make the results quantitative. Fatty acids can also be identified in this way but there are simpler ways of doing this.

5.4.3 Phospholipids

In studying the phospholipids present in an oil or biological extract, interest may lie in total phospholipid, individual phospholipid classes, or the molecular species present in the sample. At one time total phospholipids were determined by measuring the level of phosphorous by classical methods and multiplying that by a factor to express phospholipid level. This has been replaced by separation of lipid classes by TLC or HPLC. Phospholipid class analysis is achieved by normal HPLC using a silica column and appropriate mixed polar solvents. For molecular species separation a reversed-phase HPLC system is used with mixed polar solvents. Phospholipids can also be determined by ^{31}P NMR since the phosphorus atom in each phospholipid class gives a distinct signal. For quantitative examination triphenyl phosphate is used as a standard.

5.4.4 Minor components (sterols and tocopherols)

Sterols in free and/or esterified form are present in vegetable and animal fats at levels of 0–3 per cent but in products with added phytosterols this may rise to 13 per cent. Lipids of animal origin contain mainly the zoosterol cholesterol, but lipids from plant sources contain phytosterols (mainly sitosterol, campesterol, stigmasterol and avenasterol, section 1.6.3). Sterols can be studied at three levels: total content of sterols after hydrolysis, proportion of free and esterified sterols, analysis of individual sterols in free sterols, esterified sterols or total sterols.

Individual sterols are determined by gas chromatography of the unsaponifiable fraction using the free sterols or their trimethylsilyl ethers. To assist in quantification an internal standard such as betulin or β-cholestanol may be added to the analyte. If sterol esters and free sterols are to be distinguished these must be separated by silica chromatography before hydrolysis, eluting sterol esters first with a less polar solvent and then free sterols with a more polar solvent.

The sample may be hydrolysed or subjected to methanolysis and total sterols subsequently separated from other components by TLC. Individual sterols are then determined by gas chromatography as such (ROH) or as trimethylsilyl ethers ($ROSiMe_3$). Fast methods of GC have been devised for routine analysis. It is usually necessary to add appropriate internal standards to obtain quantitative information.

Gel permeation chromatography (GPC) can be used without saponification to separate sterols, sterol esters, squalene and tocopherols and each class of

compounds can be examined further by gas chromatography. Appropriate internal standards are added for each class.

Up to four tocopherols and four tocotrienols (section 1.6.4) may be present in a lipid sample. For analysis, the sample may be saponified when the tocopherols concentrate along with sterols in the unsaponifiable fraction, but saponification is not always necessary. Individual tocopherols can be separated by gas chromatography when the order of elution is α-tocopherol, α-tocotrienol, β-tocopherol, β- tocotrienol, γ-tocopherol, γ-tocotrienol, δ-tocopherol and δ-tocotrienol (though there is some doubt about δ-tocotrienol). Tocopherols can also be separated from one another by HPLC and are best measured with a fluorescence detector or with a coulometric electrochemical array detector. In this method saponification is not necessary because of the high sensitivity of the fluorescence detector.

Bibliography and references

Adlof, R. (2003) *Advances in Lipid Methodology – Five*, The Oily Press, Bridgwater.

Bligh, E.G. & Dyer, W.J. (1959) A rapid method for total lipide extraction and purification, *Canad. J. Biochem. Physiol.* **37**, 911–917.

Christie, W.W. (2003) *Lipid Analysis – Isolation, Separation, Identification and Structural Analysis of Lipids*, The Oily Press, Bridgwater.

Christie, W.W. (1992) *Advances in Lipid Methodology One*, The Oily Press, Dundee.

Christie, W.W. (1993) *Advances in Lipid Methodology Two*, The Oily Press, Dundee.

Christie, W.W. (1996) *Advances in Lipid Methodology Three*, The Oily Press, Dundee.

Christie, W.W. (1997) *Advances in Lipid Methodology Four*, The Oily Press, Dundee.

Christie, W.W. & (in part) Gunstone, F.D. <www.lipid.co.uk>

Currie, G. J. & Kallio, H. (1993) Triacylglycerols of human milk: rapid analysis by ammonia negative ion tandem mass spectrometry, *Lipids,* **28**, 217–222.

Folch, J., Lees, M. & Sloane, S.G.H. (1957) A simple method for the isolation and purification of total lipids for animal sources, *J. Biol. Chem.*, **226**, 497–509.

Knothe, G. (2002) Structure indices in fatty acid chemistry. How relevant is the iodine value? *J. Amer. Oil Chem. Soc*., 79, 847–854.

Knothe, G. (2003) Quantitative analysis of fatty compounds by ^{1}H-NMR, *Lipid Technology*, **15**, 111–114.

McLean, B. & Drake, P. (2002) Review of methods for the determination of fat and oil in foodstuffs, Campden and Chorleywood FRA Group, Review No 37, 1–52.

FTIR spectroscopy <www.agrenv.mcgill.ca.foodsci.irg.irhomepg.htm>.

6 Physical properties

6.1 Polymorphism, crystal structure and melting point

In the solid state, long-chain compounds frequently exist in more than one crystalline form and consequently have more than one melting point. This property of polymorphism is of both scientific and technical interest. Understanding this phenomenon is essential for the satisfactory blending and tempering of fat-containing materials such as cooking and confectionery fats, which must attain a certain physical appearance during preparation and maintain it during storage. Problems of graininess in margarine and bloom in chocolate, for example, are both related to polymorphic changes (sections 10.2 and 10.6). The experimental methods used most extensively to examine melting and crystallisation involve dilatometry, low-resolution pulsed ^{1}H NMR spectroscopy, differential scanning calorimetry, infrared spectroscopy and X-ray diffraction (Larsson, 1994).

X-ray investigations indicate that the unit cell for long-chain compounds is a prism with two short spacings and one long spacing, as indicated in Fig. 6.1. When the long spacing is less than the molecular dimension calculated from known bond lengths and bond angles, it is assumed that the molecule is tilted with respect to its end planes. Sometimes, however, the length is such as to indicate a dimeric or trimeric unit for the most stable form. The molecules assume the angle of tilt at which they are most closely packed. This will give the greatest stability and the highest melting point.

6.1.1 Alkanoic and alkenoic acids

The melting points of long-chain acids and their methyl esters are listed in Table 3.1. These values show alternation with increasing chain length, a phenomenon commonly displayed by the physical properties of long-chain compounds in the solid state and related to the arrangement of molecules in the crystals. The melting points of acids with an even number of carbon atoms in the molecule and their methyl esters plotted against chain-length fall on smooth curves lying above similar curves for the odd acids and their methyl esters. Odd acids melt lower than even acids with one less carbon atom. The two curves for saturated acids converge at 120–125ºC.

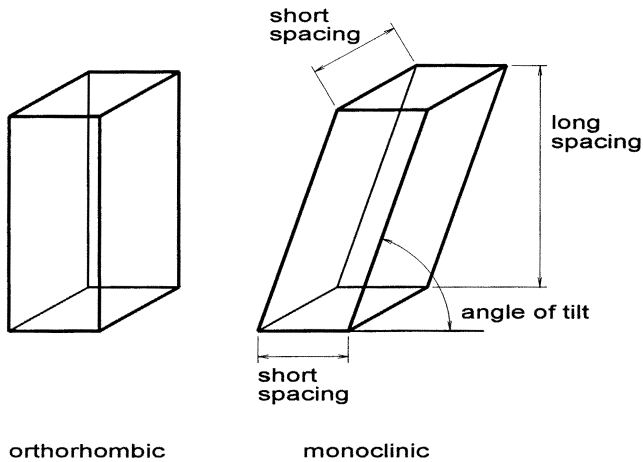


Figure 6.1 The unit cell of long-chain compounds (kindly supplied by my colleague Dr C.M. Scrimgeour).

The melting points of unsaturated acids depend on chain length and on the number, position, and configuration of the unsaturated centres. For example stearic (70ºC), oleic ($\Delta 9c$, 11ºC) elaidic ($\Delta 9t$, 45ºC) and stearolic acids ($\Delta 9a$, 46ºC) have the melting points shown. Among polyunsaturated acids those with conjugated unsaturation are higher melting than their methylene-interrupted isomers (Table 6.1).

Table 6.1 Melting points (ºC) of some mono- and poly-unsaturated acids

Monoenes	
16:1 (9*c*)	0.5
18:1 (9*c*)	16.3
20:1 (9*c*)	25
22:1 (13*c*)	33.4
Polyenes with methylene-interrupted unsaturation	
18:2 (9*c*12*c*)	−5
18:2 (9*c*12*t*)	−3
18:2 (9*t*12*t*)	29
18:3 (9*c*12*c*15*c*)	−11
18:3 (9*t*12*t*15*t*)	30
Polyenes with conjugated unsaturation	
18:2 (9*c*11*t*)	22
18:2 (9*t*11*t*)	54
18:3 (9*c*11*t*13*c*)	44
18:3 (9*c*11*t*13*t*)	49
18:3 (9*t*11*t*13*c*)	32
18:3 (9*t*11*t*13*t*)	71

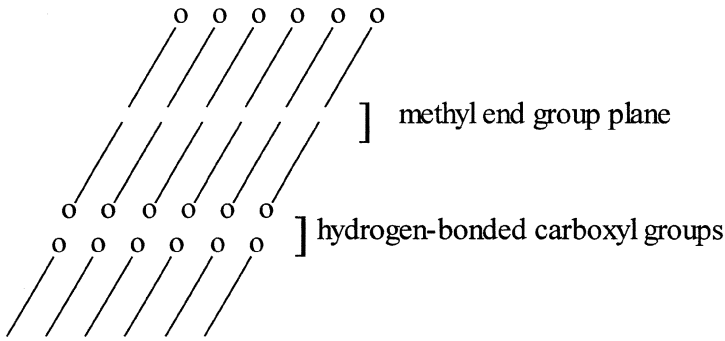


Figure 6.2 Schematic arrangement of alkanoic acid molecules in the crystalline form. o represents the polar head group (COOH) and the line represents the alkyl chain which will assume a zig-zag arrangement of successive carbon atoms.

Alkanoic acids exist in three polymorphic forms designated A, B and C for acids with an even number of carbon atoms. Form C has the highest melting point and is the most stable (physically). It is obtained by crystallisation from the melt or from polar solvents. Crystallisation from nonpolar solvents gives form A or forms B and C. The molecules crystallise in dimeric layers. Alternation of melting point for odd and even chain-length compounds results from the fact that the methyl groups in the end group plane interact differently in the odd and even series.

6.1.2 Glycerol esters

For most technical purposes the melting behaviour of triacylglycerols is more important than that of fatty acids. It has long been known that fats show multiple melting points, and as far back as 1853 glycerol tristearate was reported to have three melting points at 52, 64 and 70ºC. When the melt of a simple triacylglycerol is cooled quickly it solidifies in its lowest melting form (α) with perpendicular alkyl chains in its unit cell (the angle of tilt is 90º). When heated slowly this melts but, held just above this melting point, it will re-solidify in the β' crystalline form. In the same way a more stable β form can be obtained from the β' form. The β form has the highest melting point and is obtained directly by crystallisation from solvent. The β' and β forms have tilted alkyl chains which permit more efficient packing of the triacylglycerols in the crystal lattice. Glycerol esters with only one type of acyl chain are easy to make and have been thoroughly studied. The results have provided useful guidance but such molecules are not generally significant components of natural fats (except perhaps after complete hydrogenation). With mixed saturated triacylglycerols such as PStP (P = palmitic, St = stearic) the β form is only obtained with difficulty and such compounds usually exist in their β'

Table 6.2 Characteristics of α, β', and β forms of crystalline triacylglycerols

Form	MP	Short spacings (nm)	Infrared absorption (cm^{-1})	Hydrocarbon chain	Subcell
α	lowest	0.4	720	perpendicular	orthorhombic
β'	intermediate	0.42–0.43 and 0.37–0.40	726 719	tilted	orthorhombic
β	highest	0.46 and 0.36–0.39	717	tilted	triclinic

form. Among triacylglycerols with saturated and unsaturated acyl chains, symmetrical compounds (SUS and USU) have higher melting (more stable) β forms but the unsymmetrical compounds (USS and UUS) have stable β' forms (S = saturated and U = unsaturated acyl chains) (Table 6.2).

The stable β form generally crystallises in a double chain-length arrangement (DCL or β_2) but if one acyl group is very different from the others in either chain length or in degree of unsaturation, the crystals assume a triple chain-length arrangement (TCL or β_3) since this allows more efficient packing of alkyl chains and head groups. These crystals have the short spacing expected of a β crystalline form but the long spacing is about 50 per cent longer than usual (Fig. 6.3).

In the DCL arrangement, the molecules align themselves (like tuning forks) with two chains in extended line (to give the double-chain length) and a third parallel to these (Fig. 6.3). Some mixed glycerol esters which have a TCL form when crystallised on their own, give high-melting (well-packed) mixed crystals with a second appropriate glycerol ester. For example CPC and PCP

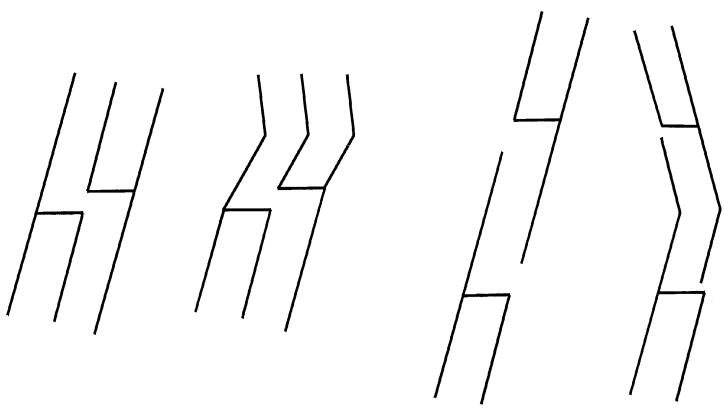


Figure 6.3 DCL and TCL structures (kindly supplied by my colleague Dr C.M. Scrimgeour).

or OPO and POP (where C = capric, P = palmitic and O = oleic). This has been described as 'compound formation'.

The methyl groups at the top and bottom of each triacylglycerol layer do not usually lie on a straight line, but form a boundary with a structure depending on the lengths of the several acyl groups. This is called the 'methyl terrace'. The molecules tilt with respect to their methyl end-planes to give the best fit of the upper methyl terrace of one row of glycerol esters with the lower methyl terrace of the next row of esters. There may be several β_2 modifications differing in the slope of the methyl terrace and in the angle of tilt.

Crystallisation occurs in two stages: nucleation and growth. A crystal nucleus is the smallest crystal that can exist in a solution and is dependent on concentration and temperature. Spontaneous (homogeneous) nucleation rarely occurs in fats. Instead heterogeneous nucleation occurs on solid particles (dust, etc.) or on the walls of the container. Once crystals are formed, fragments may drop off and either re-dissolve or act as nuclei for further crystals. The latter is not desirable in fat crystallisation so agitation should be kept to the minimum required to facilitate heat transfer. Nucleation rates for the different polymorphs are in the order $\alpha > \beta' > \beta$ so that α and β' crystals are more readily formed in the first instance, even though the β polymorph is the most stable and is favoured thermodynamically. Crystal nuclei grow by incorporation of other molecules from the adjacent liquid layer at a rate depending on the amount of supercooling and the viscosity of the melt (Mori, 1988; Timms in Gunstone & Padley, 1997; Sato, 2001a,b; Lawler & Dimick, 2002).

In the production of margarines and shortenings the β' crystalline form is preferred to the β form. β' Crystals are relatively small and can incorporate a large amount of liquid. This gives the product a glossy surface and a smooth texture. β Crystals, on the other hand, though initially small, grow into needle-like agglomerates. These are less able to incorporate liquids and produce a grainy texture. Margarines and shortenings, made from rape/canola, sunflower, or soybean oil after partial hydrogenation, tend to develop β crystals. This can be inhibited or prevented by the incorporation of some hydrogenated palm oil or palm olein which stabilise the crystals in the β' form. These changes in crystallisation pattern are linked with the larger amount of palmitic acid in the palm products. Glycerol esters with C_{16} and C_{18} acyl chains are more likely to be stable in the β' form than glycerol esters with three C_{18} chains.

Because of the importance of its melting behaviour, the polymorphism displayed by cocoa butter has been thoroughly investigated. This material is particularly rich in three 2-oleo-1,3-disaturated glycerol esters namely POP, POSt and StOSt. The solid fat has been identified in six crystalline forms designated I-VI with the melting points and double/triple chain length nature indicated in Table 6.3. Of these, form V (β_2) is the one preferred for

Table 6.3 Polymorphism in cocoa butter

	I	II	III	IV	V	VI
MP (ºC)	17.3	23.3	25.5	27.3	33.8	36.3
Chain length	D	D	D	D	T	T

D = double chain length, T = triple chain length.

chocolate. This crystalline form gives good demoulding characteristics, has a stable gloss, and shows a favourable snap at room temperature. Two procedures have been employed to promote the formation of this particular crystalline form. The most extensively used is tempering, i.e. putting molten chocolate through a series of cooling and heating processes. This optimises the production of the appropriate polymorph. An alternative procedure requires seeding of the molten chocolate with cocoa butter already prepared in form V (β_2) or VI (β_1) but this method is restricted by the difficulty of obtaining adequate supplies of these crystalline forms.

The synthetic glycerol ester 2-oleo-1,3-dibehenin (BOB, O = 18:1, B = 22:0) may be added to cocoa butter to prevent bloom formation by keeping it in its form V at temperatures above 30ºC (section 10.6).

Oils rich in saturated acids contain high-melting triacylglycerols which may crystallise from the oil when stored. When this is considered to be undesirable, the oil is subjected to winterization. The oil is chilled gradually and kept at around 5ºC for several hours before being filtered. The liquid fraction should then remain clear at ambient temperature. This process is applied to cottonseed oil and to partially hydrogenated soybean oil.

6.2 Spectroscopic properties

6.2.1 Ultraviolet spectroscopy

The use of ultraviolet spectroscopy in the study of lipids is confined to systems containing or generating conjugated unsaturation. It is therefore of value in the study of natural acids with conjugated unsaturation such as the dienes, trienes, tetraenes and acetylenic compounds described in section 3.7. Conjugated dienes such as CLA have a UV maximum around 230–240 nm and trienes show triple peaks around 261, 271 and 281 nm. Methylene-interrupted polyenes do not show any interesting UV absorption until double bonds migrate to form conjugated systems. This happens during autoxidation (section 7.2.2), alkali isomerisation (section 7.8) and other reactions involving doubly allylic methylene groups. UV spectroscopy is also used in the study of carotenoids with extended conjugated systems (Young & Hamilton in Hamilton & Cast, 1999; Angioni *et al*. in Dobson, 2002).

6.2.2 *Infrared spectroscopy*

Infrared spectroscopy has been applied to solid lipids to provide information about polymorphism, crystal structure, conformation and chain-length (section 6.1.2) but the commonest use of traditional IR spectroscopy has been the recognition and quantitation of *trans* unsaturation in acids and esters where unsaturation is predominantly *cis,* using neat liquids or solutions. One *trans* double bond absorbs at 968 cm^{-1}. Additional *trans* centres increase the intensity but do not change the frequency unless they are conjugated when small changes are reported.

There is no similar diagnostic absorption for a *cis* olefin but Raman spectra show strong absorption bands at 1665 ± 1 cm^{-1} (*cis* olefin), 1670 ± 1 cm^{-1} (*trans* olefin), and 2230 ± 1 cm^{-1} and 2291 ± 2 cm^{-1} (acetylenes) for the type of unsaturation indicated.

Carbonyl compounds have a strong absorption band in the region 1650–1750 cm^{-1}. The wavelength varies slightly with the nature of the carbonyl compound as in the following saturated and $\alpha\beta$-unsaturated compounds: aldehydes (1740–1720 and 1705–1680 cm^{-1}), ketones (1725–1705 and 1685–1665 cm^{-1}), acids (1725–1700 and 1715–1690 cm^{-1}) and esters (1750–1730 and 1730–1715 cm^{-1}).

Most oils with the usual mixture of saturated and unsaturated acids have similar infrared spectra. Additional bands associated with less common functional groups include hydroxyl (3448 cm^{-1}), keto (1724 cm^{-1}), cyclopropene (1852 and 1010 cm^{-1}), epoxide (848 and 826 cm^{-1}), allene (2222 and 1961 cm^{-1}), vinyl (990 and 909 cm^{-1}) and conjugated enyne systems (952 cm^{-1}).

The analytical uses of FTIR and NIR have been discussed in section 5.3.6. For more information see Chapman & Goni (1994), Ismail *et al*. in Hamilton & Cast (1999), van de Voort *et al*. in Dobson (2001) and Mossoba *et al*. in Dobson (2001).

6.2.3 *Electron spin resonance (ESR) spectroscopy*

ESR spectroscopy is used for the study of free radicals (odd electron species) but finds only limited use in lipid studies. It has been used in the study of autoxidation which occurs through a free radical mechanism (section 7.2.2) (Anderson & Skibsted in Dobson, 2002) and is also used in the study of membranes after incorporation of spin-labelled material such as 5-doxylstearic acid (shown below) (McPhail, 2003).

6.2.4 *^{1}H NMR spectroscopy*

^{1}H NMR spectroscopy is used in two ways in the study of lipids. With wide-line (low resolution) (pulsed) instruments, it is possible to determine the

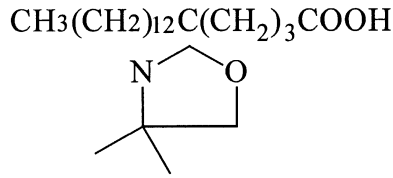


Figure 6.4 Structure of 5-doxylstearic acid.

proportion of solid and liquid in a fat and the content of oil in a seed. High-resolution spectrometers, on the other hand, are used to examine solutions and give information about the solute, which may be an individual compound or a mixture, such as a natural oil or fat.

6.2.4.1 Low resolution NMR spectroscopy

Low-resolution ^{1}H NMR or time-domain NMR is used extensively in quality control laboratories for the measurement of solid fat content, simultaneous determination of oil and moisture content, in the study of oil and water droplet size distribution and in measurements to be made through packaging. The technique has been reviewed by Meeussen in Hamilton & Cast (1999) and Todt *et al.* (2001). See also section 5.3.5.

6.2.4.2 High resolution NMR spectroscopy

A typical ^{1}H spectrum is shown in Figure 6.5. It contains signals that can be distinguished by chemical shift, coupling constant, splitting pattern and area. The last of these provides quantitative information that can be presented as mol%. The remaining parameters give structural information (Diehl, in Dobson, 2001; Knothe, 2003).

A saturated long-chain methyl ester has five signals with the following chemical shifts (ppm), number of hydrogen atoms, and splitting pattern:

• CH_3	0.90	3	triplet
• $(CH_2)_n$	1.31	2n	broad (many overlapping signals)
• -C*H*$_2$CH$_2$COOCH$_3$	1.58	2	quintet
• -CH$_2$C*H*$_2$COOCH$_3$	2.30	2	triplet
• -CH$_2$CH$_2$COOC*H*$_3$	3.65	3	singlet

Such a spectrum indicates the presence of a straight-chain saturated methyl ester but does not distinguish between homologues in a mixture. In olefinic esters there are additional signals corresponding to the:

- olefinic hydrogen atoms (-C*H*=C*H*- 5.35 ppm, 2H for oleate, 4H for linoleate, 6H for linolenate),
- allylic hydrogen atoms (C*H*$_2$CH=CHC*H*$_2$- 2.05 ppm, 4H),
- doubly allylic hydrogen atoms (=CHC*H*$_2$CH= 2.77 ppm, 2H for linoleate and 4H for linolenate).

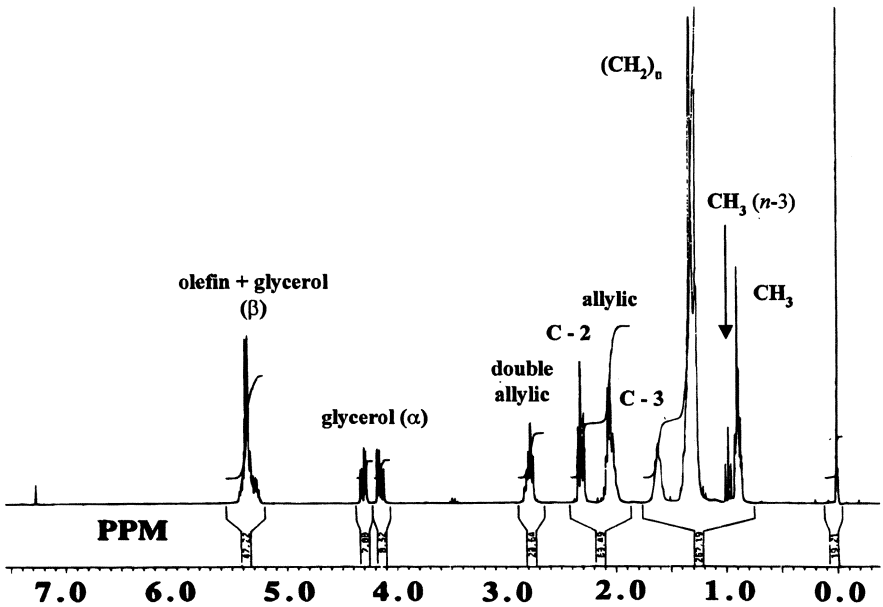


Figure 6.5 Typical ^{1}H NMR spectrum of a vegetable oil – see text for discussion (kindly supplied by my colleague Dr C.M. Scrimgeour).

For linolenate and other n-3 esters the proximity of a double bond affects the C*H*$_3$ signal which then appears at 0.98 ppm. This makes it possible to distinguish the n-3 esters from other common fatty esters with their C*H*$_3$ signal at 0.90 ppm. Glycerol esters have five hydrogen atoms associated with the glycerol unit. There is a one-proton signal at 5.25 ppm (C*H*OCOR) overlapping with olefinic signals and a four-proton signal split between 4.12 and 4.28 ppm (C*H*$_2$OCOR).

There is a growing interest in using ^{1}H NMR spectroscopy to obtain information about the composition of triacylglycerol mixtures occurring naturally. The recognition and measurement of total n-3 acids and of Δ-4 acids (DHA) in fish oils has been discussed in section 5.3.5.

For vegetable oils containing the usual mixture of saturated acids and C_{18} unsaturated acids useful information can be obtained by ^{1}H NMR procedures that are non-destructive and require no chemical reactions. The signal at 2.30 ppm (α-methylene function) provides a measure of all the acyl groups. The signals at 0.89 and 0.98 ppm distinguish linolenate (n-3) from all other esters. Signals at 2.77 ppm are a combined measure of triene (linolenate) and diene (linoleate) and those at 2.05 ppm relate to all of linolenate, linoleate and oleate. The intensity of these signals can be used to calculate the composition (%mol) in terms of oleic, linoleic, linolenic and total saturated acids.

6.2.5 *^{13}C NMR spectroscopy*

^{13}C NMR spectra are based on natural ^{13}C atoms present at a level of 1.1 per cent in organic compounds. The spectra provide two kinds of information: the chemical shift of each signal (usually around 50 signals in a natural mixture) and their relative intensities. The former is of qualitative value and permits identification of important structural features. The latter, with appropriate safeguards, provides quantitative information of analytical value. Chemical shifts may vary slightly with concentration of the solution under study and (rather more) with the solvent employed. Most measurements are made with solutions of about 1M concentration and $CDCl_3$ is the solvent most commonly used. Other solvents include mixtures of CD_3OD and $CDCl_3$, $(CD_3)_2SO$, D_2O and C_6D_6. Figure 6.6 shows a typical spectrum of vegetable oils.

The chemical shift of a carbon atom depends on its total environment to a distance of six or more atomic centres. For example, in glycerol trioleate, the signals for the olefinic carbon atoms (C_9 and C_{10}) differ from one another and also to a small extent depending on whether the oleate is an α or β chain (attached to primary or secondary glycerol hydroxyl groups). The C-1 signal is also slightly different for saturated and $\Delta 9$ unsaturated chains. In these examples, the difference is produced by structural changes up to 11 atomic centres away. This makes the spectrum more complex, but also more informative when all the chemical shifts have been assigned.

In another example, the methyl groups at the end of the acyl chains in glycerol tripalmitate give one signal at around 14.1 ppm which is well separated from other signals and hence easily recognised. The difference

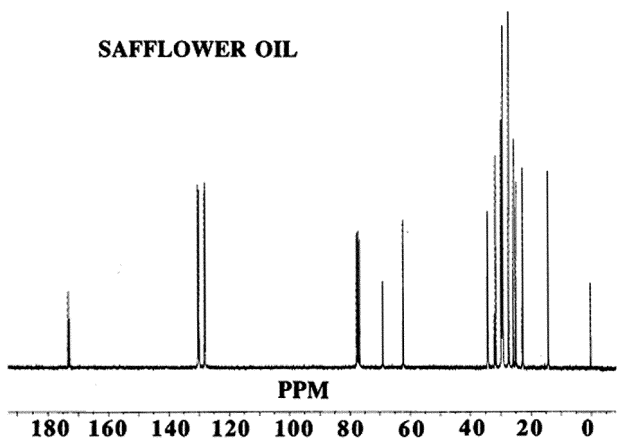


Figure 6.6 ^{13}C NMR spectrum of safflower oil – for assignment of signals see text. The signals around 80 ppm come from the solvent. (Taken with permission from <www.lipid.co.uk>.)

between α and β chains for this signal in this molecule is too small to be observed but in a vegetable oil containing saturated and unsaturated chains, the resonance at 14.1 ppm appears as a cluster of two or more signals. Each is indicative of a different environment for the methyl group and may result from *n*-3, *n*-6 or n-9 acids where the closest double bond affects the chemical shift of the methyl signal.

To obtain quantitative data attention has to be given to the protocol for obtaining the spectrum. In particular, the problem of relaxation has to be overcome either by adding a relaxation agent such as $Cr(acac)_3$ (chromium acetonylacetonoate) and/or by including a delay time between successive scans of the spectrum. This will add to the time required to collect the spectrum. Spectrometers now available operate at a frequency for ^{13}C of 68MHz or more and spectra are generally obtained using an NOE (nuclear Overhauser effect) suppressed, inverse-gated, proton-decoupled technique.

Exciting pulses have a 45–90º pulse angle and acquisition times (including delay times) are 1–20 sec per scan. The number of scans is usually 1000 or more. The sample size for a routine ^{13}C NMR spectrum is normally 50–100 mg and the spectrum is obtained in 20–30 minutes. With smaller samples, high quality spectra can be obtained with as little as 1 mg but with a correspondingly longer acquisition time.

In using ^{13}C NMR data (chemical shifts and intensities), the first step is to assign as many of the chemical shifts as possible. If the substance under study is a mixture, many individual signals will appear as clusters. This makes interpretation more difficult, but eventually provides additional information. It is wise to ignore signals in the methylene envelope (29.4–29.9 ppm) resulting from mid-chain carbon atoms that are not greatly influenced by nearby functional groups. Instead, examine the easily-recognised shifts associated with the following carbon atoms: ω1 (around 14.1 ppm), ω2 (22.8), ω3 (32.1), C-1 (174.1), C-2 (34.2), C-3 (25.1), glycerol (68.9 and 62.1), olefinic (127–132) and allylic (27.3 and 25.6).

Assignments of chemical shift are often made on the basis of available knowledge. Existing information has been built up over the past 30 years assisted by the study of ^{2}H-containing compounds and the use of chemical shift reagents (Gunstone, website). Where the necessary information is not available more advanced spectroscopic procedures will assist assignment. These can also be made on the basis of line-width and relaxation measurements. Easily recognised carbon atoms present in most triacylglycerols have been cited above. This provides enough information to make a preliminary assignment to the signals in a spectrum such as that of sunflower seed oil (Fig. 6.6).

From the peak areas of appropriate signals, the average number of double bonds per triacylglycerol molecule and the average molecular weight can be calculated and hence the iodine value (excluding unsaponifiable material). These are based on signals at 24.85 (C-3, a measure of total acyl chains),

25.62 (L11, a measure of linoleic acid), 27.15 (O8, O11, L8, L14, monoenes and dienes) and the multiplet at 29.45 ppm (mid-chain methylene) (Ng & Gee, 2001).

In magic-angle spinning mode it is possible to examine solid samples and to determine the fatty-acid composition of seeds individually or in small batches. For example, the composition of a transformed canola seed was calculated from the five signals at 21.1 (Ln 17), 23.3 (C-17 for O, L, and sat), 25.6 (C-3 all acyl groups), 26.2 and 26.05 (L11, Ln11 and 14), 28.0 ppm (O8 and 11, L8 and 14, Ln8) (Hutton *et al*., 1999). ^{31}P NMR spectroscopy is discussed in section 5.4.3.

6.2.6 Mass spectrometry

Mass spectrometry is a procedure used to determine the structure of individual molecules. Originally, these had to be isolated by standard procedures, but it is now more usual to combine the mass spectrometer with GC or HPLC so that individual components of a mixture can be separated by chromatography and identified by mass spectrometry. If the compound is already known, then its mass spectrum can be compared with that already reported and contained in a data bank (Christie, website). If the compound is novel it may be possible to identify it by application of the basic principles of mass spectrometry. When a chromatographic separation precedes mass spectrometry then it is also desirable to quantify the data so that the proportion of each molecular species is also known. This is usually achieved by measurement of the total ion current, but accurate quantification requires calibration with standards or the use of isotopic internal standards. In the combined GC-MS procedures, it is also necessary to select derivatives that combine good chromatographic properties (satisfactory separation under simple GC or HPLC conditions) with good spectroscopic properties (molecular and/or fragment ions that lead to easy recognition of the molecule). This last may require a selection of the appropriate spectroscopic procedure.

When a molecule is ionised (electronically or chemically) it forms a molecular ion (M^+). This may fragment to give one charged (A^+) and one uncharged (B) particle and a mass spectrometer is a device for producing and examining the charged particles. These are separated according to their mass to charge ratio (*m/z,* where *z* is usually 1). With high-resolution instruments, this value can be measured with such accuracy as to indicate the molecular formula of each ion. The intensity of each peak is related to that of the base peak (largest) which is given a value of 100.

$$M \rightarrow M^+ \rightarrow A^+ + B$$

Electron ionisation (EI) is the most widely used ionisation technique. This occurs through an exchange of energy between electrons emitted by a glowing

filament (usually at 70 eV) and vaporised sample molecules. Under these conditions the molecular ion usually fragments in one or more ways.

Chemical ionisation (CI) results from gas phase reactions between a small amount of sample and a large amount of reactant gas (such as methane, ammonia, or isobutene) itself ionised by EI-producing reactant gas ions (CH_5^+, NH_4^+, $C_4H_9^+$). CI is usually softer than EI, with the result that more of the molecular ion is available for detection and fragmentation is less extensive. This usually makes interpretation simpler. The following CI techniques are used by lipid analysts and have been discussed in section 5.3.7:

- atmospheric pressure chemical ionisation (APCI),
- fast atom bombardment (FAB),
- collision induced dissociation (tandem mass spectrometry, MS/MS).

For the structural identification of fatty acids, MS procedures linked to GC or HPLC have replaced the older classical methods. Mass spectrometry was first carried out on methyl esters but these are not very satisfactory because under EI the double bonds migrate along the chain and cannot be located unequivocally. Several methods of 'fixing' the double bond were devised but only one of these, applied mainly to monoene esters, remains in use. For both mono and polyunsaturated acids, the methyl esters have been replaced by other acid derivatives that give useful structural information. Appropriate fatty-acid derivatives are generally examined by EI and triacylglycerols by one of the CI methods.

Olefinic esters react with dimethyldisulfide (MeSSMe) and iodine to give a bis(methylthio) derivative the mass spectrum of which shows a molecular ion and two or three large fragment ions that together clearly indicate the position of the SMe groups and hence, the double bond.

$$-CH{=}CH- \rightarrow -CH(SMe)CH(SMe)-$$

For example, methyl oleate gives a molecular ion at 390 ($C_{21}H_{42}O_2S_2$) and two large fragment ions at 173 ($C_9H_{18}SMe$) and 217 ($C_{10}H_{18}O_2SMe$). A third fragment ion at 185 corresponds to the loss of methanol (32 mass units) from the peak at 217. These clearly show that methyl oleate is Δ9-18:1 but do not indicate the configuration of the double bond. However *cis* and *trans* monoenes form *threo* and *erythro* adducts respectively and although these have similar mass spectra they are separated by gas chromatography. The procedure is less satisfactory with polyunsaturated acids but there are other ways of examining these. Nevertheless, the method was used to identify a 21:5 acid as the Δ6,9,12,15,18 isomer. The acid was subjected to partial reduction with hydrazine and hydrogen peroxide (section 7.1.2) and the monoene fraction was isolated by silver ion chromatography. Mass spectrometric examination of the bis(methylthio) adducts then showed key fragments at *m/z*

257 and 175 for the Δ6 acid, 215 and 217 (Δ9), 173 and 259 (Δ12), 131 and 301 (Δ15) and 89 and 343 (Δ18).

Polyunsaturated acids are better examined as picolinyl esters or as 2-alkyl-4,4-dimethyloxazoles (DMOX).When these molecules are ionised, the charge is carried on the nitrogen atom and double bond ionisation and isomerisation are minimised. Radical-induced cleavage occurs evenly along the chain and gives a series of relatively abundant ions of high mass resulting from the cleavage of each C-C bond. When a double bond or other functional group is reached then diagnostic ions appear (section 5.3.7).

The picolinyl esters are made from the free acids and picolinyl alcohol either via the acid chloride (reaction with oxalyl chloride) or through interaction with 1,1′-carbonyldiimidazole in dichloromethane in the presence of 4-pyrrolidinopyridine as catalyst. Another method involves interesterification of triacylglycerols or phospholipids with potassium *tert*-butoxide and 3-hydroxymethylpyridine for two minutes at room temperature.

The spectrum shows some fragments of low mass characteristic of picolinates resulting from $ArCH_2^+$ (93), $ArCH_2O^+$ (108), $ArCH_2OC(OH)=CH_2^+$ (151) and $ArCH_2OC(O)=CH_2^+$ (164) where Ar is C_5H_4N or C_5H_5N. In addition there is a molecular ion peak and a series of other high mass fragments which, correctly interpreted, will indicate a structure for the picolinate. For example, the ester from α-linolenic acid has peaks at 369 (M+, the 18:3 picolinate which is a C_{24} compound), 354 (M^+-15), 340, 326, 312, 298, 272, 258, 232, etc. Most of these ions differ by 14 mass units from their neighbour, representing loss of CH_2, but something different happens between 298 and 272 and between 258 and 232 where there is a loss of 26 mass units (C_2H_2 representing a –CH=CH– unit). These fragments indicate the presence of double bonds at Δ9 and Δ12. The double bond nearest to the carboxyl group is not always easily spotted, but with experience it is not too difficult to define (Fig. 6.7).

Comparison of the fragment ions for stearic and oleic picolinates (Table 6.4) show how it is possible to determine the double-bond position. The features of note are: the C_{10} to C_{17} fragments are two mass units lower for oleate than for stearate while those up to C_8 have the same mass; there are enhanced signals for the C_{11} and C_{12} fragment ions related to allylic groups at C_8 and C_{11}; and, most significantly, the C_9 fragment is missing and the C_8 and C_{10} fragments differ by 26 mass units. These concepts can be extended to linoleate and linolenate. With yet more double bonds, the interpretation becomes more complex, but it is usually possible to recognise the unsaturated centre closest to the methyl group. It may then be assumed that the remaining unsaturated centres have the usual methylene-interrupted pattern.

DMOX derivatives are made by heating the lipid with 2-amino-2-methyl-1-propanol in a nitrogen atmosphere at 180ºC (2 hours for free acids, 18 hours for methyl or glycerol esters). Their spectra show peaks at 113 and 126

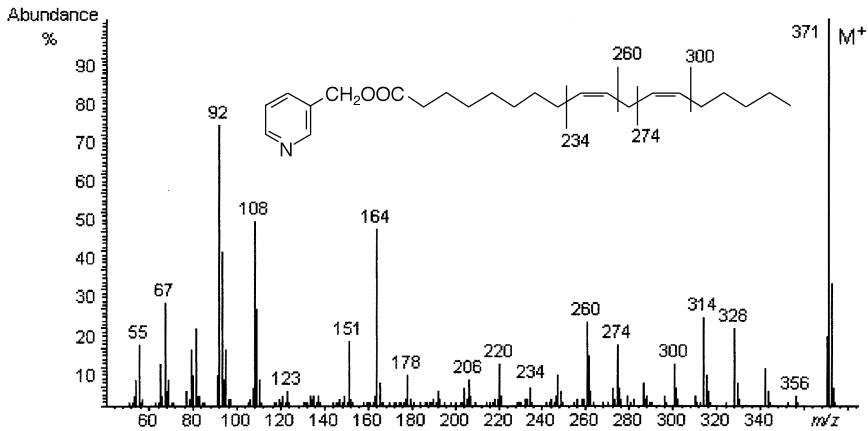


Figure 6.7 Mass spectrum of linoleic acid as the picolinate. (Taken with permission from <www.lipid.co.uk>.)

common to all DMOX derivatives, along with a molecular ion and a series of fragments differing by 14 mass units except that some pairs differ by only 12 mass units. These are indicative of olefinic centres and are interpreted according to Christie (2003, p. 289): 'if there is an interval of 12 mass units between the most intense peaks of clusters of ions containing n and n-1 carbon atoms then there is a double bond between carbon n+1 and n in the acyl chain'. Spectra of DMOX derivatives of many acids are available on Christie's website.

MS procedures, combined with a chromatographic separation system, also give valuable insight into the structure and composition of triacylglycerol mixtures such as milk fats (Currie & Kallio, 1993), vegetable oils (Byrdwell, 1998, 2001; Neff *et al*., 2001) and fish oils. In general, identification depends on molecular ions that define the number of both carbon atoms and double

Table 6.4 Significant fragment ions in the mass spectra of stearic, oleic, linoleic and linolenic picolinates

		Number of carbon atoms in the acyl chain of the fragment ions													
Ester	[M]+	17	16	15	14	13	12	11	10	9	8	7	6	5	4
18:0	375	360	346	332	318	304	290	276	262	248	234	220	206	192	178
18:1	373[a]	358	344	330	316	302	288[a]	274[a]	260	*	234	220	206	192	178
18:2	371[a]	356	342	328	314	300	*	274	260	*	234	220	206	192	178
18:3	369[a]	354	340	*	314	300	*	274	260	*	234	220	206	192	178

[a] Enhanced signal.
* Missing fragment, gap of 26 mass units between adjacent signals, gap of 40 mass units between somewhat enhanced signals.

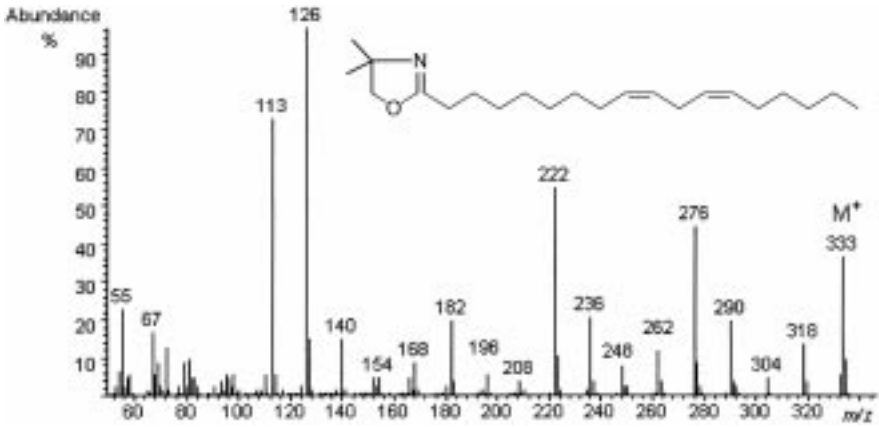


Figure 6.8 Mass spectrum of linoleic acid as the DMOX derivative. (Taken with permission from <www.lipid.co.uk>.)

bonds in each triacylglycerol molecule. In addition fragment ions indicate the nature of each acyl group in terms of its number of carbon atoms and unsaturated centres and in some cases will define the distribution of fatty-acyl residues between the primary (*sn*1/3) and secondary (*sn*-2) glycerol positions. Quantitative determination of mixtures is still a problem because the MS responses of triacylglycerols vary with the molecular structure. This topic is intensively reviewed by Laakso & Manninen in Hamilton & Cast (1999) and Laakso in Dobson (2002).

In measuring the molecular distribution of triacylglycerols using MALDI-TOF, a mixture of analyte and matrix such as α-cyano-4-hydroxycinnamic acid [4-$HOC_6H_4CH{=}C(CN)COOH$] is deposited on a stainless steel plate and placed in the ion source of the MS. Sodium ions are often present in the matrix or are added to the sample as sodium acetate. When a laser beam is directed at the sample, ions are formed by cationisation of triacylglycerol molecules $(M+Na)^+$. With soybean oil, for example, a series of peaks corresponding to 54:n (n = 2–9) and 52:n (n = 2–5) are apparent in the spectrum. Most of these can be identified on the basis of the known fatty-acid composition (Ayorinde, 2000).

Reverse phase HPLC followed by APCI MS gives a molecular ion $(M+H)^+$ and fragment ions corresponding to M-RCOOH. For example, the StLO fraction gives peaks at 885.6 $(M+H)^+$, 605.4 $(StO)^+$, 603.5 $(StL)^+$ and 601.4 $(OL)^+$. Among the DAG the 1,3 isomer is less intense than the 1,2 and 2,3 isomers and this makes it possible to identify the fatty acid at the 2 position (Byrdwell 1998, 2001; Neff *et al*., 2001).

Mass spectrometry of lipids has also been reviewed by Christie (1998), Laakso & Manninen (1999); Roach *et al*. in Hamilton & Cast (1999); Dobson & Christie (2002); Laakso (2002) and Korachi *et al*. in Dobson (2002).

6.3 Other physical properties

6.3.1 Density

Density may not seem an exciting physical property to many technologists, but it is very important in the trading of oils since shipments are sold on a weight basis but measured on a volume basis. These two values are related by density, so it is important to have correct and agreed values for this unit. This is not the same for all oils. It depends on fatty-acid composition and minor components as well as on the temperature. An equation taking these variables into account is based on iodine value, saponification value and temperature (Pantzaris, 1985).

$$d = 0.8543 + 0.000308(SV) + 0.000157(IV) - 0.00068t$$

where d = apparent density (g/ml or kg/L), SV = saponification value, IV = iodine value, and t = temperature (ºC).

Density can be defined in various ways and the correct form must be used when relating volume to weight.

- Density (absolute density or density in vacuum) is: Mass in vacuum of a volume of oil at t°*C* ÷ volume of the oil at the same temperature expressed in g/mL or kg/L.
- Apparent density (density in air, weight-by-volume, or litre-mass) is: Mass in air of a volume of oil at t°*C* ÷ volume of the oil at the same temperature expressed in g/mL or kg/L.
- Relative density (specific gravity, density in relation to water) is: Mass in air of a given volume of oil at t_1ºC ÷ mass in air of same volume of water at t_2ºC. This is a ratio without units. It is important to note that two temperatures are involved and the value is meaningless unless both figures are cited. This is the value most usually employed and equations exist to connect these three expressions.

Further information is given by Gunstone (2000).

6.3.2 Viscosity

Viscosity can be reported as kinematic viscosity or dynamic viscosity with the two values being related through density. The viscosity of a vegetable oil depends on its chemical composition (summarised in the iodine value and saponification value) and the temperature of measurement. Equations have been derived which permit calculation of viscosity from knowledge of the other three parameters. These have been developed empirically from observation with a range of oils at different temperatures (Duff & Prasad, 1989; Toro-Vazquez & Infante-Guerro, 1993). Coupland & McClements (1997) and

Fisher (1998) have related viscosity with density, refraction, surface tension and other physical properties.

The relation between temperature and viscosity for selected oils has been described by several authors (Timms, 1985; Ibemesi & Igwe, 1991; Lang *et al*., 1992; Noureddini *et al*., 1992; Tasioula-Margari & Demetropoulos, 1992).

6.3.3 Refractive index

The refractive index is easily measured on small amounts of material. Refractive index increases with chain length (though not in a linear fashion) and with increasing unsaturation. Geometric isomers differ from one another and methylene-interrupted polyenes differ from those with conjugated unsaturation. Triacylglycerols have higher values than free acids. Values for commercial oils are cited in Table 6.5.

6.3.4 Solubility of gases in oils

A recent discussion (Hilder in Gunstone & Padley, 1997) on the solubility of gases in oils includes the data presented in Tables 6.6 and 6.7 for oxygen, nitrogen and air. When an oil is in contact with air, the dissolved gases will depend on their individual solubility as well as their concentration in air. The high solubility of the monatomic argon enhances its concentration so that one per cent in air becomes three per cent of the gases in the oil.

Koetsier in Gunstone & Padley (1997) has summarised data on the solubility of hydrogen in vegetable oil. This information is obviously important for hydrogenation. He cites solubility values (maximum concentration in oil at a given temperature and pressure) from two sources at 1 bar and 100–200ºC of 2.60–3.36 mol/m^3 and 2.76–3.40 mol/m^3. The concentration of hydrogen is thus much lower than the concentration of unsaturated centres and for a fish oil of iodine value hydrogenated at 5 bar and 180º Koetsier gives concentrations of around 7000 mol/m^3 and 16 mol/m^3 respectively for the olefinic groups and the hydrogen.

6.3.5 Other physical properties

Gross heats of combustion (HG) for saturated and unsaturated triacylglycerols can be related to the number of valence electrons (EN). The following equations have been derived.

$$HG = -109.20 + 26.39\ EN \qquad \text{saturated triacylglycerols}$$
$$HG = 115.87 + 25.88\ EN \qquad \text{unsaturated triacylglycerols}$$
$$HG = 1\ 896\ 000/SN - 0.6\ IV - 1600$$

Coupland & McClements (1997) reported several physical properties

Table 6.5 Physical and chemical properties of selected commodity oils and fats

	Specific gravity (temperature ºC)	Ref index (40ºC)	Ref index (25ºC)	Iodine value	Saponi–fication value	Titre (ºC)	Unsaponifiable (%)	Mp (ºC)
Cocoa butter	0.973–0.980 (25/25)	1.456–1.458	–	32–40	192–200	45–50	0.2–1.0	31–35
Coconut	0.908–0.921 (40/20)	1.448–1.450	–	6–11	248–265	–	<1.5	23–26
Corn	0.917–0.925 (20/20)	1.465–1.468	1.470–1.473	107–128	187–195	–	1–3	–
Cottonseed	0.918–0.926 (20/20)	1.458–1.466		100–115	189–198	–	<2	–
Linseed	0.930–0.936 (15.5/15.5)[d]	1.472.1.475	1.477–1.482	170–203	188–196	19–21	0.1–2.0	–
Olive	0.910–0.916 (20/20)	–	1.468–1.471	75–94	184–196	–	1.5	–3–0
Palmkernel	0.899–0.914 (40/20)	1.452–1.488	–	14–21	230–254	–	<1.1	24–26
Palm	0.891–0.899 (50/20)	1.449–1.455[e]	–	50–55	190–209	–	<1.4	33–40
Palm olein	0.899–0.920 (40/20)	1.459–1.459	–	>55	194–202	–	<1.4	–
Palm stearin	0.881–0.891 (60/20)	1.447–1.451	–	<49	193–205	–	<1.0	–
Peanut	0.914–.0.917 (20/20)	1.460–1.465	–	86–107	187–196	–	<1.1	–
Rape[a]	0.910–0.920 (20/20)	1.465–1.469	–	94–120	168–181	–	<0.21[f]	–
Rape[b]	0.914–0.920 (20/20)	1.465–1.467	–	110–126	182–193	–	<0.21[f]	–
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	1.472–1.476	118–145	188–194	–	<1.6 (max 2.0)	–
Sunflower[c]	0.915–0.920 (20/20)	–	1.467–1.469	75–90			0.8–1.0 (max 2.0)	–

[a] High-erucic rape seed oil.
[b] Low-erucic rape seed oil.
[c] High-oleic sunflower seed oil.
[d] Also 0.924–0.930 (25/25).
[e] 50ºC.
[f] These values are correctly copied from the source but they are in error. Better values are 0.5–1.2 per cent.
Source: AOCS (1997).

Table 6.6 Solubility of oxygen and nitrogen in oils

Temp (ºC)	Oxygen (ppm, 1 bar)	Nitrogen (ppm, 1 bar)
0	170	80
25	180	85
50	185	90
75	190	95
100	200	105
125	*	110
150	*	115

Source: Hilder in Gunstone & Padley (1997).
* Oxygen solubilities at higher temperatures are not reliable because oxidation occurs.

Table 6.7 Gas content of oil saturated with air

	Solubility (ppm)	Air dissolved in oil (ppm)
Oxygen	180	38
Nitrogen	85	66
Argon	270	3

Source: Hilder in Gunstone & Padley (1997).

(density, viscosity, adiabatic expansion coefficient, thermal conductivity, specific heat, ultrasonic velocity and ultrasonic attenuation coefficient) for a number of liquid oils. Timms (1978) reviewed and significantly extended information on the heats of fusion of glycerides. He derived an equation for the heat of fusion of mono acid glycerides in the β polymorph form and showed how this could be adapted to calculate the heat of fusion of most glycerides of commercial interest. Chumpitaz *et al*. (1999) recently reported the surface tension of lauric, myristic, palmitic and oleic acids and of tricaprylin and tripalmitin, over a range of temperatures. These data are important for processes involving gas-liquid contact such as distillation and stripping columns, deodorisers, reactors and equipment for physical refining.

Bibliography and references

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

Byrdwell, W.C. (1998) APCI-MS for lipid analysis, *Inform,* **9**, 986–997.

Byrdwell, W.C. (2001) Atmospheric pressure chemical ionisation mass spectrometry for analysis of lipids, *Lipids*, **36**, 327–346.

Chapman, D. & Goni, F.M. (1994) Physical properties: optical and spectral characteristics. In: *The Lipid Handbook*, 2nd edn (eds F.D. Gunstone, J.L. Harwood & F.B. Padley), Chapman and Hall, London, pp. 487–560.

Christie, W.W. (1997) Structural analysis of fatty acids. In*: Advances in Lipid Methodology*, ed

W. W. Christie, Oily Press, Dundee, pp. 119–169.
Christie, W.W. (1998) Gas chromatography – mass spectrometry methods for structural analysis of lipids, *Lipids*, **33**, 343–353.
Christie, W.W. (2003) *Lipid Analysis – Isolation, Separation, Identification and Structural Analysis of Lipids*. The Oily Press, Bridgewater.
Christie, W.W. <www.lipid.co.uk>.
Chumpitaz, L.D.A., Coutinho, L.F. & Meirelles, A.J.A. (1999) Surface tension of fatty acids and triglycerides, *Journal of the American Oil Chemists' Society*, **76**, 379–382.
Coupland, J.N. & McClements, D.J. (1997) Physical properties of liquid edible oils, *Journal of the American Oil Chemists' Society*, **74**, 1559–1564.
Currie, G. & Kallio, H. (1993) MS milk fats, *Journal of the American Oil Chemists' Society*.
Dobson, G. (2001) Spectroscopy and spectrometry of lipids – Part 1, *European Journal of Lipid Science and Technology*, **103**, 815–840.
Dobson, G. (2002) Spectroscopy and spectrometry of lipids – Part 2, *European Journal of Lipid Science and Technology*, **104**, 36–68.
Duff, N.V.K. & Prasad, D.H.L. (1989) Inter-relationships among the properties of fatty oils, *Journal of American Oil Chemists' Society*, **66**, 701–703.
Firestone, D. (ed.) (1997) *Physical and Chemical Characteristics of Oils, Fats and Waxes*, AOCS Press, Champaign, Ill.
Fisher, C.H. (1998) Correlating viscosity with temperature and other properties. *Journal of the American Oil Chemists' Society*, **75**, 1229–1232.
Gunstone, F.D. (2000) Composition and properties of edible oils in *Edible Oil Processing* (eds W. Hamm & R.J. Hamilton), Sheffield Academic Press, Sheffield, pp. 1–33.
Gunstone, F.D. <www.lipid.co.uk>.
Gunstone, F.D. & Padley, B.F. (1997) *Lipid Technologies and Applications*, Dekker, New York
Hamilton, R.J. & Cast, J. (1999) *Spectral Properties of Lipids,* Sheffield Academic Press, Sheffield.
Hutton, W.C., Garbow, J.R. & Hayes, T.R. (1999) Nondestructive NMR determination of oil composition in transformed canola seeds, *Lipids*, **34**, 1339–1346.
Ibemesi, J.A. & Igwe, I.O. (1991) Anomalous viscosity behaviour of fatty acid esters in solution, *Journal of the American Oil Chemists' Society*, **68**, 147–152.
Lang, W., Sokhansanj, S. & Sosulski, F. W. (1992) Modelling the temperature dependence of kinematic viscosity for refined canola oil, *Journal of the American Oil Chemists' Society*, **69**, 1054–1055.
Larsson, K. (1994) *Lipids: Molecular Organisation, Physical Functions, and Technical Applications*, The Oily Press, Dundee.
Knothe, G. (2003) Quantitative analysis of mixtures of fatty acids by 1H-NMR, *Lipid Technology*, 15, 111–114.
Lawler, P.J. & Dimick, P.S. (2002) Crystallisation and polymorphism in fats. In*: Food Lipids: Chemistry, Nutrition, and Biotechnology,* (2nd edn, eds C.C. Akoh & D.B. Min), Marcel Dekker, New York, pp. 275–300.
McPhail, D. (2003) ESR spectroscopy: applications in lipid research*, Lipid Technology*, **15**, 88–90.
Mori, H. (1988) Solidification problems in the preparation of fats. In *Crystallisation and Polymorphism of Fats and Fatty Acids* (eds N. Garti & K. Sato), Marcel Dekker, New York, pp. 423–442.
Neff, W.E., List, G.R. & Byrdwell, W.C. (2001) New tool for triacylglycerol analysis in food oils, *Lipid Technology*, **13**, 15–17.
Ng, S. & Gee, P.T. (2001). Determination of IV of palm and palmkernel oil by ^{13}C, *European Journal of Lipid Science and Technology*, **103,** 223–227.
Noureddini, H., Teoh, B.C. & Clements, L.D. (1992) Viscosities of oils and fatty acids. *Journal of the American Oil Chemists' Society*, **69**, 1189–1191.
Pantzaris, T.P. (1985) The density of oils in the liquid state, *PORIM Technology*, **12**.
Sato, K. (2001a) Crystallisation behaviour of fats and lipids – a review. *Chemical Engineering Science*, **56**, 2255–2265.
Sato, K. (2001b) Uncovering the structure of β' fat crystals: what do the molecules tell us? *Lipid Technology*, **13**, 36–40.

Tasioula-Margari, M. & Demetropoulos, I.N. (1992) Viscosity-structure relationship in dilute triglycerides' solutions. Correlation to retention time in reversed-phase liquid chromatography. *Journal of the American Oil Chemists' Society*, **69**, 1112–1117.

Timms, R.E. (1978) Heats of fusion of glycerides. *Chemistry and Physics of Lipids,* **21**, 113–129.

Timms, R.E. (1985) Physical properties of oils and mixtures of oils. *Journal of the American Oil Chemists' Society*, **62**, 241–248.

Toro-Vazquez, J.F. & Infante-Guerrero, R. (1993) Regressional models that describe absolute viscosity. *Journal of the American Oil Chemists' Society*, **70**, 1115–1119.

7 Chemical properties related to unsaturated centres

All fatty acids contain a carboxyl group and many also contain one or more double bonds, and the chemistry of these molecules involves one or both of these functionalities. The industrial chemistry of fatty acids and their esters is concerned mainly (90%) with the acid/ester group, and with the double bond only to a minor extent. This claim may be true but it is misleading. In addition to the fact that the double bond is the basis of much interesting chemistry that has not yet been exploited on a commercial scale, the chemistry of partial hydrogenation and of oxidation have been overlooked. The former is part of food chemistry rather than oleochemistry, and the latter is directed to the understanding of a reaction that has to be avoided rather than exploited.

7.1 Hydrogenation

7.1.1 Catalytic hydrogenation

Each year, millions of tonnes of soybean and other unsaturated vegetable oils containing oleic, linoleic and linolenic acids (also fish oils with more complex patterns of unsaturation) are subjected to partial hydrogenation using a heterogeneous catalyst which is almost always nickel. Hydrogenation may be carried out in three ways. Very light hydrogenation (called brush hydrogenation) is used to reduce the levels of linolenic acid in soybean oil and in rapeseed oil to produce oils with longer shelf life that are still liquid (section 2.3.3). Extensive hydrogenation is used to produce a hard fat for interesterification with a soft oil. But the most common form of partial hydrogenation is carried out to convert an oil into a plastic (deformable) solid which can be used to make a spreading fat. As explained in section 10.3.1, this must contain an appropriate blend of solid and liquid components at refrigerator temperature, room temperature, and mouth temperature. The changes occurring during partial hydrogenation are complicated by the fact that hydrogenation on a heterogeneous catalyst is reversible and double bonds remaining in a partially hydrogenated fat may have changed position and configuration. Hydrogenation processes affect melting behaviour and have nutritional consequences. These changes take place in triacylglycerols with

mixed fatty acids, but our understanding of hydrogenation processes has come mainly from the study of individual methyl esters.

Partial hydrogenation of methyl oleate (9*c*-18:1) gives a mixture of stearate, unchanged oleate and several isomeric *cis* and *trans* 18:1 esters with the latter having a higher melting point than the former (section 6.1.1).

etc. $\Delta 11 \rightleftharpoons \Delta 10 \rightleftharpoons \Delta 9 \rightleftharpoons \Delta 8 \rightleftharpoons \Delta 7$ etc.

Depending on the reaction conditions, methylene-interrupted dienes like methyl linoleate (9*c*12*c*-18:2) react 5–100 times faster than the monene methyl oleate, This relative reaction rate has an important influence on the selectivity of the process occurring with mixed triacylglycerols. Dienes are converted to monoenes faster than monoenes are reduced to saturated acyl chains. The reaction is complex in that a great variety of dienes and monoenes can be formed and each unsaturated centre can exist in *cis* or *trans* form. Dienes present may include those with conjugated unsaturation, such as the $\Delta 9c11t$ and $\Delta 10t12c$ isomers, methylene interrupted dienes (mainly $\Delta 9,12$ isomers) and non-methylene-interrupted dienes (such as the $\Delta 8,12$- and $\Delta 9,13$ compounds). The rate of reaction of these diene types is:

conjugated > methylene-interrupted >> non-methylene-interrupted

with the result that the level of conjugated dienes in the final product is generally quite low, since these are readily reduced to monoenes.

Copper chromite catalysts promote the reduction of methylene-interrupted and conjugated dienes only. Monoenes and non-methylene-interrupted dienes are not reduced under these conditions. With this catalyst, partial hydrogenation of linolenate (9*c*12*c*15*c*-18:3) gives rise to a wide range of dienes and monoenes.

The complexity described above results from the fact that the reversible process involves half hydrogenated species produced by reaction between an adsorbed hydrogen molecule and an absorbed olefinic centre. As already reported (section 6.3.4) it has been calculated that the concentration of hydrogen is much lower than that of the olefinic centre.

The reaction occurs through the following stages which are elaborated in section 2.3.3.

- Hydrogen and olefinic ester are adsorbed on active sites on the surface of the metallic catalyst.
- Reaction between olefin and hydrogen atoms gives half-hydrogenated molecules represented as MH and DH for monoenes and dienes respectively.
- If these react with a second atom of hydrogen then the olefin has been reduced and may desorb from the catalyst surface
- Alternatively, the second stage may be reversed and half hydrogenated

molecules will regenerate monoenes or dienes by loss of a hydrogen atom. This need not be the hydrogen atom added in stage 2 and depending on which atom is lost the product may have changed configuration and/or changed double-bond position.

Nickel usage has fallen from 700 ppm a century ago to 100–150 ppm today. The reasons for this include the following: the soybean and rapeseed oils hydrogenated today require less catalyst than the fish oils used in former years, there is less poisoning of the catalyst because of the improved refining procedures for the oils, batch reactors are now more efficient and the fact that today's products are now more liquid (less hydrogenated) (Berben, 2002). These factors have been accompanied by improvements in catalyst preparation. The nickel was first supported on kieselguhr, then on synthetic silicates, and now on alumina. The last produces catalysts with pore diameters up to 100 Ångstrom (10 nm). The use of other metals such as Pd, Pt, Ru, or Rh leads to products with less *trans* compounds. Nickel remains the catalyst of choice for hydrogenation of vegetable oils but it is expected that palladium will slowly replace nickel for fatty acid hydrogenation (Berben, 2002). Insofar as partial hydrogenation is replaced by interesterification there will be a demand for more extensively hydrogenated oils which can be prepared in a (cheaper) fixed-bed reactor.

7.1.2 Other chemical reductions

Complete chemical reduction converts an unsaturated ester to its perhydro derivative. This is conveniently accomplished in the laboratory using palladium charcoal or a homogeneous catalyst such as Wilkinson's catalyst [$(Ph_3P)_3RhCl$]. Deuterated molecules can be conveniently made in this way by using deuterium in place of hydrogen. The problems of double bond migration and stereomutation occur only with heterogeneous catalysts and the location of the deuterium atoms at the erstwhile olefinic carbon atoms is ensured with a homogeneous catalyst.

Partial hydrogenation of acetylenic compounds using appropriate catalysts gives olefinic compounds of defined configuration. These are important processes in the laboratory synthesis of unsaturated acids through acetylenic intermediates (section 4.1.3).

Non-catalytic hydrogenation can be carried out by hydrazine (N_2H_4) in the presence of oxygen or some other oxidising agent. The reactive species (N_2H_2) effects *cis* addition without stereomutation or double bond migration. If hydrazine is replaced by tetadeuterohydrazine (N_2D_4) then *erythro* dideutero compounds are formed from *cis* olefins and the corresponding *threo* compounds from *trans* isomers.

The reduction of acids/esters to alcohols is discussed in section 8.5.1.

7.1.3 Biohydrogenation

Ruminant animals (cows, sheep) ingest polyunsaturated fatty acids from seed meals and from grass but their depot fats and milk fats are rich in saturated and monoene acids and contain only low levels of polyene acids. The monoene acids are mixtures of positional and configurational isomers and cow milk fat contains Δ6*c/t* through Δ16*c/t*-18:1 acids. These are the products of biohydrogenation of polyene acids formed in the rumen through the activity of rumen bacteria which promote lipolysis and esterification, double-bond isomerisation, and hydrogenation and dehydrogenation.

There are two major dietary sources of *trans* acids: those found in spreading and cooking fats resulting from partial hydrogenation of unsaturated vegetable oils with heterogeneous catalysts (section 7.1.1) and those in dairy and meat products of ruminant origin resulting from partial biohydrogenation of similar polyunsaturated fatty acids. These two sources contain a similar range of *trans* isomers but in differing proportions.

In the mammary gland most of the C_4–C_{14} saturated acids and about one half of the C_{16} acids of ruminants are produced by *de novo* synthesis while the rest of the C_{16} and the saturated and unsaturated C_{18} acids are derived from dietary sources or by mobilisation of body fat reserves during early lactation. Only this latter half of milk fat fatty acids is subject to change by modification of dietary intake. Further, free unsaturated acids are subject to biohydrogenation in the rumen, it is necessary to protect such acids during their passage through the rumen if these are to be incorporated unchanged in the milk fat. This was first achieved through coating the oil/fat (soybean, linseed, rape/canola), but two other methods are now more commonly employed. In the first, calcium salts are used as the 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 supplement contains appropriate proportions of n-9, n-6, and n-3 unsaturated acids and that it is over 75 per cent protected from metabolism in the rumen. As a result of these dietary changes both the milk fats and the meat fats are more unsaturated. This, in turn, affects oxidative stability and the flavours developed during cooking.

Rumenic acid (9*c*11*t*-18:2) is produced by ruminants through enzymatic isomerisation of linoleic acid in the rumen. This is followed by hydrogenation leading first to vaccenic acid (11*t*-18:1) and finally to stearic acid.

In ruminants and non-ruminants, there is an alternative pathway to CLA resulting from the action of the ubiquitous Δ9 desaturase on 11*t*-18:1 and

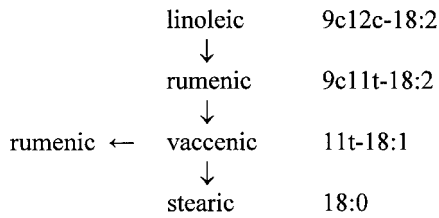


Figure 7.1 Two routes to rumenic acid involving isomerisation of linoleic acid or Δ9 desaturation of vaccenic acid.

other *trans* monoenes. These *trans* monoene acids are products of rumen biohydrogenation of linoleic acid or they are present in the diet as partially hydrogenated vegetable oils (Fig. 7.1).

Rumenic acid is the major conjugated 18:2 acid in ruminant and other animal fats but it is accompanied by other CLA at lower concentrations such as the 7t9c diene acid. This has complicated biological studies since it is not always clear which isomer is responsible for each of the several physiological properties observed. The term conjugated linoleic acid (CLA) is used to describe the mixed conjugated dienes obtained from both biological and chemical sources (section 7.3).

7.2 Oxidation through reaction with oxygen

7.2.1 Introduction

All olefinic compounds are prone to reaction with oxygen and since this generally leads to deterioration such as the breakdown of rubber and of paints as well as to the development of rancidity in fat-containing foods much effort has been put into understanding these reactions. Even if it is not possible to prevent these undesirable changes they can at least be inhibited through the use of appropriate antioxidants.

The oxygen molecule exists in two forms – in its normal ground state it is a triplet form ($^3O_2$) and in the excited state it exists in a singlet form ($^1O_2$). Both react with olefinic systems and while they share some similarities, there are some important differences between the reactions of these two forms of oxygen. Triplet oxygen is a diradical •O–O• and reacts mainly at allylic centres to give allylic hydroperoxides. In contrast, singlet oxygen is an electrophilic substance reacting with electron-rich olefinic systems, but also producing allylic hydroperoxides (section 7.2.4). It is more reactive than triplet oxygen by 22.4 kcal/mole and has a lifetime of only 50–700 μs.

Lipid oxidation may occur through enzymic and non-enzymic processes and the latter may involve oxygen in its triplet or singlet form. Oxidation can

olefinic acds/esters (methyl oleate, linoleate etc., glycerol esters, oils and fats)

↓

allylic hydroperoxides (highly reactive species)

↓

- Volatile compounds of lower molecular weight (aldehydes etc) which provide odour and flavour, often at low concentration.
- Compounds with the same chain length: rearrangement products, products of further oxidation, and products of reaction with other components in the reaction system
- Compounds of higher molecular weight such as dimers and polymers

Figure 7.2 Formation and further reactions of allylic hydroperoxides.

be promoted by heat, light, metals, several initiators and can be inhibited by antioxidants acting in different ways (section 7.2.7). There are small but significant differences between the oxidation of monoenes and methylene-interrupted polyenes. The initial products are generally allylic hydroperoxides with their unsaturation still intact and these undergo further reactions that are important in the development of off-flavours and rancidity (Fig. 7.2).

7.2.2 Autoxidation

Reaction between olefinic esters and triplet oxygen is a radical chain process involving three stages of initiation, propagation and termination (Fig. 7.3). In the initiation stage, an allylic hydrogen atom is removed and a resonance-stabilised radical is produced. The nature of this initiation step is not fully understood and three suggestions have been made: metal-catalysed decomposition of pre-formed hydroperoxides (it is almost impossible to obtain a sample of olefinic material that is not already oxidised to some small extent), formation of hydroperoxide by photo-oxidation (section 7.2.3), or thermally in a heated fat. The initiation step and the propagation sequence depend on the ease with which a hydrogen atom can be removed from a methylene group. The energy required to remove hydrogen from a saturated methylene group, an allylic methylene group, and a doubly-allylic methylene group as at C-11 in linoleate is 100, 75 and 50 kcal, respectively. These values are related to the relative ease of oxidation of saturated esters, oleate, and linoleate (Table 7.1).

In the two-step propagation sequence, given an adequate supply of oxygen, conversion of alkyl radical to peroxy radical is fast and the conversion of peroxy radical to hydroperoxide is rate-determining. This sequence will continue as long as there is a supply of reactants but, as indicated in the termination reactions, there is some loss of alkyl and peroxy radicals through dimerisation to form stable products which do not promote the reaction further.

Initiation	$RH \longrightarrow R\bullet$	resonance-stabilised alkyl radical
Propagation	$R\bullet + O_2 \longrightarrow RO_2\bullet$	fast reaction to a peroxy radical
	$RO_2\bullet + RH \longrightarrow RO_2H + R\bullet$	rate-determining step
Termination	$RO_2\bullet + RO_2\bullet \longrightarrow$ stable products	
	$RO_2\bullet + R\bullet \longrightarrow$ stable products	
	$R\bullet + R\bullet \longrightarrow$ stable products	

Figure 7.3 Olefin autoxidation. RH represent an olefinic compound in which H is attached to an allylic carbon atom.

Peroxy radicals, however formed, can react further in four different ways:

1. Reaction with a hydrogen atom converts the peroxy radical to a hydroperoxide. The hydrogen atom may come from an allylic group in the same or another olefin molecule or from antioxidant acting as a hydrogen donor. Such products are formed under kinetic control.
2. The peroxy radical may undergo β-scission. This is equivalent to the reversal of its formation and generates oxygen and alkyl radical. Scission is more likely to occur when the alkyl radical is itself resonance-stabilised as with linoleate rather than oleate. The alkyl radical will then exist in its resonance hybrid forms and may also undergo a configurational change – usually from *cis* to the more stable *trans* form. The hydroperoxides from this modified radical will be those resulting from themodynamic control.
3. Rearrangement, involving the reaction shown below and accompanied by a change of configuration of the double bond from *cis* to *trans*.

   (*cis*) $RCH{=}CHCH(OO\bullet)CHR' \rightleftharpoons$ (*trans*) $RCH(OO\bullet)CH{=}CHR'$

4. If the peroxy radical contains a β-olefinic system these may interact to produce a molecule containing both cyclic peroxide and hydroperoxide functions (section 7.2.4). These structural features are present only in intermediates formed in the autoxidation of molecules with three or more double bonds. However, in photo-oxidation, they may also be formed from dienes (linoleate).

Table 7.1 Relative rates of autoxidation and photo-oxidation of oleate, linoleate and linolenate

Reaction	Oxygen	18:1	18:2	18:3
Autoxidation	triplet	1	27	77
Photo-oxidation	singlet	3×10^4	4×10^4	7×10^4
Ratio		30 000	1500	900

The results described in the previous paragraphs have been obtained from the study of bulk esters, i.e. samples containing only alkyl or glycerol esters of olefinic acids. The situation is different when the unsaturated compounds are physically organised in emulsions as in biological systems and in most foods.

The oxidative stability of polyunsaturated acids in bulk or in organic solvent is inversely proportional to the number of bis-allylic positions but reaction in aqueous systems does not follow preconceptions of oxidative stability based on studies in the bulk phase or in organic solvents. In aqueous micelles, stability increases with increasing unsaturation. In emulsions, lipid oxidation is greatly affected by the properties of the interface between emulsifier and lipid. In aqueous micelles of PUFA at 7ºC linoleic acid is the most susceptible to oxidation (50% lost after 13 hours), but EPA and DHA with five and six double bonds respectively are surprisingly stable. Even after 2000 hours between 80 per cent and 90 per cent of these two acids remain unoxidised. There is also a marked difference between ALA and GLA. Oxidative stability of polyunsaturated fatty acids in aqueous micelles rises with the number of bis-allylic centres. This unexpected result is closely related to the conformation of polyunsaturated fatty acids in aqueous micelles. Similar results are obtained with their ethyl and glyceryl esters where stability is in the order DHA > linolenic > linoleic. The monohydroperoxides of DHA in aqueous micelles are more stable than those from linolenic and linoleic esters and therefore, promote oxidation less readily. Similar results are observed with glycerol esters. Tuna triacylglycerols with an average number of 3.72 bis-allylic positions per triacylglycerol molecule are more stable than soybean triacylglycerols (1.94 bis-allylic groups) in water at 37ºC.

In the oxidation of olefinic lipids there is normally an induction period during which reaction is very slow and deterioration is not significant, followed by a quicker stage of undesirable oxidation. One purpose of antioxidants is to extend this induction period. Autoxidation can be inhibited by hindering the initiation process or by promoting the termination process thereby reducing the length of the propagation sequence. A more detailed account of the nature of the autoxidation products is given in section 7.2.4. Porter *et al*. (1995) have provided an excellent review.

7.2.3 Photo-oxidation

Because of the greater reactivity of singlet oxygen, photo-oxidation is a quicker process than autoxidation and there is less difference between the reactivity of monoenes and polyenes. Some typical figures are given in Table 7.1 from which it is apparent that photo-oxidation of linoleate is 1500 times quicker than autoxidation and that with oleate this ratio is 30 000.

Singlet oxygen can be formed by enzymic, chemical, and photochemical pathways. In the present context, singlet oxygen is formed from ordinary

$$cis\ \ RCH{=}CHCH_2R' + {}^1O_2 \rightarrow trans\ RCH(OOH)CH{=}CHR'$$

Figure 7.4 Reaction of olefin with singlet oxygen to give allylic hydroperoxides with double bonds in different position and of changed configuration.

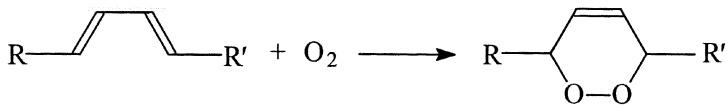


Figure 7.5 1,4-Cycloaddition reaction of conjugated diene with singlet oxygen to give an endoperoxide (1,4-cycloadition).

triplet oxygen and light in the presence of a sensitiser such as chlorophyll, riboflavin, myoglobin, erythrosine, rose bengal or methylene blue. The sensitiser absorbs energy from a photon and this energy is eventually passed to oxygen, converting it from the triplet to the singlet state. Singlet oxygen reacts with double bonds by an ene reaction to give an allylic hydroperoxide and with conjugated dienes to give endoperoxides (Figs 7.4 and 7.5).

Despite some similarities, photo-oxidation and autoxidation show some important differences. Photo-oxidation:

- is an ene reaction between electrophilic singlet oxygen and an electron-rich double bond whereas autoxidation is a radical chain reaction,
- displays no induction period in contrast to autoxidation,
- is unaffected by the antioxidants used to inhibit autoxidation but is inhibited by singlet oxygen quenchers such as carotene,
- is a reaction occurring at olefinic carbon atoms accompanied by double bond migration so that the product is an allylic hydroperoxide with *trans* configuration (section 7.2.4),
- gives reaction products that are similar to, but not identical with, those resulting from autoxidation. The distinct hydroperoxides can furnish short-chain aldeydes with their own characteristic flavours and odours,
- is a quicker reaction than autoxidation, especially for monoene esters (Table 7.1), and is related to the number of olefinic centres rather than to the number of 1,4-pentadiene units,
- gives hydroperoxides, which once formed, promote the alternative autoxidation route.

7.2.4 Hydroperoxide structures

The more important hydroperoxides produced from oleate, linoleate and linolenate and some of their breakdown products are listed in Tables 7.2 and 7.3.

Table 7.2 The major hydroperoxides (%) produced from oleate, linoleate and linolenate during autoxidation and photo-oxidation

Ester	Hydro-peroxide	Double bonds	Auto-oxidation[a]	Photo-oxidation[a]
Oleate	8	9 (*c*/*t*)	27	–
	9	10 (*t*)	24[b]	50[c]
	10	8 (*t*)	23[b]	50[c]
	11	9 (*c*/*t*)	26	–
Linoleate	9	10*t*12*c*[†]	52	32
	10	8*t*12*c*	–	17
	12	9*c*13*t*	–	17
	13	9*c*1t*t*[†]	48	34
Linolenate	9	10,12,15[†]	32	23
	10	8,12,15*	–	13
	12	9,13,15*	11	12
	13	9,11,15*	11	14
	15	9,12,16*	–	13
	16	9,12,14[†]	46	25

[a] Yields (%) are based on monohydroperoxides only; unoxidised and more extensively oxidised material may also be present (see text). Yields vary with reaction parameters such as temperature.
[b] Mainly *trans.*
[c] Entirely *trans*.
[†] Contain conjugated diene systems. These have *cis, trans* configuration when formed under kinetic control but change to *trans, trans* isomers under thermodynamic control.
* Can form a cyclic peroxide (see text).

7.2.4.1 Methyl oleate (Fig. 7.6)

Photo-oxidation of methyl oleate gives a 1:1 mixture of two products (9-OOH 10*t* and 10-OOH 8*t*). Reaction is confined to the olefinic carbon atoms and the double bond migrates and assumes a *trans* configuration. Autoxidation produces a more complex mixture of products. This results from the fact that the two alkyl radicals first produced are themselves resonance stabilised (Fig. 7.6) so that the hydroperoxide function may be attached to C-8, C-9, C-10 or C-11 in approximately equal amounts. Additionally the double bond may have *cis* or *trans* configuration as indicated in Table 7.2. For analytical and structural studies, the hydroperoxide group is usually reduced by triphenylphosphine to produce more stable hydroxy esters for chromatographic and spectroscopic examination.

The most important of the short-chain compounds resulting from the oleate hydroperoxides are aldehydes (8:0, 9:0, 10:1 and 11:1) and ω-oxo esters (8:0, 9:0, 10:1 and 11:1) (section 7.2.5).

Pure methyl oleate is oxidised slowly only after a long induction period (Table 7.1) but the situation is different with oils having oleic acid since these

Table 7.3 Short-chain compounds resulting from breakdown of the hydroperoxides of oleate, linoleate, and linolenate. Most, if not all, of these compounds have been observed

Compound	Oleate	Linoleate	Linolenate
Aldehydes	8:0	6:0	3:0
	9:0	7:1 (2*t*)	4:1 (2*t*)
	10:0	9:1 (3*c*)	6:1 (3*c*)
	10:1 (2*t*)	10:2 (2*t*4*c*)	7:2 (2*t*4*c*)
	11:1 (2*t*)		9:2 (3*c*6*c*)
			10:3 (2*t*4*c*7*c*)
ω-oxo esters	8:0	9:0	9:0
	9:0	10:1 (8)	10:1 (8)
	10:0	12:1 (9)	12:1 (9)
	10:1 (8)	13:2 (9,11)	13:2 (9,11)
	11:1 (9)		15:2 (9,12)
			16:3 (9,12,14)
Hydrocarbons	7:0	5:0	2:0
	8:0	8:1 (2)	5:1 (2)
			8:2 (2,5)
Alcohols	7:0	5:0	2:0
	8:0	8:1 (2)	5:1 (2)
			8:2 (2,5)
Methyl esters	7:0	8:0	8:0
	8:0	11:1 (9)	11:1 (9)
			14:2 (9,12)

probably contain pro-oxidants and antioxidants along with linoleic acid which oxidises faster to hydroperoxides whose breakdown can promote autoxidation of oleate.

7.2.4.2 Methyl linoleate

Photo-oxidation of methyl linoleate gives four major hydroperoxides through reaction at each of the four olefinic carbon atoms accompanied by double bond migration and change of configuration from *cis* to *trans* (Table 7.2). The hydroperoxide may be attached to carbon 9, 10, 12 or 13. The 9- and 13-hydroperoxides are described as 'outer', their diene system is conjugated, they represent a higher proportion of total hydroperoxides, and they are identical with products formed from autoxidation. The 10- and 12-hydroperoxides are described as 'inner', their diene system is not conjugated, represent a lower proportion of the total hydroperoxides, and such compounds are formed by photo-oxidation only and not by autoxidation. Only the inner hydroperoxides contain a β-hydroxy alkene system and are therefore capable of conversion to

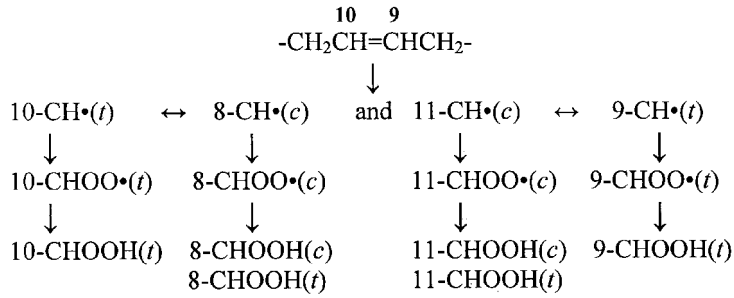


Figure 7.6 Autoxidation of methyl oleate via alkyl and peroxy radicals to hydroperoxides. The symbols *c* and *t* indicate the configuration of the double bond. These are products formed under kinetic control when there is an adequate supply of hydrogen donor to convert peroxy radical to hydroperoxide. The hydrogen donor may be oleate itself or an antioxidant. Not shown in the above scheme is the conversion of 10-CHOO•(*t*) to 8-CHOOH(*t*) and of 9-CHOO•(*t*) to 11-CHOOH(*t*) formed via reversion of peroxy radical to alkyl radical. These are significant when there is only a limited supply of hydrogen donor and are produced under thermodynamic control.

hydroperoxy peroxides. This may account for the lower levels of inner hydroperoxides (Fig. 7.7).

Autoxidation of methyl linoleate occurs 20–40 times faster than the reaction with oleate because linoleate contains a doubly-activated methylene function at C-11 from which it is easier to remove a hydrogen atom (section 7.2.2). In contrast, oleate contains two methylene groups each activated by one double bond but has no doubly activated methylene groups. Linolenate contains two such CH_2 groups (C-11 and C-14) and is autoxidised about twice

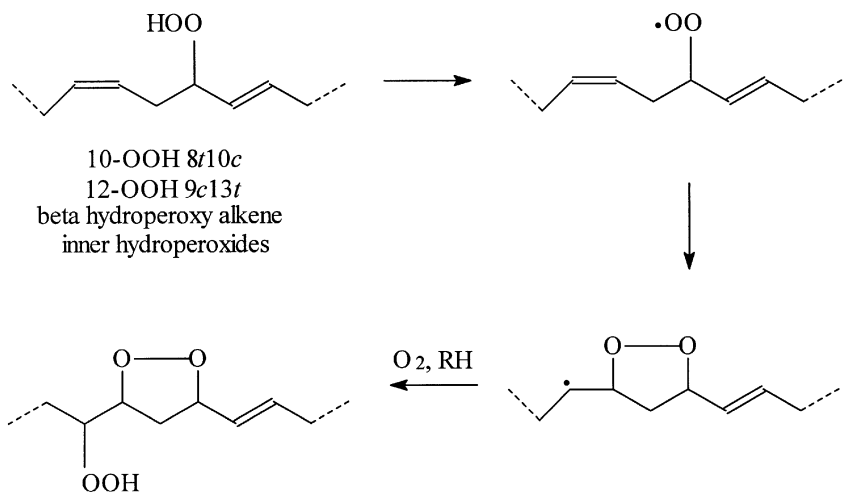


Figure 7.7 Conversion of inner HPO to hydroperoxy peroxides.

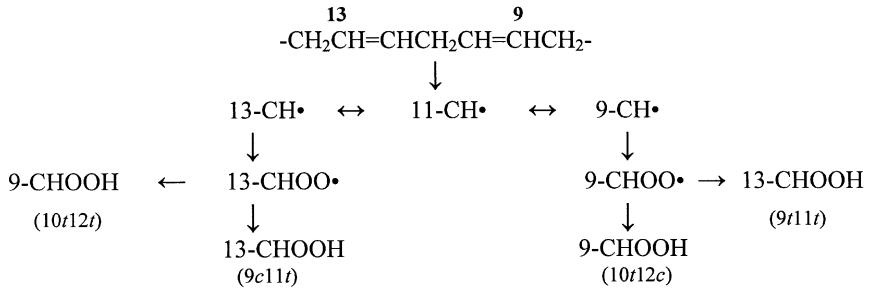


Figure 7.8 Autoxidation of methyl linoleate via alkyl and peroxy radicals to the 9-and 13-hydroperoxides (see discussion in text for conversion of *cis,trans* to *trans, trans* forms).

as quickly as linoleate. This relationship is continued in the more highly-unsaturated polyunsaturated fatty acids such as arachidonic acid (20:4), eicosapentaenoic acid (20:5) and docosahexaenoic acid (22:6). The relative rate of autoxidation of these in bulk is related to the number of such CH_2 groups. In contrast, non methylene-interrupted dienes which do not have doubly activated methylene groups autoxidise more slowly like oleate. It has already been shown (section 7.2.2) that these generalisations do not apply when the acids/esters are organised in emulsions.

Autoxidation of methyl linoleate first gives two products (9-OOH 10*t*12*c*-18:2 and 13-OOH 9*c*11*t*-18:2) both of which have conjugated diene systems. These are the products formed under kinetic control when there is an adequate source of hydrogen donors from linoleate or antioxidant. Under appropriate conditions these initial products are converted to their *trans*, *trans* isomers which are the products of thermodynamic control. The reaction is such that the *cis, trans* 9-hydroperoxide gives the *trans*, *trans* 13-hydroperoxide and the 13 hydroperoxide is converted to its 9 isomer. This depends on the fate of the initially-formed peroxy radicals (Fig. 7.8).

If the 9- and 13-peroxy radicals are not quickly converted to hydroperoxides they undergo β-cleavage regenerating the alkyl radicals which change from *cis, trans* to *trans*, *trans* conformers. These are the precursors of the 13- and 9- *trans*, *trans* hydroperoxides.

All these hydroperoxides break down to the short-chain compounds indicated in Table 7.3. The most important are the volatile aldehydes, hexanal from the 13-hydroperoxide and decadienal from the 9-hydroperoxide. The hydrocarbon pentane, also formed from the 13-hydroperoxide, can be detected in exhaled breath and is used as a measure of biological oxidation of linoleate and other n-6 acids.

7.2.4.3 Methyl linolenate

Photo-oxidation of methyl linolenate gives six hydroperoxides with the hydroperoxy group attached to the olefinic carbon atoms C-9, C-10, C-12,

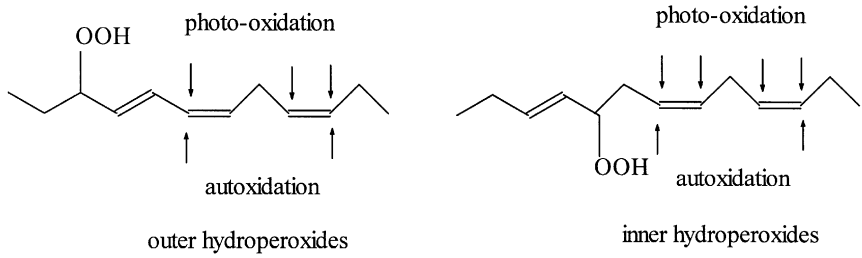


Figure 7.9 The possibilities of further oxidation of outer and inner linolenate hydroperoxides by autoxidation and by photo-oxidation.

C-13, C-15, or C-16 and one double bond moved in position and changed in configuration. Only four of these have conjugated unsaturation and a different four can form hydroperoxy peroxides. The two outer hydroperoxides are present in larger amounts than the four inner hydroperoxides (Table 7.2).

Autoxidation of linolenate is best considered in terms of the two pentadiene units (9,12 and 12,15) present in this molecule. The former gives 9- and 13-hydroperoxides and the latter gives 12- and 16-hydroperoxides. Some of these products still contain a pentadiene unit and can be oxidised further to give bis-hydroperoxides either by photo-oxidation or by autoxidation as indicated in Fig. 7.9.

The volatile aldehydes produced from linolenate (and other n-3 acids) include propanal (3:0), hexenal (6:1), nonadienal (9:2) and decatrienal (10:3) from the 16-, 13-, 10- and 9- hydroperoxy products, respectively. The aldehydes from linolenate have lower sensory threshold values than those from linoleate and this, combined with the faster oxidation of linolenate, accounts for the quicker development of rancidity in foods containing n-3 polyunsaturated fatty acids.

2-Pent-2′-enyl- and 2-pentyl-furan have been identified among the oxidation products of soybean oil. These result from the 10-hydroperoxy compounds from linolenate and linoleate respectively which are products of photo-oxidation but not of autoxidation. The reaction with linoleate is formulated below. This reaction follows a similar pathway with the $\Delta 15$ double bond unaffected and remaining unchanged in the unsaturated C_5 side chain (Fig. 7.10).

Typically the major odour-producing compounds formed in crude herring oil include 1-pent-3-enone, hexanal, 4*c*-heptenal, 2*t*,4*t*-heptadienal, 2*t*,6*c*-nonadienal, and nonanal resulting from n-3, n-6 and n-9 acyl chains. Key odorants formed by autoxidation of arachidonic acid (n-6 20:4) are hexanal, 1-octen-3-one, 2*t*,4*c*- and 2*t*,4*t*-decadienal, *trans*-4.5-epoxy-2*t*-decenal and 2*t*,4*c*,7*c*-tridecatrienal.

7.2.4.4 Cholesterol

The possible link between cholesterol-oxidation products and coronary heart disease and other disease states, makes it appropriate to discuss the source and

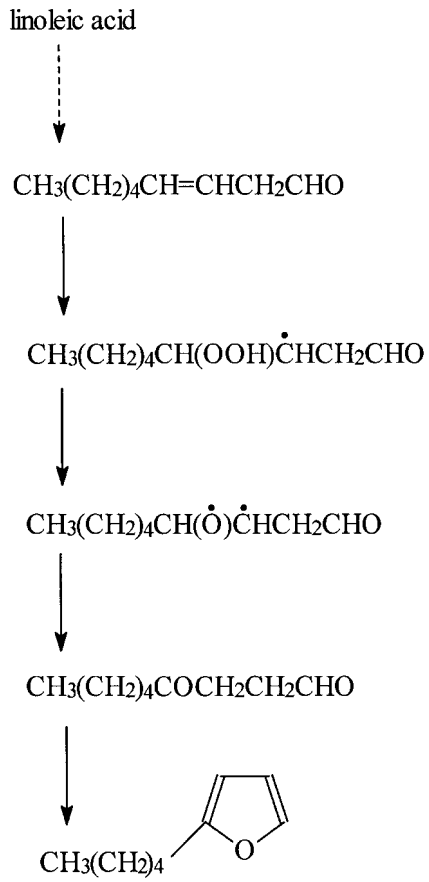
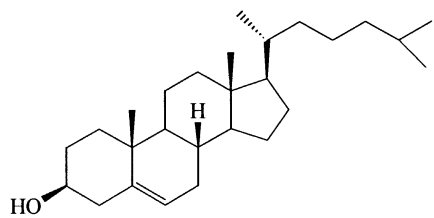


Figure 7.10 The formation of 2-pentylfuran from linoleic acid via the 10-hydroperoxide produced by photo-oxidation and the nonenal formed from this.

formation of such compounds. Cholesterol, with the structure shown, contains a cyclic double bond



(Δ5) and some tertiary carbon atoms in its side chain (C-20 and C-25): all sites where oxidation may occur. Cholesterol oxides are produced as part of

the normal metabolism of cholesterol but at higher levels they affect human health by contributing to the development of atherosclerosis. When cholesterol oxides replace cholesterol in the cell membrane they alter its fluidity, permeability, stability and other properties.

Oxidised animal-based foods represent a primary source of oxidised cholesterol. Such products are not present in fresh foods but are formed during handling prior to consumption, mainly through autoxidation. Between 0.5 and one per cent of dietary cholesterol may be oxidised and the levels increase with unsaturation of associated phospholipids. The primary oxidation products include 7-α-hydroxy-, 7-β-hydroxy, and 7-keto-cholesterol, cholesterol α- and β-epoxides, 3,5,6-trihydroxycholesterol and 20- and 25-hydroxycholesterol (Cuppett, 2003).

7.2.5 Decomposition of hydroperoxides to short-chain products

The hydroperoxides formed by the oxidation processes are not directly responsible for the off-flavours and odours associated with rancidity. They are however, unstable compounds readily decomposing to volatile short-chain molecules which may be aldehydes, ketones, alcohols, acids, esters, lactones, ethers and hydrocarbons. The resulting flavours are sometimes considered as desirable, as in many cooked foods, but on other occasions are described as off-flavours and associated with deterioration of the food product. Rancidity depends on the blend of short-chain compounds, on their concentration, and to some extent on cultural acceptance. These compounds vary markedly in their flavour threshold values and the levels of short-chain aldehydes are particularly significant.

The major breakdown pathway is related to the weakness of the RO-OH bond with an activation energy of only 44kcal/mol producing hydroxy (HO•) and alkoxy (RO•) radicals. The latter may cleave in two ways, giving aldehydes and an alkyl radical which is itself converted to a hydrocarbon or an alcohol. Some of these products still retain a double bond (with *cis* or *trans* configuration) and while some are volatile, others are still attached to the remainder of the glycerol ester and are described as core aldehydes (Kuksis, 2000; Sjovall *et al*., 2002) (Figs 7.11 and 7.12).

Many of these short-chain compounds have very low flavour threshold values and display an olfactory effect at very low concentrations. For example, the 9-hydroperoxide from linoleate gives 2,4-decadienal with a deep fried flavour at a concentration of 5×10^{-10} molar, equivalent to only 0.5 ppb. Many short-chain breakdown products have been identified (Tables 7.3 and 7.4). They result from the large number of hydroperoxides which can be formed, especially from a partially hydrogenated oil with many olefinic compounds, from the large number of decomposition pathways, and from further oxidation of the short-chain compounds themselves. For example,

Table 7.4 Flavour-threshold values of some aldehydes and their sources from oxidised fats

Aldehyde	Threshold value (ppb)	Hydroperoxide source
6:1 (3)	90	18:3 (13-HPO)
7:1 (4*c*)	0.5–1.6	cod muscle
7:2 (2*t*4*c*)	55	18:3 (12-HPO)
9:1 (6*t*)	0.3	18:2 (9,15) (10-HPO)
9:2 (3*t*6*c*)	1.5	18:3 (10-HPO)
9:2 (2*t*6*c*)	2	18:3 (10-HPO)
10:3 (2*t*4*c*7*c*)	150	18:3 (9-HPO)

Also 1,5*c*-octadien-3-one (0.02 ppb) from butter fat.

polyfunctional molecules, such as the very reactive C_8 and C_9 hydroperoxy-2-enals formulated below, are formed in aqueous solution under physiological conditions.

$CH_3(CH_2)_nCH(OOH)CH{=}CHCHO$ where n = 3 or 4

Core aldehydes based on triacylglycerols, phospholipids and sterol esters have been identified and can be produced in the laboratory by reaction of appropriate olefinic compounds with tert butylhydroperoxide in the presence of ferrous sulfate (Sjovall *et al*., 2002). In these products one or more of the unsaturated acyl groups is oxidised to a short-chain unit containing an aldehyde function such as the C_9 and C_{12} compounds shown below as well as epoxy aldehydes. The fate of these reactive molecules *in vivo* is not fully known, but it has been demonstrated that the aldehydes react readily with NH_2

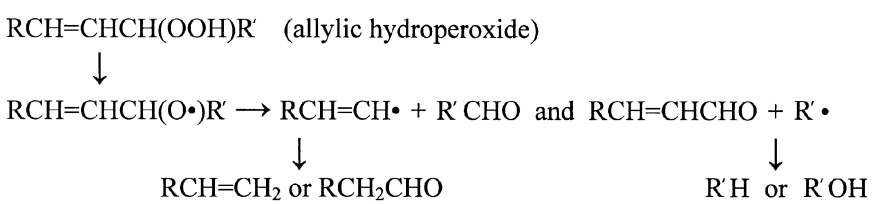


Figure 7.11 Homolytic breakdown of allylic hydroperoxides to short-chain compounds. The alkyl radicals are converted to hydrocarbons or alcohols by reaction with H• or HO•. The aldehyde on the last line is a 2-enol written in 'keto' form.

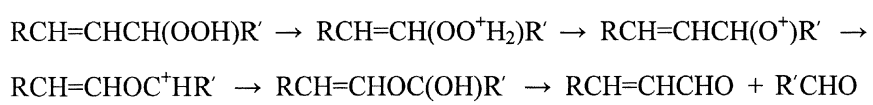


Figure 7.12 Acid catalysed heterolytic breakdown of allylic hydroperoxides to short-chain aldehydes via a hemiacetal.

groups in phospholipids, amino acids, or polypeptides with consequent modification of the properties of these important biological molecules.

$OHC(CH_2)_7COX$ and $OHCCH{=}CHCH(OH)(CH_2)_7COX$

where X represents the remainder of the triacylglycerol, phospholipid or sterol ester molecule.

7.2.6 Other secondary reaction products

Hydroperoxides are readily converted to a range of compounds of the same chain length by reaction with water or other solvents. These include alcohols, ketones, epoxides, triols, and keto compounds which may also contain OH, OMe, SEt, and OCOR groups arising from water or other reactants (Fig. 7.13).

The oxygenated molecules also form dimers and oligomers linked through C-O-O-C, C-O-C or C-C bridges. They can be detected by gel permeation chromatography (section 5.3.3) and have been found in processed fish oil capsules at levels around two per cent. Higher levels (30–40%) have been observed in oils oxidised at 35ºC in air and light.

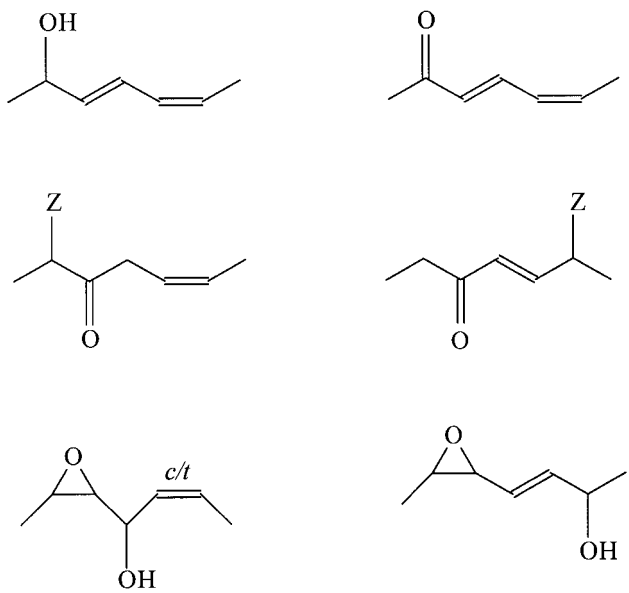


Figure 7.13 Partial structures of secondary oxidation products from linoleate hydroperoxides including hydroxy and keto dienes, α- and γ-ketols in which Z = OH, OMe, SEt or OCOR and hydroxy epoxides along with the related trihydroxy compounds.

7.2.7 Antioxidants – introduction

Antioxidants play an important role in the preservation of foods and other materials that deteriorate through oxidative changes. This discussion is concerned with compounds already present or those added to foods to extend their usable lifetime by inhibiting lipid oxidation. Their presence in biological systems is also important because several diseases or conditions are related to the activity of reactive intermediates formed during the oxidation process. Oxidative deterioration is a chemical process that can be inhibited by removal of reactants (oxygen present in air), by removal of compounds and conditions that promote the reaction (light, metal ions, temperature) and by addition of compounds that slow down (inhibit) the oxidation process. As already indicated (section 7.2.5), rancidity is linked to the appearance of short-chain compounds (mainly aldehydes) which are formed as a consequence of several reactions. Antioxidants may therefore act at one or more stages in the total process including:

- initiation of autoxidation,
- propagation of the autoxidation process,
- formation of singlet oxygen,
- breakdown of hydroperoxides to short-chain compounds with undesirable odour and flavour,
- stages at which hydroperoxides or peroxy radicals rearrange to give isomeric products that furnish different breakdown products and so influence flavour.

Antioxidants can be classified according to which of the above stages are influenced and also on whether they are considered to be natural or synthetic. The synthetic compounds are cheaper and often more effective, but there is a growing demand for natural compounds. However, the supply of natural antioxidants is not sufficient to meet the total demand for antioxidants for food, feed, cosmetics, and pharmaceuticals. Much of the large and growing food industry would not be possible without antioxidants of some kind.

The synthetic compounds that can be used are strictly controlled as is the level at which they may be used. The matter is complicated in that not all countries have agreed to the same list of acceptable compounds. This becomes important for materials that are to be traded between countries having different permitted antioxidants. Obviously the antioxidants must be non-toxic and that must apply also to the final products produced from them as a result of their antioxidant activity (section 7.2.8).

Vegetable oils generally contain antioxidants. Nature provides its own safeguards to accompany the polyunsaturated fatty acids. Attention has to be given to what happens to these during the refining processes (section 4.2). How much is removed and how much is retained in the refined oil? Some of the

antioxidants that are removed are trapped in the deodoriser distillate and this provides a valuable starting point for the recovery of these materials (section 1.6).

In comparing antioxidant activity several factors have to be considered.

1. Effects vary with different oils and fats because of their varying fatty acid composition and the differing levels of antioxidants already present.
2. Results obtained at different temperatures may not be directly comparable because mechanisms of hydroperoxide formation and breakdown change with temperature, as does the volatility of the antioxidants.
3. Results vary with the method of assessment: some measure primary products (hydroperoxides) and others measure secondary products (carbonyl compounds and/or volatile compounds).
4. Mixtures of antioxidants are influenced by synergistic effects and it is difficult to disentangle these.
5. Solubility factors have to be considered, especially when there is a distribution between aqueous and lipid phases.

7.2.8 Primary and secondary antioxidants

Primary antioxidants, also described as chain-breaking, are radical acceptors or radical scavengers. They affect the initiation stage by trapping alkyl (R•) radicals and the propagation sequence by trapping the peroxy radicals (ROO•). They do this either by having a readily available hydrogen atom (as in phenols and amines) or by reacting additively with the radical (as with highly unsaturated molecules such as carotene). In both cases, the final products are stable enough not to be involved in further oxidation processes. The induction period is a time in which radicals are being generated and antioxidants consumed.

$$ROO\bullet + AH \rightarrow ROOH + A\bullet \ldots \rightarrow \text{products}$$
$$ROO\bullet + B \rightarrow ROOB\bullet \ldots \rightarrow \text{products}$$

AH = amines or phenols B = polyunsaturated compounds such as β-carotene.

Antioxidants behave in a sacrificial manner and the induction period ends when all the antioxidant is exhausted. Some antioxidants are able to stop two or more propagation sequences, because the products first formed from the antioxidants still have antioxidant activity. Sometimes it is possible to incorporate something into the antioxidant package that will regenerate the active antioxidant and so extend its period of activity. A common example is the addition of palmityl ascorbate to vitamin E (section 7.2.9). The spent antioxidants are mainly quinones or dimers with C-C or C-O links.

Secondary antioxidants are mainly metal chelators such as ethylene diamine tetra-acetic acid (EDTA), citric acid, phosphoric acid and certain amino acids. These remove the metal ions (mainly iron and copper) that

promote the initiation step in autoxidation. They are often used along with primary antioxidants.

Antioxidants do not prevent oxidation but they slow it down, thereby extending the induction period and hence the shelf-life of fat-containing foods. Since autoxidation is an autocatalytic process, it is important to add the antioxidant as soon as possible after extraction. It is also desirable to avoid as far as possible those conditions of heat, light and access to air that promote oxidation. Photo-oxidation is not inhibited by the antioxidants used for auto-xidation but by singlet oxygen quenchers of which the best known is β-carotene.

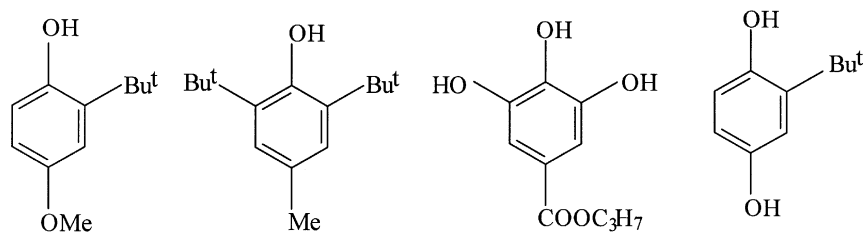
7.2.9 Synthetic and natural antioxidants

Four important synthetic antioxidants with structures and E-numbers are shown in Fig. 7.14. They are solid compounds and may be conveniently used as solutions in propylene glycol, monoacylglycerols or vegetable oils. The synthetic antioxidants are mono or dihydric phenols and react with a peroxy radical to give a phenoxy radical (ArO•), stabilised by extensive delocalisation of the odd electron over the aromatic system (Fig. 7.14).

Butylated hydroxyanisole (BHA) shows good solubility in fat and reasonable stability in fried and baked products. It is very effective with animal fats but less so with vegetable oils. It shows marked synergism with butylated hydroxytoluene and propyl gallate and can be used at a maximum level of 200 ppm.

Butylated hydroxytoluene (BHT) is less soluble than BHA and is not soluble in the propylene glycol frequently used as a solvent for antioxidants. It is synergistic with BHA but not with propyl gallate and can be used to a maximum level of 200 ppm.

Propyl gallate (PG) is less soluble than BHA or BHT. It does not generally survive cooking as it decomposes at 148ºC. Nevertheless, it is effective when used with BHA and may be used up to 100 ppm.



BHA (E320) BHT (E321) PG (E310) TBHQ (no E number)

Figure 7.14 The structures and E-numbers of synthetic antioxidants. TBHQ has no E number because it is not a permitted antioxidant in EU-15.

Tertbutyl hydroquinone (TBHQ) is acceptable in the United States and many other countries, but not in EU-15. It is very effective with vegetable oils, has good solubility and is stable at high temperatures. It is frequently used for oil transport and storage and is completely removed during deodorisation.

The best-known and most widely used natural antioxidants are the tocols (tocopherols and tocotrienols) which are widely distributed in plant products but not in those which are animal-derived. There are eight natural tocols – four tocopherols with a phytyl side chain and differing from one another in the number and disposition of methyl groups and four tocotrienols which are similar to the corresponding tocopherols but have a tri-unsaturated side chain (for structural details see section 1.6.4). The levels of these in a range of crude vegetable oils are given in Table 7.5. Natural tocol mixtures are usually used at levels up to 500 ppm along with ascorbyl palmitate (200–500 ppm) which has a sparing activity on vitamin E. Above around 1000 ppm α-tocopherol acts as a pro-oxidant. Since most vegetable oils already contain tocols at levels of 200–800 ppm further addition shows little effect. In contrast, the oxidative stability of lard, with little or no natural antioxidant, is markedly enhanced with tocopherol. Lard has an induction period of only 2.5 hours when heated to 100ºC with blown air but this is extended to 18 hours with added tocopherol (0.01%).

The various tocols differ in their vitamin E activity and total activity is often presented as α-tocopherol equivalents (mg α-tocopherol/mg compound) based on equivalence values for tocopherols: α (1.0), β (0.5), γ (0.1) and δ (0.03), and tocotrienols: α (0.3) and β (0.05).

Table 7.5 Vitamin E content (mg/100g) of some vegetable oils and of butter and lard

	Tocopherols					Tocotrienols						
Oil	α	β	γ	δ	total	α	β	γ	δ	total	grand total	IU
Soybean	10	–	59	26	96					0	96	24
Corn	11	5	60	2	78					0	78	20
Rapeseed	17		35	1	53					0	53	30
Sunflower	49		5	1	55					0	55	73
Groundnut	13		22	2	37					0	37	23
Cottonseed	39		39	78						0	78	64
Safflower	37		17	24	80					0	80	61
Palm	26		32	7	65	14	3	29	7	53	118	49
Coconut	tr		tr		1	tr		2	tr	3	4	1
Olive	20	1	1		22					0	22	30
Wheat germ	121	65	24	25	235	2	17			19	254	233
Rice	12	4	5		21	18	2	57		77	98	30
Butter	2			2							2	3
Lard	1			1	1					1	2	2

Adapted from Stone & Papas in Gunstone (2003).

Some plants have other natural antioxidants in their leaves or seeds. Familiar examples include oat oil with α-tocopherol, α-tocotrienol and avenathramides, sesame oil with sesamin, sesamolin, sesaminol, all of which are derivatives of sesamol (3,4-methylenedioxyphenol), and ricebran oil with tocotrienols, avenasterols and oryzanols which are sterol esters of ferulic acid.

4-OH-3-OMeC_6H_3CH=CHCOOH ferulic acid

Antioxidants are also present in herbs and spices and while these can sometimes be used as extracts, their food use is limited by their strong flavours which may or may not be acceptable in other foods. Tea leaves are a rich source of antioxidants (catechins) as are many fruits and vegetables containing flavonoids. Dietary consumption of these as whole foods provides a good source of the antioxidants required by the body to counter oxidative damage to protein and to DNA mediated through radicals produced through lipid oxidation. This applies also to vegetables containing carotenes. Rosemary leaves contain some powerful antioxidants such as carnosic acid, carnosol and rosmarinic acid and rosemary extracts are available for use as antioxidants.

Vitamin C (ascorbic acid) acts as an oxygen scavenger, removing traces of residual oxygen in a packed and sealed product. It is water-soluble but can be used in a lipid-soluble form as ascorbyl palmitate.

Phospholipids show ill-defined antioxidant activity possibly through activity as a chelating agent and/or emulsifier.

7.3 Biological oxidation

7.3.1 α-Oxidation

α-Oxidation provides routes to α-hydroxy acids and to acids with one less carbon atom (*nor*-acids) both of which are produced via α-hydroperoxyacids thus:

$$\begin{array}{l} RCH_2COOH \rightarrow RCH(OOH)COOH \rightarrow RCHO \rightarrow RCOOH \\ \qquad\qquad\qquad\quad \downarrow \\ \qquad\qquad\qquad\quad RCH(OH)COOH \end{array}$$

α-Hydroxy acids are present in sphingolipids and in wool wax and sometimes in seed oils. For example, the seed oil from *Salvia nilotica* contains oleic, linoleic and linolenic acids, the corresponding C_{18} α-hydroxy acids and three unsaturated C_{17} homologues.

$$18{:}2(9c12c) \xrightarrow{a} 12{:}2(3c6c) \xrightarrow{b} 12{:}2(2t6c) \xrightarrow{a} 10{:}1(4c) \xrightarrow{c} 10{:}2(2t4c) \xrightarrow{d} 10{:}1(3t)$$

$$\xrightarrow{b} 10{:}1(2t) \xrightarrow{a} 2{:}0$$

Figure 7.15 β-Oxidation of linoleic acid as its CoA ester. a = β-oxidation (several cycles), b = enoyl-CoA isomerase, c = acyl-CoA dehydrogenase, d = 2,4-dienoyl-CoA reductase.

7.3.2 β-Oxidation

β-Oxidation is the major pathway by which by fatty acids are degraded, two carbon atoms at a time, to acetate that is finally metabolised to carbon dioxide and water. Energy liberated in this process is trapped as ATP for use in biological systems. The saturated acid RCH_2CH_2COOH is degraded to RCOOH via the reaction sequence:

$$RCH_2CH_2COOH \rightarrow (\textit{trans})\ RCH{=}CHCOOH \rightarrow RCHOHCH_2COOH \rightarrow RCOCH_2COOH \rightarrow RCOOH + CH_3COOH$$

With unsaturated acids, other enzymes are also involved as in the sequence for linoleic acid, shown in Fig. 7.15.

7.3.3 ω-Oxidation

Long-chain acids are sometimes oxidised at, or close to the methyl end of the carbon chain. In ω-oxidation, the product is a dibasic acid produced via the ω-hydroxy acid. Once formed the dibasic acid may be subject to β-oxidation at either or both ends of the molecule. Attempts are being made to produce dibasic acids on an industrial scale by ω-oxidation of appropriate fatty acids with enzyme preparations.

$$CH_3(CH_2)_nCOOH \rightarrow HOCH_2(CH_2)_nCOOH \rightarrow OHC(CH_2)_nCOOH \rightarrow HOOC(CH_2)_nCOOH$$

7.3.4 Lipoxygenase

Lipoxygenases which occur widely in plant and animal systems are non-haeme iron proteins. In plants they promote oxidation of C_{18} acids – particularly linoleic and linolenic. For both substrates, reaction is initiated at C-11 and involves removal stereospecifically of one of the two hydrogen atoms. Removal of one C-11 hydrogen atom from linoleic acid leads to the 9(*S*)-OOH 10*t*12*c*-18:2 and removal of the other C-11 hydrogen atom leads to 13(*S*)-OOH 9*c*11*t*-18:2. The fatty acids are probably the preferred substrate, but reactions can occur with phospholipids and triacylglycerols.

$$R_2CH_2 \rightarrow R_2CH\bullet \rightarrow R_2CHOO\bullet \rightarrow R_2CHOO^- \rightarrow R_2CHOOH$$

Figure 7.16 Enzymic oxidation of polyunsaturated fatty acids. CH_2 represents a doubly allylic methylene function.

Some plant lipoxygenases give a single product, others give a mixture of two. Enzymic reactions using commercial soybean lipoxygenase have been applied routinely in the laboratory to prepare pure stereospecific hydroperoxides. Linolenic acid gives 9- and 13-hydroperoxides with an additional 15*c* double bond. Some products from linolenic acid still contain a 1,4-pentadiene unit and can be oxidised a second time. All the hydroperoxides act as precursors for a range of other products.

Enzymic hydroperoxidation occurs through a peroxy radical and a peroxy anion as shown in Fig. 7.16. In the production of the alkyl radical, Fe^{3+} is reduced to Fe^{2+} and in the conversion of peroxy radical to peroxy anion, Fe^{2+} is oxidised to Fe^{3+}.

Lipoxygenase-derived products from linoleic and linolenic acids form substrates for several different enzyme families leading to over 150 different fatty acid derivatives Under the influence of peroxygenases, the hydroperoxides are converted to epoxy and dihydroxy acids. Another enzyme (allene oxide synthase, AOS) yields allene oxides (Fig. 7.17). These are reactive compounds leading to a wide range of compounds including ketols, cyclopentanones, 12-oxophytodienoic acid (a precursor of the jasmonates), divinyl ethers (see colneleic and colnelenic acids in section 3.9.2), and short-chain aldehydes and oxo-acids. Many of these show physiological activities though these are not fully understood.

Plants are able to convert fatty acids to compounds which are toxic to certain pathogens or are able to act as signalling molecules (e.g. jasmonates). For example, through the action of lipoxygenase and other enzymes, the rice plant converts linoleic acid and linolenic acid to a range of C_{18} molecules containing hydroxy groups and/or epoxy groups. These compounds retain their unsaturation though double bonds may be changed in position and configuration.

There is an ALA cascade in plants which is the C_{18} equivalent of the arachidonic acid (C_{20}) cascade leading to eicosanoids in animals (section 7.3.5). The plant-derived products include jasmonates (with a cyclopentanone

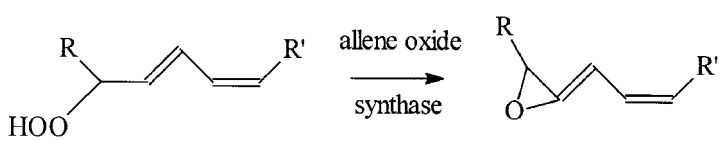


Figure 7.17 Reaction by which 13(*S*)-OOH 9*c*11*t*-18:2 is converted to the allene oxide 12,13(*S*)-epoxy-9*c*11-18:2.

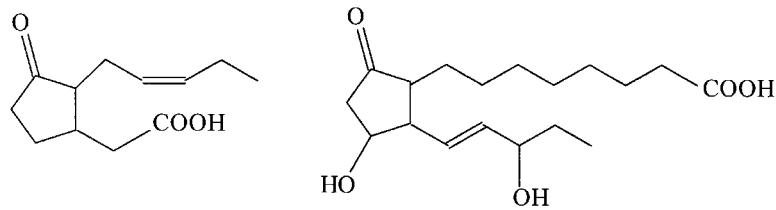


Figure 7.18 Structure of jasmonic acid (C_{12}) and phytoprostane E1 (C_{18}).

ring), phytoprostanes E1 (hydroxy keto cyclopentane ring) and phytoprostanes F_1 (dihydroxycyclopentane ring). Except jasmonic acid itself (C_{12}) the remaining compounds are C_{18} acids (Fig. 7.18). Jasmonates are essential for plant development and defence.

Microbial isolates specifically oxidise unsaturated acids. For example oleic acid has been oxidised to 10-hydroxy 8*t*-18:1 and 7,10-dihydroxy 8*t*-18:1, and linoleic acid to 12,13-dihydroxyoleate, 12,13,16 (and 17)-trihydroxyoleates, and to the 13,16-epoxide and the 12,17; 13,17-bisepoxide and their 7-hydroxy derivatives.

Other micro-organisms convert olefinic acids to 10-hydroxy (and oxo) acids by a specific hydration (not oxidation) of the double bond, for example, linoleic acid gives 10-hydroxy 12*c*-18:1.

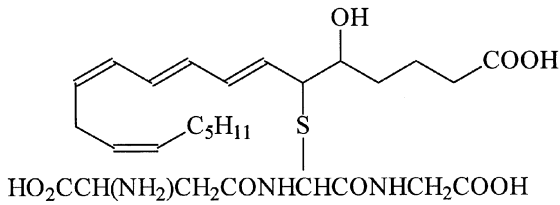
7.3.5 Production and function of eicosanoids

In animal systems, unsaturated C_{20} acids are the preferred substrates for enzyme-promoted oxidation. Arachidonic acid (20:4), liberated from phospholipid (particularly from phosphatidylinositols) by phospholipase A2, is the precursor of several important enzymatic oxidation products, though similar reactions occur with 5,8,11-20:3 and 5,8,11,14,17-20:5. These eicosanoids are produced in animal systems on demand, have very short life-cycles (seconds or minutes), and show important hormone-like properties. Examples include the prostaglandins produced from cyclic endoperoxides under the influence of cyclooxgenase and the leukotrienes produced from hydroperoxy fatty acids under the influence of lipoxygenase (Figs 7.19 and 7.20). These compounds are classed generally as eicosanoids showing their origin from C_{20} acids.

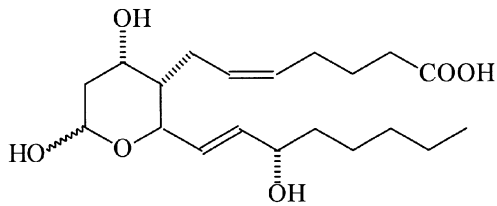
7.4 Other oxidation reactions

7.4.1 Epoxidation

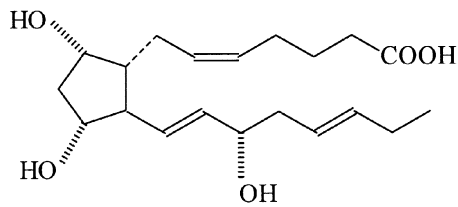
Epoxidation is the reaction by which olefinic compounds are converted into (three-membered) cyclic ethers called epoxides. Long-chain epoxy acids



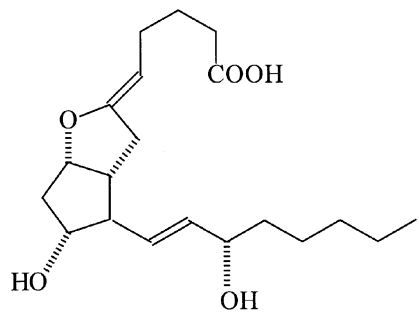
Leukotriene C4



TXB2 (thromboxane)



PGF2α (prostaglandin)



PGI2 (prostacyclin)

Figure 7.19 Structures of selected eicosanoids.

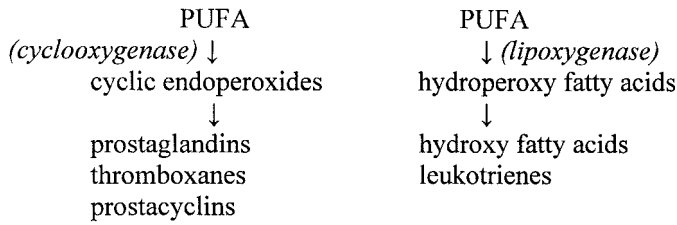


Figure 7.20 Biosynthetic pathways to the eicosanoids from 20:4 and also from 20:3 and 20:5.

occur naturally (section 3.9.2) and are formed through bio-oxidation, both as intermediates and as products (section 7.3.4). Other compounds containing a three membered ring are those in which the oxygen in replaced by S (epithio compounds), NH (aziridines) and CH_2 (cyclopropanes and cyclopropenes (section 3.8).

7.4.1.1 Preparation of epoxides

Epoxidation is an important reaction carried out in the laboratory and as an industrial process. Epoxidised vegetable oils, particularly soybean oil and linseed oil produced up to 200 000 tonnes a year, are used as plasticisers and stabilisers for PVC. Other uses of epoxy acids and esters, now being developed, are discussed later. The epoxidation of vegetable oils is probably the biggest single use of hydrogen peroxide (Fig. 7.21).

Epoxidation is most commonly effected by reaction of olefinic acid, alkyl ester, or glyceryl ester with an organic peroxy acid [RC(=O)OOH] which is generally prepared *in situ* by reaction of hydrogen peroxide with a carboxylic acid [RC(=O)OH] and, when necessary, an acidic catalyst. The carboxylic acid may be replaced by an anhydride or acid chloride. Industrial epoxidation is most often carried out with peroxyformic or peroxyacetic acids but other peroxy acids may be more convenient in the laboratory. These include the peroxy acids derived from trifluoroacetic, lauric, benzoic, and 3-chloro-benzoic acids, and monoperoxy acids derived from succinic, maleic, and phthalic anhydrides. Some of these can be safely stored at 0–20ºC and are convenient for small-scale reactions.

Epoxidation is exothermic and high concentrations of peroxy acid should always be avoided. The reaction is a *cis* addition process, so oleic acid gives *cis*-9,10-epoxy stearic acid and linoleic gives a mixture of two *cis, cis*

Figure 7.21 Conversion of an alkene to an epoxide with a peroxy acid (RCO_3H).

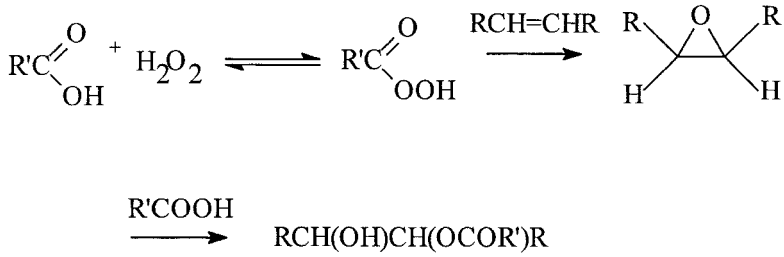


Figure 7.22 Epoxidation of an alkene by peroxy acid produced *in situ* and reaction of the epoxide with carboxylic acid.

diastereoisomeric bis- epoxides. The reaction is near-quantitative, but there is some loss of product through a ring-opening reaction between epoxide and carboxylic acid (see section 7.4.1.2). Peroxyformic acid results from an equilibrium reaction between formic acid and hydrogen peroxide. Peroxyacetic acid is formed similarly but requires an acidic catalyst such as sulfuric acid (Fig. 7.22). The relative rates of epoxidation for oleic, linoleic and linolenic acids are 1.0, 1.0 and 1.7, respectively. The triacylglycerols are slightly more reactive than the acids with values of 1.9, 3.0 and 4.9, respectively.

Other methods of preparing epoxides involve reaction with dimethyl dioxirane (DMDO), with methyltrioxorhenium (MTO, $MeReO_3$) or with tungstates. DMDO is made from potassium peroxymonosulfate ($KHSO_5$) and acetone. MTO reacts with hydrogen peroxide to give organorhenium peroxo complexes. This latter method shows promise as an alternative industrial epoxidation process.

Enzymatic procedures have also been explored. The lipase from *Candida antarctica,* available in immobilised form as Novozyme 435, with hydrogen peroxide solutions promotes conversion of acids or esters to peracids which then effect epoxidation of olefinic groups present in the reaction mixture. Sunflower, soybean, and linseed oils have been satisfactorily epoxidised by this route using 35 per cent hydrogen peroxide with 5 mol% of free fatty acid (Fig. 7.23).

7.4.1.2 Physical and chemical properties

Epoxides have characteristic ^{1}H and ^{13}C NMR spectra which can be used for both qualitative and quantitative analysis (Gunstone, website). Partly by reason of the steric strain in a three-membered ring, epoxides are reactive ethers and this reactivity is enhanced in acidic solution when the epoxide function is protonated. Whether protonated or not, epoxides react with nucleophiles to give ring-opened bifunctional molecules. If the nucleophile is itself difunctional, then more complex products of higher molecular weight may be produced. Typical reagents include water, alcohols, carboxylic acids

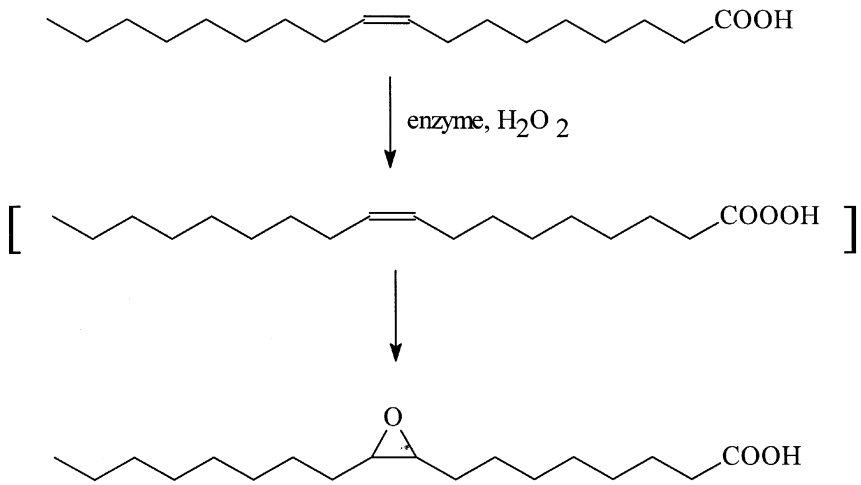


Figure 7.23 Enzymatic epoxidation of olefinic acids with hydrogen peroxide via an intermediate peroxy acid.

and amines to give the products indicated in Table 7.6. Some of these will react with isocyanates to produce polyurethanes. Attention must also be paid as to whether the reagent can also react with the carboxyl or ester group present in the epoxide. With sodium iodide and an appropriate solvent, epoxy esters are converted to keto esters ($RCOCH_2R'$ and RCH_2COR').

7.4.1.3 Applications

Reference has already been made to the major use of epoxidised oils as stabilisers and plasticisers in PVC. The first of these properties depends on the ability of epoxides to react with the hydrogen chloride produced when PVC decomposes and thereby to inhibit further deterioration. Epoxy esters can be used as diluents in paints and so replace volatile organic solvents. Epoxy esters are being actively investigated as lubricants and there is evidence that these can be improved by reaction of the epoxide function with linear and

Table 7.6 Products formed from epoxides by reaction with nucleophilic reagents. With unsymmetrical epoxides such as that from oleic acid the products will be mixtures of two regioisomers

Reagent	Product name	Product structure
Water	diol	-CH(OH)CH(OH)-
Alcohol, ROH	alkoxy hydroxy	-CH(OH)CH(OR)-
Carboxylic acid, RCOOH	acyloxy hydroxy	-CH(OH)CH(OCOR)-*
Amine, RNH_2	alkylamino hydroxy	-CH(OH)CH(NHR)-

* Also called hydroxy estolides, especially when R is medium or long chain.

branched-chain alcohols. Reaction with acrylic acid (CH_2=CHCOOH) gives an acrylate which can be cured (polymerised) by radiation. Polyhydroxy compounds produced by reaction of epoxides with diols and triols (glycerol) serve as a source of polyurethanes through reaction with isocyanates.

Natural vernolic acid (12,13-epoxyoleic acid) is being examined as an anti-rust additive (as its C_4–C_6 amides) and as a source of dibasic acids and of high-value products such as the pheromone bombykol and the plant wound hormone traumatic acid.

bombykol	$CH_3(CH_2)_2CH{=}CHCH{=}CH(CH_2)_8CH_2OH$
traumatic acid	$HOOC(CH_2)_8CH{=}CHCOOH$

7.4.2 Hydroxylation

An alkene can be converted to a diol by reagents which effect *cis* or *trans* hydroxylation. The acyclic diols produced from aliphatic compounds are correctly described as *erythro* and *threo* diols (and not as *cis* or *trans*). The relation between these is set out in Fig. 7.24 along with the reagents commonly employed. Of these, hydrolysis of epoxide is the most convenient, especially on a large scale.

Oleic acid yields two racemic diols: the *threo* (MP 95ºC) and *erythro* (MP 132ºC) isomers. There are eight 9,10,12,13-tetrahydroxystearic acids related to the Δ9,12 diene acids and 32 hexahydroxystearic acids related to the Δ9,12,15 triene acids. Procedures have been described whereby these polyhydroxy acids can revert stereospecifically to the unsaturated acids.

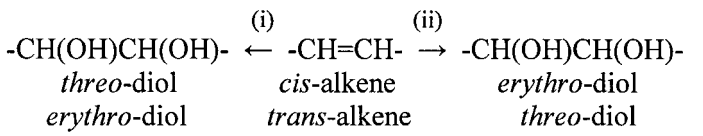


Figure 7.24 Conversion of alkenes to diols. Reagents: (i) *trans*-hydroxylation by I_2 and AgOCOPh (anhydrous) or epoxidation followed by hydrolysis, (ii) *cis*-hydroxylation by dilute alkaline $KMnO_4$, AgOAc/AcOH (moist), OsO_4 alone or OsO_4 in the presence of an oxidising agent.

7.4.3 Oxidative fission

Oxidation of unsaturated acids at the double bonds gives mono and dibasic acids. The identification of these acids as a standard method of determining double-bond position has now been replaced by mass spectrometric and NMR processes.

Polyfunctional molecules such as dibasic acids are required on an industrial scale as monomers in the production of polyesters and polyamides. Some important dibasic acids are products of the petrochemical industry and others

are conveniently produced from unsaturated fatty acids. Sebacic acid (C_{10}) is made from ricinoleic acid from castor oil (sections 1.3.1 and 11.9) and azelaic acid (C_9) is made from oleic acid (or other $\Delta 9$ unsaturated acids) by ozonolysis. This procedure would also give adipic acid (C_6) from petroselinic acid and brassylic acid (C_{13}) from erucic acid but only the production of azelaic (~20 000 tonnes) is carried out through industrial ozononolysis.

Olefinic compounds (RCH=CHR′) react with ozone to first give a cyclic compound (molozonide) which forms two zwitterions (RCH^+OO^- and $R'CH^+OO^-$) the fate of which depends on whether they react with aldehydes (also formed during the reaction) or alcohols or short-chain acids used as solvents to give ozonides, alkoxyhydroperoxides or acyloxyhydroperoxides, respectively. All these intermediates react further to give alcohols, aldehydes, acids, esters/acetals or amines depending on the reagent selected (Table 7.7). Starting with a mono-olefinic acid there are two products, one monofunctional and the other bifunctional as in the conversion of oleic acid to nonanoic acid and nonanedioic (azelaic) acid.

$$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH \rightarrow CH_3(CH_2)_7COOH + HOOC(CH_2)_7COOH$$

Ozonolysis of unsaturated acids is a convenient procedure in that it requires no catalyst and there is no spent oxidant to be handled when the reaction is complete. Oxidation is not usually complete with ozone alone so ozonolysis is generally followed by reaction with oxygen at elevated temperatures. In a typical process, commercial grade oleic acid is diluted with nonanoic acid and run countercurrent with ozone at 25–40ºC to produce ozonides of oleic acid. After ozonisation, the temperature is raised to 60–100ºC while oxygen is passed through the system. Under these conditions the ozonide is split to carboxylic acid and aldehyde and the latter is oxidised to carboxylic acid. The reaction may be effected with nonanoic acid without other solvent or in the presence of solvents such as methanol or pentanes (Rebrovic & Gunstone, 1996).

Industrial ozonolysis presents some difficulties and alternative procedures have been examined, though there is no evidence that these are being used on a large scale. One employs oxygen in the presence of aldehyde as an oxidising

Table 7.7 Reagents used to produce short-chain compounds via ozonolysis

Product type	Reagent
Alcohol	$LiAlH_4$, $NaBH_4$, H_2/Ni, H_2/Pt
Aldehyde	Zn/acid, Ph_3P, Me_2S, H_2/Lindlar's catalyst
Acid	peroxy acid, Ag_2O
Ester/acetal	MeOH
Amine	NH_3/Ni

agent and proceeds via the epoxide. The second is oxidation with sodium hypochlorite in a three-stage process involving emulsification of oleic acid in water, non-catalytic oxidation with sodium hypochlorite and alkali and cleavage of the resulting diol with ruthenium chloride ($RuCl_3$) or other ruthenium salt as catalyst. Some of these oxidation processes work better with Δ-1 alkenes so it has been suggested that methyl oleate be converted to 9-decenoate by ethenolysis (section 7.7) before oxidation to azelaic mono ester.

7.5 Halogenation

The reaction of olefinic molecules with halogens is part of classical organic chemistry and was of interest to lipid chemists when measurement of an iodine value was a routine analytical procedure. This was based on reaction with iodine monochloride. Today it is less important and the subject is treated only briefly.

Bromination is a two-step process involving the formation of a cyclic bromonium cation. Normally this reacts with bromide anion to give a dibromide in a *trans* addition process. This second stage can be diverted by alternate nucleophiles such as water, alcohols (ROH), and carboxylic acids (RCOOH). The products then contain structural units such as –CHBrCHX– where X is Br, OH, OR or OCOR, respectively. Other halogens react similarly.

Dibromides can be debrominated with zinc or with sodium iodide. This reaction is a *trans* elimination process so the recovered alkene has the same configuration as the alkene from which the dibromide was prepared. Dehydrobromination (twice) with base gives an acetylenic compound from the *threo* dibromide but allenic compounds from the *erythro* isomer.

$$-CH_2CHBrCHBrCH_2-$$

erythro dibromide $\rightarrow$ $-CH{=}C{=}CHCH_2-$ and $-CH_2CH{=}C{=}CH-$

threo dibromide $\rightarrow$ $-CH_2C{\equiv}CCH_2-$

When the double bond in an olefinic acid is appropriately placed with respect to a free carboxyl group then neighbouring group participation occurs between the nucleophilic carboxyl group and the intermediate halonium cation to give a halogeno lactone. This has been studied most extensively with $KHCO_3$-I_2-KI. EPA and AA (Δ5 acids) and DHA (Δ4 acid) react in this way to give products of the type formulated in Fig. 7.25. The γ-lactones are more easily formed and are more stable while the δ-lactones are formed less readily but are more reactive once formed. Where both may be formed, the six-membered ring (δ-lactone) will be the product of kinetic control and the γ-lactones are formed under thermodynamic control. The unsaturated acid can be regenerated by reaction of the iodolactone with trimethylsilyl iodide and this reaction has been used to isolate AA, EPA and DHA from natural mixtures containing these acids.

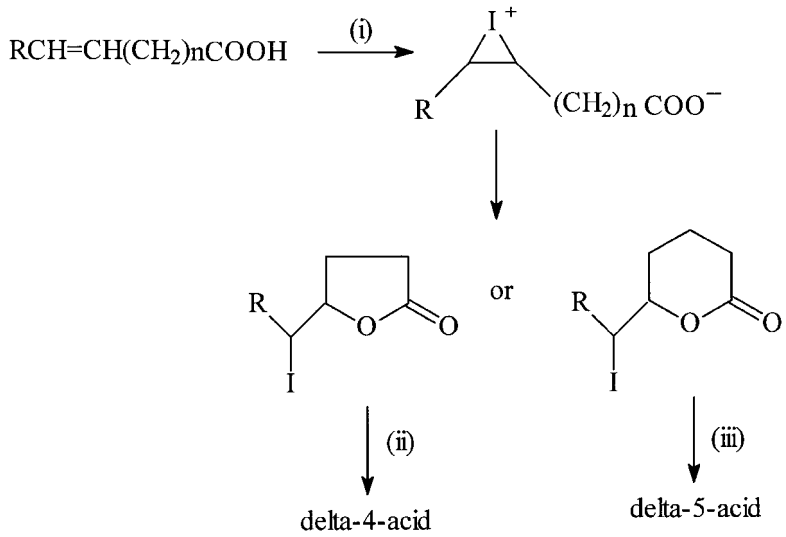


Figure 7.25 Iodolactonisation of acids with unsaturation at Δ4 (as in DHA) or Δ5 (as in EPA or AA). (i) I_2, KI, $KHCO_3$; EtOH at 25ºC or tetrahydrofuran at −2 to +6ºC; (ii) and (iii) TMSCl and NaI in CH_3CN.

7.6 Stereomutation

Fatty acids occur naturally almost entirely in their *cis* form but they can be converted by choice or inadvertently to their *trans* isomers. Generally, the product will be an equilibrium mixture of all the possible isomers but since the *trans* forms are thermodynamically more stable they usually predominate in about a 4:1 ratio. Thus, oleic acid (*cis*) gives a mixture rich in elaidic acid (*trans*). Linoleic acid will produce a mixture of four isomers – the original *cis,cis* acid will be accompanied by the *trans,trans* isomer and by two *cis,trans* isomers. The *cis* and *trans* isomers have different physical and nutritional properties (section 9.3). In particular, the *trans* isomers are higher melting and this is important in the partial hydrogenation of unsaturated vegetable oils for the production of fat spreads.

In any reaction involving double-bond migration, such as partial hydrogenation, biohydrogenation, autoxidation, or photo-oxidation migration will be accompanied by stereomutation so that *cis* olefins become mainly *trans* isomers (see sections 7.1 and 7.2). Stereomutation also occurs when polyunsaturated fatty acids are exposed to high temperatures as in deodorisation where temperatures up to 250ºC may be employed. Linolenic acid is isomerised about 14 times faster than linoleic acid, so this is a problem mainly

for oils such as soybean and rapeseed containing up to 10 per cent of the triene acid. As much as 30 per cent of the linolenic acid originally present may be converted to *trans* isomers, especially the *tcc* and *cct* esters, during deodorisation. This process should be carried out at as low a temperature as practicable to minimise these undesirable changes.

Cis and *trans* isomers can be interconverted by reactions proceeding through several stereospecific stages. These frequently occur via the corresponding epoxides. There are also several reagents which convert the natural *cis* isomers to their *trans* forms in an equilibrium mixture. The predominant higher-melting less-soluble *trans* isomer can then be isolated by crystallisation or by silver ion chromatography. Conjugated polyene acids isomerise more readily and more extensively than their non-conjugated isomer as, for example, with iodine and light.

Reagents commonly employed for stereomutation include selenium (190–200ºC), sodium nitrite/nitric acid as a source of NO_2 (effective at 40ºC in a few minutes), and sulfur compounds such as 3-mercaptopropionic acid, 2-mercaptoethanoic acid, 2-mercaptoethylamine, thiophenol, arylsulfonic acids, and toluenesulfinic acid. Reaction proceeds through a reversible addition that allows rotation about the erstwhile double bond before its regeneration. *Cis* and *trans* isomers can be distinguished qualitatively and quantitatively by chromatographic and spectroscopic procedures.

7.7 Metathesis

Metathesis of natural oils and fats and their derivatives is a catalytic reaction by which oleochemical feedstocks can be converted into valuable chemical products. The reaction is used with benefit in the petrochemical industry but has not yet found commercial application in the oleochemical industry. The reaction has been studied with olefinic acids (as methyl or glyceryl esters) and alcohols (as acetates or trimethylsilyl ethers).

With an appropriate catalyst the following reaction occurs:

$$2R^1CH{=}CHR^2 \rightleftharpoons R^1CH{=}CHR^1 + R^2CH{=}CHR^2$$

As is apparent, there is an exchange of the alkyl groups attached to the olefinic group. The product is an equilibrium mixture of three compounds, and in the process, the olefinic centres undergo stereomutation so that most of the product has *trans* configuration.

Self-metathesis of methyl oleate gives an equilibrium mixture of the starting ester (mainly as *trans* isomer) and of C_{18} hydrocarbon (9-octadecene) and C_{18} dibasic acid (9-octadecenedioic acid) in a molecular ratio of 2:1:1. The unsaturated dibasic acid is a useful starting material for the synthesis of the fragrance civetone (*cis*-9-cycloheptadecen-1-one). Methyl 10-undecenoate

(available from castor oil – section 11.9) undergoes metathesis to give ethane and the unsaturated C_{20} dibasic acid.

Cross-metathesis can be exploited to give esters of various chain lengths. As examples the following olefinic methyl esters can be made from the starting materials indicated: 9-dodecenoate from oleate and 3-hexene, 10-tridecenoate from 10-undecenoate and 3-hexene, 9-tetradecenoate from oleate and 5-decene and 13-triacontenoate from erucate and 1-octadecene. Ethenolysis is a special example of cross metathesis used in the conversion of methyl oleate and erucate to C_{18}, and C_{26} dibasic acids respectively. It provides a procedure for dimerising the $=CH(CH_2)_nCOOMe$ fragment of the olefinic ester as in the following example:

$$RCH{=}CH(CH_2)_{11}COOMe + CH_2{=}CH_2 \rightleftharpoons CH_2{=}CH(CH_2)_{11}COOMe \rightleftharpoons MeOOC(CH_2)_{11}CH{=}CH(CH_2)_{11}COOMe$$

More complex reactions occur with polyunsaturated fatty acids. With glycerol esters, new links are made between olefinic chains either intramolecularly or intermolecularly.

Metathesis occurs under the influence of an appropriate homogeneous or heterogeneous catalyst. The former generally consist of derivatives of transition metals such as tungsten, molybdenum or ruthenium. Heterogeneous catalysts are usually transition metal oxides or chlorides supported on an inorganic oxide such as Re_2O_7/Al_2O_3. Many such combinations have been described including some with high activity (Mol, 2002).

7.8 Double bond migration and cyclisation

The unsaturated centres in olefinic compounds can be moved along a chain under the influence of acid or alkali or by heating. Under these reaction conditions, the natural *cis* olefinic compounds will be converted to equilibrium mixtures of the *cis* and *trans* isomers with the latter predominating. For example, methylene-interrupted dienes are converted to their more stable conjugated isomers by treatment with alkali (see commercial production of CLA, section 3.7). Whenever triacylglycerols containing linoleic and linolenic acids are treated with alkali, as in hydrolysis or methanolysis, harsh conditions should be avoided. Products with conjugated polyene systems are easily recognised by their characteristic ultraviolet absorption (section 6.2.1) and within the history of lipid analysis there was a time when this reaction was exploited to measure levels of linoleic and linolenic acids. The major products are shown in Fig. 7.26.

When heated, conjugated dienes undergo sigmatropic rearrangement. This mechanism explains the changes observed when *cis trans* dienes are heated at 200ºC for 13 hours in an inert atmosphere. The diene system moves along the

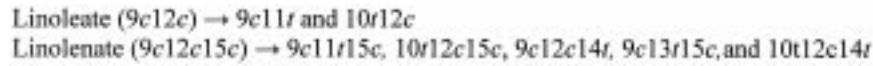


Figure 7.26 Conjugated dienes and trienes formed from linoleic and linolenic acids by alkali isomerisation. Only the major products are shown, others will also be formed.

chain but only in one direction. Hydrogen is transferred only from the *cis* allylic group to the other end of the diene system. Double bond movement is accompanied by a change of configuration so that the 9c11t ester gives an equilibrium mixture with the 8t10c diene only with no 10,12 diene. Similarly the 9*t*11*c* ester gives an equilibrium mixture with the 10*c*12*t* diene and the 10*t*12*c* ester gives an equilibrium mixture with the 11*c*13*t* diene.

$$\underset{\textit{cis}\quad\textit{trans}}{\text{–CH}_2\text{CH=CHCH=CH–}} \rightleftharpoons \underset{\textit{trans}\quad\textit{cis}}{\text{–CH=CHCH=CHCH}_2\text{–}}$$

Under acidic conditions double bond migration occurs through a series of protonation/deprotonation processes:

$$\text{–CH}_2\text{CH=CH–} \rightleftharpoons \text{–CH}_2\overset{+}{\text{C}}\text{HCH}_2\text{–} \rightleftharpoons \text{–CH=CHCH}_2\text{–}$$

With perchloric acid oleic acid gives several 18:1 isomers and the γ-lactone (five-membered ring) of stearic acid. This last is formed by interaction of the carbocation on C-4 with the carboxyl group. It should also be possible to form the δ-lactone (six-membered ring). Starting with 5*c*-20:1 (from meadowfoam oil) a careful study of the lactonisation process has shown that it is possible to produce the γ and/or δ-lactones. With perchloric acid at 100ºC 5-eicosenoic acid gives the γ-lactone in 91 per cent yield. This is the more stable lactone formed under thermodynamic control with these vigorous reaction conditions. Under milder reaction conditions, such as in refluxing dichloromethane the product, obtained in 90–100 per cent yield, was a mixture of γ and δ lactones in a ratio of 1:38. The less stable δ-lactone is also the more reactive of the two lactones and readily forms hydroxy esters and hydroxy amides acids by reaction with alcohols or amines.

Polyene acids, especially with three or more double bonds, undergo cyclisation at elevated temperatures, a process which may be facilitated by alkali to promote double-bond movement and by sulfur or iodine to assist stereomutation. Tall oil contains an unusual triene acid [5,9,12-18:3] and some bicyclic acids which may be derived from this acyclic triene acid as in the reaction scheme in Fig. 7.27.

Extended heat treatment at 200–275ºC of unsaturated oils such as sunflower (linoleic-rich) and linseed (linolenic-rich) produces acids with cyclic structures. After hydrogenation (to simplify the investigation) several 1,2-disubstituted derivatives of cyclopentane and cyclohexane were identified.

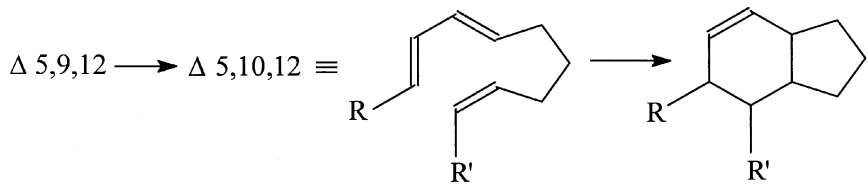


Figure 7.27 Cyclisation of unusual triene acid present in tall oil.

Compounds of this type have also been found at low levels (up to ~0.6%) in deep frying fats.

7.9 Dimerisation (dimer acids, isostearic acid, estolides, Guerbet alcohols and acids)

In this section, the term dimerisation is used loosely to cover any reaction in which the product has twice as many carbon atoms as the staring material.

One dimerisation process is a reaction of unsaturated acids carried out on a commercial scale to produce two useful products – dimer acids and isostearic acid. These products are not single compounds but mixtures with interesting properties which can be exploited. The reaction can occur at high temperatures (260–400ºC) without a catalyst, but on a commercial scale the desired changes take place at around 230ºC over 4–8 hours using montmorillonite (4%) as a cationic catalyst. Subsequent distillation gives a monomer concentrate (isostearic acid) and a dimer concentrate containing some trimer.

Reaction occurs with either monoene or diene acids, and tall oil containing oleic and linoleic acid is frequently used for this purpose. Monoenes give mainly acyclic and monocyclic dimers while dienes give mono and bicyclic dimers. Neither the mechanism nor the structures of the products is completely understood, but they are branched and/or cyclic compounds with 36 carbon atoms and two carboxyl groups. Typical structures are shown, but these are only part of the total product. Hydrogen exchange converts cyclic olefins to derivatives of cyclohexane and benzene. Hydroxy acids, estolides, tetrahydrofurans and tetrahydropyrans have all been recognised as possible intermediate products.

The dimer acids are used mainly as polyamides. The acids react with diamines such as ethylene diamine to give polyamides with excellent adhesive properties. These are used as hot-melt adhesives in shoe making and in printing inks and coatings. Reaction of the dimer acids with polyamines such as the triamine [$H_2N(CH_2)_2NH(CH_2)_2NH_2$] give polyamides with a free NH_2 group, which can act as a curing agent for epoxy resins. Dimer acids are also used as imidazoles in corrosion inhibitors and as esters in lubricants. Heat-treated vegetable oils are used in letter press inks. Short-path distillation gives

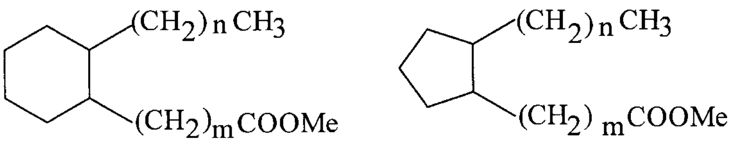


Figure 7.28 Possible structures of cyclised C_{18} fatty acids. The compounds exist as *cis* and *trans* isomers. In the cyclohexanes m = 6–9 and n = 10-m and in the cyclopentanres m = 5–9 and n = 11-m.

dimer acids of higher purity (95–99%) used in polymer applications as hydrophobic blocks.

The monomer remaining after dimerisation is a complex mixture of saturated and unsaturated and straight- and branched-chain acids. After hydrogenation and separation, a concentrate of saturated branched-chain acids is obtained. This low-melting product is designated isostearic acid. Based on its low melting point and oxidative stability appropriate esters are used in lubricants and in cosmetics.

Dimerisation is usually carried out in the presence of a little water (1–2%). At higher water levels (9–10%) mono-estolides are produced. These have the typical structure shown below. They are dimeric in size but contain only one free acid group with the two momomer units linked as esters. With acidic catalysts more estolide is formed including compounds of higher molecular weight with up to ten ester functions and one carboxyl function. Mixtures of oleic acid and a saturated acid produce a range of estolides with differing numbers of ester function from the oleic acid, but finally capped by reaction with a molecule of saturated acid as in the simple example shown below.

$CH_3(CH_2)_8CH(CH_2)_7COOH$
|
$OCO(CH_2)_7CH{=}CH(CH_2)_7CH_3$

Typical mono-estolide from oleic acid.

$CH_3(CH_2)_8CH(CH_2)_7COOH$
|
$OCO(CH_2)_nCH_3$

Simple capped mono-estolide from oleic acid and a saturated acid.

Estolides of a different type are made by acylation of the free hydroxyl groups in castor or lesquerella oil. These oils react with oleic acid without a catalyst at 175–250°C under vacuum or in an inert atmosphere.

Guerbet alcohols provide another type of dimeric molecule with double the number of carbon atoms present in the starting alcohol but only one functional group. These are produced from saturated alcohols by reaction with potassium hydroxide at 200–300°C. Reaction occurs via an aldehyde which undergoes

self-condensation. The product is a dimeric saturated branched-chain alcohol that can be oxidised to the corresponding dimeric, branched-chain acid and then converted to esters. These compounds have good oxidative stability and low melting points with a wide liquid range. They are used in cosmetics and as plasticisers, lubricants and solvents or solubilisers for printing colours and inks. Alcohols and acids with 16 to 36 carbon atoms are easily produced from alcohols with 8 to 18 carbon atoms respectively. Mixtures of alcohols give interesting mixtures of products (Knothe, 2002).

$$\begin{array}{l} \qquad\qquad\qquad\quad R \qquad\qquad\qquad\quad R \\ \qquad\qquad\qquad\quad | \qquad\qquad\qquad\quad | \\ RCH_2CH_2OH \rightarrow RCH_2CH_2CHCH_2OH \rightarrow RCH_2CH_2CHCOOH \\ \qquad\qquad\quad \text{Guerbet alcohol} \qquad\quad \text{Guerbet acid} \end{array}$$

7.10 Neighbouring group participation

Natural fatty acids usually contain a carboxyl group and one or more double bonds with *cis* configuration. Occasionally, there is additional functionality such as hydroxy or epoxy groups. For the most part, these functional groups react independently of each other but when two reactive sites are close enough, then reaction at one may be influenced by the presence of the other. Sometimes this influence is merely regiospecific, i.e. instead of two products formed in equal amounts one becomes dominant. At other times, both functional groups are involved in the formation of the final product. Neighbouring group participation (NGP) generally leads to novel products: these may be of interest and of potential value or they may be tiresome by-products. The new products frequently contain carbocyclic or heterocyclic (O, N, S) systems and their yields are often solvent-dependent. Thus, by appropriate selection of conditions their formation can be maximised or minimised. For example, there are several reactions (bromination, oxymercuration, epoxidation) of olefinic hydroxy and epoxy compounds in which the additional oxygen function reacts intramolecularly with an intermediate formed at the double bond leading to an oxygen-containing heterocyclic compound (tetrahydrofuran or tetrahydropyran) (Fig. 7.29).

Interesting features of these reactions include the following:

- Intramolecular processes will generally dominate over intermolecular reactions.
- When the solvent is also the reagent, the reaction may be modified by using a non-reactive solvent thus allowing intramolecular reactions only.
- The reaction is dependent on the relative position of the double bond and the oxygenated function and may also give different results for *cis* and

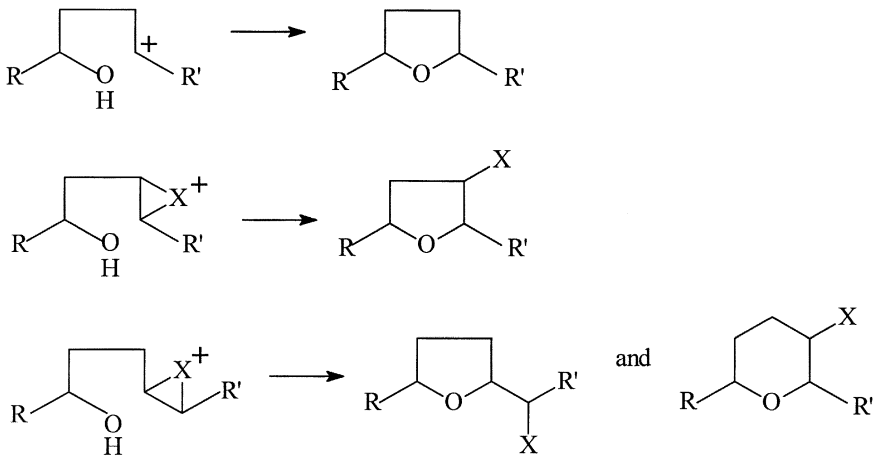


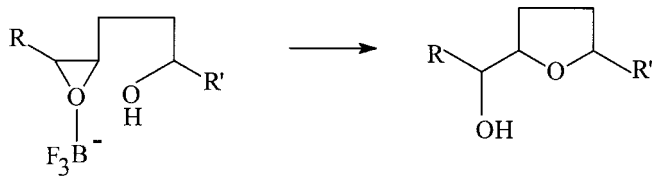
Figure 7.29 Reactions leading to tetrahydrofurans and tetrahydropyrans. The last two sequences are reactions occurring during bromination, oxymercuration, and epoxidation where X is Br, HgOAc and O respectively. (Adapted from Gunstone (1999, p. 6)).

trans olefinic centres. Ricinoleic acid contains a β-hydroxyalkene unit and isoricinoleic acid contains a γ-hydroxyalkene unit. These generally behave differently as do their *cis* and *trans* isomers.

–CH(OH)CH_2CH=CH– the β-hydroxyalkene unit present in ricinoleic acid
–CH(OH)CH_2CH_2CH=CH– the γ-hydroxyalkene unit present in isoricinoleic acid

Hydroxy epoxides from these compounds can be converted to hydroxy-tetrahydrofurans but in some cases the hydroxy epoxide cannot be isolated and passes directly to a hydroxytetrahydrofuran. Methyl ricinoleate, epoxidised with 3-chloroperoxybenzoic acid, gave the expected *cis*-epoxide which was converted by BF_3 to a 10-hydroxy-9,12-tetrahydrofuran. Similar reaction with methyl isoricinoleate followed a different course: the expected 12,13-epoxide was not isolated but was transformed directly to a mixture of diastereoisomeric 13-hydroxy-9,12-tetrahydrofurans. Simililarly 12,13-dihydroxyoleate was converted directly to the 9,12-dihydroxy-10,13-tetrahydrofuran. These results illustrate the difference between β- and γ-hydroxy alkenes. The former (e.g. methyl ricinoleate) give epoxides which can be converted to tetrahydrofurans whilst with the latter (e.g. methyl isoricinoleate) it is not possible to isolate the epoxide which goes directly to a tetrahydrofuran. The 12,13-dihydroxyoleate is both a β and a γ hydroxy alkene and the reaction is controlled by the more reactive γ-hydroxy group (Fig. 7.30).

With appropriate reagents, heterocycles containing sulfur or nitrogen can also be made by reactions involving neighbouring group participation.



Hydroxy alkene (C_{18})	Hydroxy epoxide	Tetrahydrofuran
12-OH 9*c*	9,10-epoxy-12-OH	10-OH 9,12-THF
9-OH 12*c*	[12,13-epoxy-9-OH]	13-OH 9,12-THF
12,13-diOH 9*c*	[9,10-epoxy-12,13-diOH]	9,12-diOH 10,13-THF

Figure 7.30 Rearrangement of hydroxy epoxides [epoxidation of hydroxy alkenes followed by rearrangement with BF_3]. (Adapted from Gunstone (1999, p. 6)).

7.11 Friedel-Crafts acylation and related reactions

The Friedel-Crafts reaction is more generally associated with aromatic compounds but it has been applied to olefinic acids, esters and alcohols such as the readily available Δ10-11:1 and Δ9-18:1 compounds. These react with acid chlorides (RCOCl, C_2–C_{16}) or anhydrides in the presence of a catalyst such as $EtAlCl_2$ at room temperature. Undecenoic acid gives the keto acid shown below, but oleic acid gives a mixture of products. In all cases *cis* and *trans* isomers are formed with the latter predominating (Biermann *et al.*, 2001).

$RCOCH_2CH{=}CH(CH_2)_7COOH$ from undecenoic acid
$CH_3(CH_2)_6CH{=}CHCH(OCOR)(CH_2)_7COOH$ and
$CH_3(CH_2)_7CH(OCOR)CH{=}CH(CH_2)_6COOH$ from oleic acid

Methyl oleate and related compounds undergo a wide range of addition processes. Many of these are acid-catalysed reactions and proceed through a carbocation. Alternatively some involve radical intermediates. Typical reagents and products are indicated though in many cases these are mixtures because the new functional group may be attached to C-9 or C-10 or even to other carbon atoms through the reversible formation of the charged intermediate:

- Benzene [–CH_2CHPh–]
- H_2/CO [–CH_2CH(CHO)–]
- H_2O/CO [–CH_2CH(COOH)–]
- CH_2O [–CH=CHCH(CH_2OH)–]
- RCN [–CH_2CH(NHCOR)–]

- ROH [–$CH_2CH(OR)$–]

None of these reactions are exploited on a commercial scale.

Bibliography and references

Akoh, C.C. & Min, D.B (eds) (2002) *Food Lipids – Chemistry, Nutrition, and Biotechnology,* 2nd edn, Marcel Dekker, New York. Several chapters devoted to oxidation.

Berben, P. (2002) Hydrogenation of fats and oils: trends in catalyst development, *Lipid Technology Newsletter*, **8**, 133–137.

Biermann, U., Furmeier, S. & Metzger, J.O. (2001) New Chemistry of oils and fats. In: *Oleochemical Manufacture and Applications*, (eds F.D. Gunstone & R.J. Hamilton) Sheffield Academic Press, Sheffield.

Cuppett, S.L. (2003) Cholesterol oxides: sources and health implications, *Lipid Technology Newsletter* **9**, 9–13.

Gunstone, F.D. (1997) Epoxidised oils. In: (eds F.D. Gunstone & F.B. Padley) *Lipid Technologies and Applications*, Marcel Dekker, New York.

Gunstone, F.D. (1999) Novel long-chain compounds produced through neighbouring group participation. In: *Recent Developments in the Synthesis of Fatty Acid Derivatives*. (eds G. Knothe & J.T.P. Derksen), AOCS Press, Champaign, Illinois, p. 6.

Gunstone, F.D. (ed.) (2003) *Lipids for Fuctional Foods and Neutraceuticals,* The Oily Press, Bridgwater. Several chapters are devoted to antioxidants.

Gunstone, F.D. NMR spectroscopy <www.lipid.co.uk>

Knothe, G. (2002) Synthesis, applications and characterization of Guerbet compounds and their derivatives, *Lipid Technology*, **14**, 101–104.

Knothe, G. & Derksen, J.T.P. (eds) (1999) Several chapters on epoxidation in *Recent Developments in the Synthesis of Fatty Acid Derivatives,* AOCS Press, Champaign, Illinois.

Kuksis, A. (2000) Core aldehydes – no longer neglected, *Inform*, **11**, 746–752.

Mol, J.C. (2002) Application of olefin metathesis in oleochemistry: an example of green chemistry, *Green Chemistry*, **4**, 5–13.

Porter, N.A., Caldwell, S.E. & Mills, K.A. (1995) Mechanisms of free radical oxidation of unsaturated lipids, *Lipids*, **30**, 277–290.

Rebrovic, L. & Gunstone, F.D. (1996) Oxidative cleavage of unsatuturated fatty acids, *Lipid Technology*, **8**, 135–137.

Shahidi, F. (ed.) (1997) *Natural Antioxidants – Chemistry, Health Effects, and Applications*. AOCS Press, Champaign, Illinois.

Sjovall, O., Kuksis, A. & Kallio, H. (2002) Formation of triacylglycerol core aldehydes during rapid oxidation of corn and sunflower oils with *tert*-butyl hydroperoxide/Fe^{2+}, *Lipids,* **37**, 81–94.

8 Chemical properties related to the carboxyl group

8.1 Introduction

Long-chain acids and their derivatives are amphiphilic molecules having a polar head group with hydrophilic properties and a non-polar alkyl chain with lipophilic properties. Changing the balance between these two conflicting sets of properties leads to a change in the properties of the compound and consequently in its subsequent use. It is generally easier to make this change in the carboxyl/ester function than in the alkyl chain, and much of this chapter is devoted to the ways in which these changes can be made and to their consequences. Changes in the alkyl chain are generally related to the selection of the starting material, of which there are three main types, depending on chain length: C_{12} (lauric oils), $C_{16/18}$ (palm, tallow etc), and C_{22} (erucic oils). Oxygenated acids such as ricinoleic are special cases.

Surface-active compounds are of several types. Anionic surfactants have a negative charge and include the salts of carboxylates (RCOOH), sulfates ($ROSO_2OH$), and sulfonates (RSO_2OH). Nonionic surfactants do not carry a charge but are polar by virtue of the presence of several oxygen-containing functions. These include monoacylglycerols, carbohydrate derivatives and ethylene oxide derivatives (hydroxy polyethers) of alcohols or amines. Cationics with a positive charge are mainly quaternary ammonium compounds ($RNMe_3X$) and zwitterionic compounds have balancing positive and negative charges. The basic oleochemicals from which other materials are made are the acids, methyl esters, alcohols and amides/amines and the relation between these is set out in Fig. 11.1.

Most of the reactions required to convert triacylglycerols to fatty acids and their derivatives require an appropriate catalyst. This will generally be acidic or basic but there is a growing interest in the potential use of enzymes that are increasingly available in stabilised forms in industrial quantities. Using these, many exciting products have been obtained in the laboratory, some on a pilot plant scale, but only a few on a commercial scale. The potential advantages of using enzymes include the following:

- Enzymic processes occur at temperatures and pressures not far from ambient. This leads to savings in energy and plant construction costs.

However, the reactions are generally slower and may require more equipment for an equivalent output of product.
- The products are often cleaner as first obtained and can be purified with less waste and less unwanted material for disposal.
- But most important of all is the selectivity of enzymic catalysts. This allows the production of high value products not easily made by ordinary chemical processes.

Reactions of the carboxyl and ester functions represent a very significant part of lipid chemistry. These may be conducted on a milligram scale for analytical purposes such as the preparation of methyl esters for gas chromatography and formation of oxazolines and picolinyl esters for mass spectrometry, on a gram scale for laboratory synthesis such as the preparation of specific triacylglycerols, or on a tonne scale for commercial manufacture of acids, methyl esters, other esters such as olestra (section 8.3.3) and monoacylglycerols. Methyl esters are used increasingly as biofuel, as a solvent and as a source of alcohols (section 8.6.1). The range of reactions is wide and includes hydrolysis, alcoholysis (especially methanolysis and glycerolysis), acidolysis, esterification, interesterification, and synthesis of structured triacylglycerols. The starting point for these reactions may be acids or esters including natural mixed triacylglycerols.

8.2 Hydrolysis

Lipid hydrolysis is usually carried out in the laboratory by refluxing oils and fats with aqueous ethanolic alkali producing a homogeneous reaction mixture. When this is acidified free acids form an upper layer and separation can be facilitated with an organic solvent such as ether or hexane. Glycerol remains in the aqueous layer but minor compounds such as hydrocarbons, long-chain alcohols and/or glycerol ethers will accompany the extracted acids. If desired, the usaponifiable material (non acidic components) can be recovered by extracting the reaction mixture while it is still alkaline (section 5.2.5).

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 believed that because the reaction produces 10 g of glycerol there will only be 90 g of fatty acids.

The conversion of oils and fats to soaps (saponification) is effected by treatment with aqueous alkali at around 100ºC and 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, in

the manufacture of greases and lubricants and are used as components for animal feeds.

Fats can also be hydrolysed by water itself in a fat-splitting process which yields free acids. This is probably a homogeneous reaction between fat and the small amount of water dissolved in the fat. This procedure is usually carried out in a continuous, high pressure (20–60 bar), uncatalysed, counter-current process at 250ºC though lower temperatures should be used for highly unsaturated oils. Under these vigorous conditions both the fatty acids and the glycerol will be discoloured and may have to be distilled.

Enzymic deacylation with liberation of free acid (1–5%) and formation of diacylglycerols and monoacylglycerols may occur *in vivo* in seeds. The free acid will be removed and recovered during refining, but will be accompanied by loss of oil. The lipase can be deactivated by heating prior to extraction. Lipolysis is an important part of fat digestion (section 9.2) and has been exploited as an analytical procedure (section 5.3.8).

More extensive 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 widely used on an industrial scale.

8.3 Esterification

Esters can be made from acids (or related acyl derivatives) or from existing esters by exchange of alkyl or acyl groups as listed in Table 8.1. These possibilities derive from the reversible nature of the esterification process.

8.3.1 Reaction between carboxylic acids and alcohols

Methyl esters can be made by reaction of acids with methanol and an acidic catalyst. This reaction usually involves a large excess of methanol to displace the equilibrium process in favour of ester.

$$RCOOH + MeOH \rightleftharpoons RCOOMe + H_2O$$

Reaction occurs at 50ºC or at reflux temperature with sulfuric acid (1–2%), anhydrous hydrogen chloride (5%, conveniently prepared from methanol and acetyl chloride), or boron trifluoride (12–14%). Diazomethane (CH_2N_2) may be used for acids which are themselves acid-sensitive such as those containing cyclopropene, epoxy or allylic hydroxy functions. The conversion of triacylglycerols to methyl esters is more conveniently achieved through methanolysis (section 8.3.3).

Methyl esters for use as biofuel have been prepared from soapstock which is an abundant by-product of alkali refining. Alkaline hydrolysis of any

Table 8.1 Routes for the preparation of esters[a]

Reactants	Reaction type
carboxylic acid[b] and alcohol	esterification
ester[c] and alcohol	alcoholysis[d]
ester[c] and acid	acidolysis
ester[c] and ester	interesterification[e]

[a] These reactions generally require a catalyst which may be acidic, basic, or enzymatic.
[b] The acid may be replaced by the more reactive anhydride or acid chloride – a catalyst is not usually then required.
[c] Reactions by which one ester is converted to a different ester may be grouped together as tranesterification processes. The original ester may be glycerol esters as in an oil or fat.
[d] Important examples include reactions with methanol (methanolysis) to give methyl esters and with glycerol (glycerolysis) to give glycerol esters.
[e] Also called ester-ester interchange.

glycerol esters present is followed by acid-catalysed esterification of the acids.

Carboxylic acids may be replaced by their more reactive acid chlorides or anhydrides and this is convenient when it is not appropriate to use a large excess of the alcohol as, for example, in making glycerol esters (section 8.4). Other methods of converting acids to esters have been described in sections 4.2.3 and 5.2.4.

The lipase from *Candida antarctica* has been used to promote the methylation of waste fatty acids. Conversions of around 95 per cent are achieved at 30ºC in 24 hours and the catalyst maintains its activity through 45 cycles.

When enzymic catalysts (lipases) are used it is possible to exploit the various specificities of the enzymes. For example, 1,3-regiospecific lipases such as that derived from *Rhizomucor miehei* (available in immobilised form as Lipozyme) will promote the acylation of glycerol to give 1-mono-acylglycerols and 1,3-diacylglycerols.

The same lipase fails to promote the reaction of acids having a double bond close to the carboxyl group and this property can be used to raise the concentration in natural mixtures of acids having double bonds at position 4 (DHA), 5 (AA and EPA) and 6 (GLA and petroselinic acid), since these acids remain unreacted while the more common saturated and Δ-9 unsaturated acids are converted to esters.

8.3.2 Acidolysis

By reaction of an ester with an acid it is possible to exchange acyl groups. This normally requires an acidic catalyst such as sulfuric acid or a metal oxide (zinc, calcium, magnesium, aluminium). For example, reaction of vegetable oils with lauric acid at around 150ºC will result in some random exchange of the common C_{16} and C_{18} acids. With a 1,3-specific enzymatic catalyst,

reaction proceeds at 50–70ºC and only the acyl groups at positions 1/3 are exchanged. This has been applied in a procedure for upgrading palm mid fraction as a cocoa butter equivalent. Palm mid-fraction contains too much palmitic acid and too little stearic acid and is therefore rich in POP but deficient in POSt and StOSt. Acidolysis of this fraction with stearic acid under the influence of a 1,3 stereospecific lipase such as that from *Rhizomucor miehei* results in some exchange of saturated acyl groups at the 1 and 3 positions, without any change in the oleic acid present at the 2 position.

$$POP + St \rightleftharpoons POSt + StOP + StOSt$$

8.3.3 Alcoholysis

Alcoholysis is more widely practised than acidolysis and includes the important reactions of esters (especially triacylglycerols) with methanol (methanolysis) or with glycerol (glycerolysis).

Methanolysis is carried out on the mg scale to convert glycerol esters to methyl esters prior to gas chromatographic examination (section 5.3.4). This can be achieved with an acidic (sulfuric acid, hydrochloric acid, boron trifluoride) or with a basic catalyst (sodium hydroxide or sodium methoxide) according to the reactions set out in Fig. 8.1. These are equilibrium processes and are pushed to completion by using a large excess of methanol. Reaction with a basic catalyst is quicker and occurs in a few minutes at around 50ºC on a mg scale. Free acids present in unrefined oil are not esterified under these conditions and may destroy the basic catalyst if present at high levels.

The conversion of oils and fats to methyl esters is also carried out on an industrial scale since the esters are used as biofuel and as an intermediate in the preparation of long-chain alcohols. (section 8.6.1). Claims have been made for the use of calcium carbonate as an environmentally acceptable catalyst.

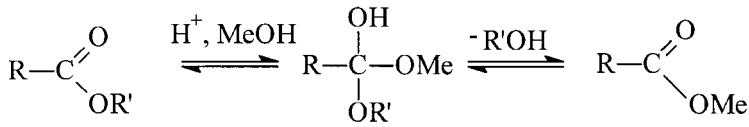
Just as hydrolysis can occur without a catalyst at an elevated temperature (section 8.2), methanolysis is also possible without a catalyst at temperatures up to 200ºC, but this is not yet used as an industrial process. Enzymic methanolysis has also been examined with a range of vegetable oils and waste edible oil using a fixed bed reactor.

Esters based on other primary alcohols such as ethanol, propanol or butanol are made by reactions of glycerol esters with the appropriate potassium alkoxide (ROK) which are themselves easily made from the alcohol (ROH) and potassium tert-butoxide ($KOBu^t$).

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 when the following equilibrium is established:

$$\text{triacylglycerol} + \text{glycerol} \rightleftharpoons \text{monoacylglycerol} + \text{diacylglycerol}$$

acidic methanolysis



basic methanolysis

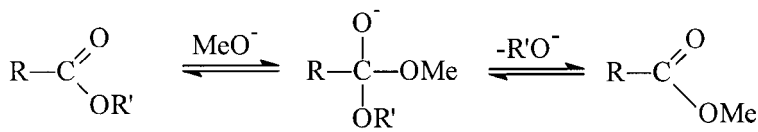


Figure 8.1 Methanolysis reaction with acidic and basic catalysts.

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 (90–95%) are produced by molecular distillation and are widely used as emulsifiers (section 10.10). A similar process has been described using enzymes as catalysts, but this has not been used on an industrial scale.

In the preparation of structured lipids, the first step is often deacylation of triacylglycerol to produce 2-monoacylglycerol and this is conveniently achieved by 1,3-specific ethanolysis using an appropriate lipase such as that from *Candida antarctica*. Applied to palm oil, this gives a 2-MAG preparation containing palmitic, oleic, and linoleic acids while tuna oil furnishes 2-MAG rich in EPA and DHA.

triacylglycerols + ethanol ⇌ 2-monoacylglycerol + ethyl esters of 1,3 fatty acids

Triacylglycerols interact with long-chain alcohols in the presences of lipases such as those from *Candida antarctica* and *Rhizimucor miehei* to produce wax esters.

Olestra is the trade name for a sucrose derivative with six to eight esterified hydroxyl groups made from sucrose (with eight free hydroxyl groups) and the methyl esters of common vegetable oils (soybean, corn, cottonseed, rapeseed, sunflower) in an alcoholysis reaction. High-grade sucrose is reacted with high-grade methyl esters in the presence of sodium or potassium soaps to improve

solubility and an alkali carbonate as catalyst. Olestra is non-toxic, non-carcinogenic, and is so poorly absorbed as to have zero calorific value. It can be used as a frying oil and can replace fats in products such as ice cream, spreads, cheese and baked goods but it is approved only for savoury foods (salty snacks, chips, crisps, extruded snacks and crackers).

8.3.4 Interesterification

With an appropriate catalyst, which may be a base or a lipase, mixtures of esters may be interesterified. This involves rearrangement of all the acyl and alkyl functions to give new esters.

Interesterification of natural oils and fats will give a different mixture of triacylglycerols with modified physical and nutritional properties. This happens when the natural non-random mixture is converted to a randomised mixture. This change will be even more marked with a mixture of two different types of oils such as a lauric oil and a non-lauric oil. This alteration is apparent in the fatty acids present in the *sn*-2 position. Before interesterification of a vegetable oil these will be mainly unsaturated acids, but after complete randomisation the fatty acids at all three positions will be the same. These changes have important effects on the physical (particularly melting behaviour) and nutritional properties of the modified fat.

Interesterification is used in newer methods of producing spreads with a reduced content of *trans* acids. Hydrogenation which can produce large amounts of *trans* acids is replaced by interesterification of a soft fat with a hard fat. Most spreads produced in Europe now have a very low level of *trans* acid, though this is less true for cooking fats and industrial spreads.

When randomisation is complete, triacylglycerol composition can be calculated from the fatty acid composition since the amount of an individual triacylglycerol (ABC) will depend only on the proportions of each of these acids (a%, b%, c% respectively). The level of this single glycerol ester will be 100 [a/100 × b/100 × c/100]% and the level of all isomers having one A, one B and one C acyl chain will be six times this figure since there are six stereoisomers meeting this requirement.

Ester-ester interchange can be achieved without a catalyst at temperatures above 200ºC but is usually carried out at 20–100ºC with a basic catalyst such as sodium hydroxide, sodium methoxide, or sodium potassium alloys. At around 80ºC, the reaction takes 30–60 minutes and may be undertaken on a multi-tonne scale (section 2.3.4). The oil should be free of water, carboxylic acid, and hydroperoxide as these compounds will destroy the catalyst. The true catalyst is a diacylglycerol anion [$ROCOCH_2CH(OCOR)CH_2O^-$] formed by interaction of a triacylglycerol molecule with sodium methoxide. The product will contain some free acid if NaOH is used as a catalyst or methyl ester if the catalyst is NaOMe and must be refined to remove these.

Directed interesterification is a modification of the normal process where the reaction is conducted at a lower temperature (25–35ºC). Under these conditions the less soluble triacylglycerols crystallise from the solution. This disturbs the equilibrium in the liquid phase which has to be re-established. The result is to raise the levels of SSS and of UUU triacylglycerols in the final product. With enzymic catalysts, interesterification leads to structured lipids which are described in the next section.

8.3.5 Structured lipids

Nutritional and other physiological properties of fats (triacylglycerols) depend on their detailed structure. Until now, use has been made of materials provided by agriculture modified in minor ways such as fractionation, partial hydrogenation, and interesterification as described in sections 2.3.2 to 2.3.5. Individual triacylglycerols can be synthesised in the laboratory, but these will not usually be suitable for human consumption. By exploiting the specificity of lipases, it is now possible to produce large quantities of oils and fats approximating to a specification designed to optimise some important physical and/or physiological property.

The cost of the enzyme is generally so high that their use is only economic for high value products but these difficulties are being overcome as enzyme producers develop immobilised enzymes of greater stability with a longer useful life. At the same time our understanding of enzyme structure allows changes to be made leading to enhanced selectivity. Beyond this there is growing willingness to pay more for fats for which approved health claims can be made.

Lipases show several different kinds of specificity which can be exploited. The most common is 1,3-regiospecificity in which reaction is confined to the *sn*-1 and 3 positions of a triacylglycerol with no change at the *sn*-2 position. Lipases may also show specificity for the fatty acids with which they associate. This may reflect the unsaturated centres and especially the position of the double bond closest to the carboxyl group or may be related to chain length.

There are many reports of preparations of structured triacylglycerols which can be described either as one-step or two-step processes. In the former an oil or fat has some of its *sn*-1/3 acyl chains replaced by different fatty acids. The acyl donor may be an ester such as an alkyl ester or triacylglycerol mixture or an acid (acidolysis). The reaction is illustrated in very simple form in the following equation. In reality the reaction is more complicated since neither reactant will be the individual species indicated in the equation.

$$\text{Gl-ABC} + \text{D (as acid or ester)} \rightleftharpoons \text{Gl-DBC} + \text{Gl-ABD} + \text{Gl-DBD}$$

The products represent racemic triacylglycerols with the acyl chains A–D with B occupying the *sn*-2 position.

Good results have been obtained with lipases such as those from *Rhizomucor miehei* (Lipozyme), *Rhizopus delemar* and *Candida antarctica*. Incorporation is usually in the range 40 to 65 per cent. This procedure is simpler than the two-step reaction but the products are less pure.

In the two-step process, triacylglycerols are selectively deacylated at the *sn*-1 and 3 positions and pure 2-monoacylglycerol is isolated. This can be achieved by enzyme-catalysed ethanolysis. The monoacylglycerol is then acylated at the free hydroxyl positions using a 1,3 specific lipase and an appropriate acyl donor which may be free acid or alkyl ester. This is a

$$\text{Gl-ABC} \rightarrow \text{Gl-(OH)B(OH)} \rightarrow \text{Gl-DBD}$$

two-step synthesis of a structured triacylglycerol proceeding through a 2-monoacylglycerol which is isolated and purified before the second step.

There is a greater control of the reaction when this is conducted in a carefully selected solvent but this involves additional handling and cost, and the aim is to produce bulk products having the desired properties as simply and cheaply as possible.

An experiment is being conducted at plant scale. A plug-in reactor ($1m^3$), containing Lipozyme TL IM prepared from *Thermomyces lanuginose* lipase is supplied by the enzyme producer. According to reports, two oils (palm oil or palm stearin and palmkernel or coconut oil) are passed through the reactor and emerge one hour later as interesterified oil. The reaction occurs at 70ºC which is 30ºC lower than for chemical interesterification, no downsteam processing is required and the product has no acids with *trans* unsaturation. Whether this is a commercially viable system remains to be demonstrated, but it illustrates one way in which enzymatic interesterification may develop into a commercial process. A second company is now using this procedure to produce spreads with a low level of *trans* acids.

Many of the laboratory experiments reported are concerned with attempts to produce triacylglycerols of the type MLM where M is an easily-metabolised fatty acid of medium-chain length (frequently C_8) and L is a long-chain acid including nutritionally important fatty acids such as EPA or DHA.

The nature of the fatty acid in the *sn*-2 position is controlled by the selection of starting material. Vegetable oils will provide sources of oleic and linoleic acid in this position and fish oils will be used for the long-chain PUFA. In a preliminary stage, the levels of these important acids may be enhanced prior to interesterification (section 2.7).

Human milk fat is unusual in that it is rich in triacylglycerols containing a saturated acid (palmitic) in the *sn*-2 position. This is unusual among natural fats so a product with this structural feature called ‘Betapol’ has been developed for addition to infant formula. In theory, tripalmitin is reacted with unsaturated acids in the presence of a 1,3-stereospecific lipase (from

Rhizomucor miehei). In practice, the reactants are a palm stearin rich in tripalmitin and a mixture of canola and sunflower oils rich in oleic acid.

8.3.6 Lactones

Oleic acid is converted to γ-stearolactone (with a five-membered ring) in 91 per cent yield by reaction with perchloric acid at 100ºC for three hours. Reaction proceeds through reversible protonation of the double bond with the carbocation moving along the carbon chain until it is in a position where it can easily be trapped by reaction with the carboxyl function to form a lactone. Formation of the δ-lactone with a six-membered ring (from the C_5 carbocation) is under kinetic control. The γ-lactone (from the C_4 carbocation), being more stable, is formed in reactions that are under thermodynamic control. Reaction of oleic acid with perchloric acid is of the latter type (Fig. 8.2).

Lactones can be made under less vigorous conditions from acids which already have appropriate functionality at C-4 or C-5 among which double bonds are the most common. Docosahexaenoic acid has a $\Delta 4$ double bond and eicosapentaenoic and arachidonic acids have a $\Delta 5$ double bond. These acids are used in iodolactonisation reactions (see section 7.5) and in the oxymercuration reaction used to convert these acids (in the form of their alcohols) to tetrahydrofurans and tetrahydropyrans. However, 5-eicosenoic acid which is readily available from meadowfoam oil provides more convenient access to the desired functionality.

5-Eicosenoic acid (20:1) is converted to its δ-lactone with perchloric acid in the presence of dichloromethane as a non-participating solvent. Reaction is at 40ºC, the yield 92 per cent, and the ratio of δ to γ lactone 12.5:1. Alternatively, the unsaturated acid is easily converted to its epoxide with hydrogen peroxide in the presence of a lipase and this reacts with sulfuric acid in toluene, ethyl acetate or acetonitrile to form the 6-hydroxy δ-lactone (Fig. 8.3). The latter compound is also produced by lactonisation of 5,6-dihydroxydocosanoic acid. The δ-lactone is much more reactive than either the free acid or the γ-lactone (5-membered ring) and reacts with alcohols (R′OH) in the presence of acids to give hydroxy esters [$RCH(OH)(CH_2)_3COOR'$], alkoxy esters [$RCH(OR')(CH_2)_3COOR'$] or olefinic esters, or with amines ($R'NH_2$) to

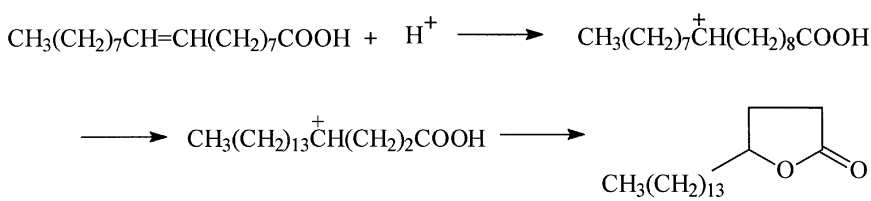


Figure 8.2 Conversion of oleic acid to γ-stearolactone.

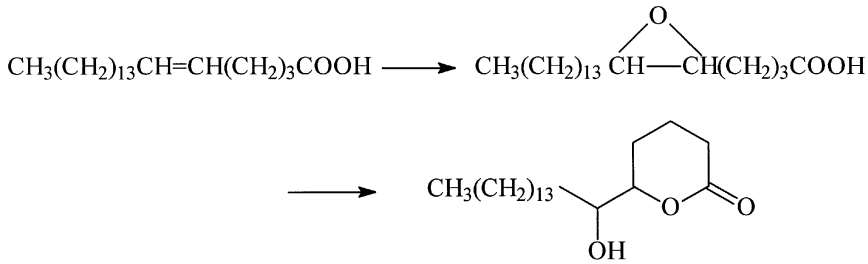


Figure 8.3 Conversion of 5-eicosenoic acid to a δ-lactone via the epoxide.

give hydroxy amides [$RCH(OH)(CH_2)_3CONHR'$] in a rapid reaction requiring no catalyst (Fig. 8.4).

Appropriate olefinic acids react with $KHCO_3$, I_2 and KI to give iodolactones (Fig. 7.25). Acids with a $\Delta 4$ double bond (such as DHA) form an iodo γ-lactone and acids with a $\Delta 5$ double bond (such as AA or EPA) form an iodo δ-lactone. There are several interesting features about these reactions. The iodolactones are neutral molecules and are therefore easily separated from unreacted acids. This provides the basis of a method for separating and isolating $\Delta 4$ and $\Delta 5$ acids from acids without unsaturation at these positions. The original unsaturated acids can be recovered from their iodolactones by reaction with trimethylsilyl iodide (or Me_3SiCl and NaI). The iodolactonisation reaction is dependent on solvent, reaction temperature, and on the ratio of iodine to iodide. For example, with ethanol as solvent and a reaction temperature of 25ºC, DHA gives a maximum yield of iodo-γ-lactone in 10 minutes but EPA requires 90 minutes to produce the iodo-δ-lactone.

8.4 Acid chlorides, acid anhydrides and ketene dimers

Acid chlorides (RCOCl) are easily prepared from acids by reaction at room temperature over 3–5 days with thionyl chloride ($SOCl_2$) for saturated acids or with oxalyl chloride (ClCOCOCl) for unsaturated acids. Other reagents such as phosphorus trichloride or phosgene ($COCl_2$) are used for large-scale reactions.

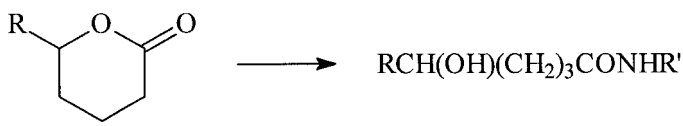


Figure 8.4 Reaction of a δ-lactone with an amine ($R'NH_2$).

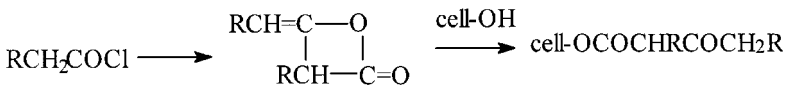


Figure 8.5 Alkyl ketene dimer (AKD) and its reaction with cellulose (cell-OH).

Acid anhdrides (RCOOCOR) are made from acids or acid chlorides by reaction with acetic anhydride or with dicyclohexylcarbodi-imide.

$$RCOOH + Ac_2O \rightleftharpoons RCOOCOCH_3 \rightleftharpoons RCOOCOR$$

$$RCOCl + Ac_2O \rightarrow RCOOCOR \leftarrow RCOOH + DCC$$

(DCC = dicyclohexylcarbodi-imide)

The acid chlorides and anhydrides are more powerful acylating agents than the acids themselves and are much used in the synthesis of triacylglycerols and of phospholipids (sections 4.2.3 and 4.3). With newer catalysts the free acids may also be used (section 4.2.3).

The alkyl ketene dimer (AKD) from hydrogenated tallow is used for sizing paper. Made from a slow reaction between water and acid chloride, the ketene dimer associates with cellulose by both physical and chemical forces (Fig. 8.5).

8.5 Peroxy acids and esters

A carboxylic acid may be considered as the acyl derivative of water. The compounds now to be considered are the mono and diacyl derivatives of hydrogen peroxide and include the peroxy acids, peroxy esters and diacyl peroxides (Fig. 8.6). Reference has been made to some of these in the discussion on epoxidation (section 7.4.1). These compounds have been widely studied and safely used on a large scale but they should always be handled with care. They should not be ground, heated in a closed container, inhaled as vapour or ingested as dust. Their reactivity in both its positive and negative aspects is linked to the weakness of the O-O bond.

Peroxy acids, formerly known as peracids, are made by interaction of a carboxylic acid or acid anhydride and hydrogen peroxide (usually 30% strength). An equilibrium process is established though it may be necessary to

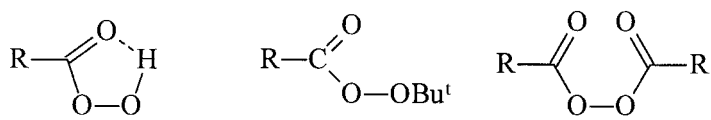


Figure 8.6 Peroxy structures.

use an acidic catalyst such as sulfuric acid or methanesulfonic acid. As indicated in section 7.4.1 peroxyformic (HCO_3H) and peroxyacetic acids (CH_3CO_3H) are generally prepared and used *in situ*. Some crystalline peroxy acids may be safely stored in the refrigerator and are convenient to use in the laboratory. These compounds are probably not pure – particularly after long storage – and it is desirable to measure their level of reactivity before use, in order to determine what quantity of material is required. 3-Chloroperbenzoic acid is commonly used for laboratory scale epoxidation.

$$RCO_2H + H_2O_2 \rightleftharpoons RCO_3H + H_2O$$

In contrast to carboxylic acids which form hydrogen-bonded dimers, the peroxy acids are hydrogen bonded internally (Fig. 8.6). This influences their properties. Acidity is reduced 1000 fold, since the hydrogen atom is more tightly bound and O-H stretching in the infrared is at $3250\,cm^{-1}$ compared with $3530\,cm^{-1}$ for carboxylic acids.

The peroxy acids are useful oxidising agents. They convert alkenes to epoxides, ketones to esters (Baeyer-Villiger reaction), thioethers to sulfoxides (R_2SO) and sulfones (R_2SO_2), and tert amines to *N*-oxides (R_3NO).

Peracids can be used as laundry bleaches. The perborate normally used is less suitable at the lower wash temperatures now favoured, but organic peroxy acids (C_8 and C_9 monobasic and C_{12} dibasic) can be used at these temperatures and are being investigated.

t-Butyl peroxy esters ($RCOOBu^t$) are made from t-butyl hydroperoxide (Bu^tOOH) and carboxylic acids or acid chlorides. Above 75°C, they decompose to give radicals ($R^\bullet$ from $RCOO^\bullet$ and $CH_3^\bullet$ from $Me_3CO^\bullet$) and can be used to initiate radical processes.

Diacyl peroxides are made from hydrogen peroxides or metal peroxides and carboxylic acids or acid chlorides. They decompose at 20–100°C and are used as radical initiators.

$$RCOOCOR \rightarrow 2RCOO^\bullet \rightarrow 2R^\bullet + 2CO_2$$

8.6 Long-chain alcohols

8.6.1 Preparation of alcohols

Long-chain alcohols (RCH_2OH) with structures similar to the better known acids, occur naturally in the free state and, more commonly, as esters. These include wax esters with long-chain alcohols and long-chain acids such as occur in jojoba and other vegetable and animal waxes and many insect pheromones which are alcohol acetates.

Alcohols are easily made in the laboratory by reduction of acids or esters with lithium aluminium hydride, a reaction which leaves the unsaturated

$$\text{RCOOR}' \xrightarrow{\text{i}} \text{RCH}_2\text{OH} \xrightarrow{\text{ii}} \text{RCH}_2\text{OMs} \xrightarrow{\text{iii}} \text{R(CH}_2)_2\text{COOH}$$

Figure 8.7 Chain extension by two carbon atoms by the malonation route. (i) $LiAlH_4$, (ii) CH_3SO_2Cl (MsCl) etc., (iii) $CH_2(COOEt)_2$ etc.

centres unchanged so that, for example, oleyl, linoleyl and linolenyl alcohols are available from their acids or esters. This reduction is frequently employed as the first step in chain-elongation sequences involving reaction of mesylate or tosylate (prepared from the alcohol) with cyanide to add one carbon atom or with malonic ester to add two carbon atoms without any change in double bond position or configuration. These sequences provide a simple route to isotopically labelled compounds as shown in the malonation sequence (Fig. 8.7).

In the laboratory, hydrogenolysis can be reversed and alcohols can be oxidised to carboxylic acids. In one route, the alcohols are converted to tetrahydropyrans by reaction with dihydropyran at 0ºC in the presence of toluenesulfonic acid and these are oxidised with CrO_3 in acetone solution at 0ºC (Jones' reagent).

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, double bonds may be reduced or be left unchanged. These processes are generally applied to natural mixtures and the products are therefore mixtures varying in chain length. C_8 to C_{14} alcohols are produced from lauric oils (coconut and palmkernel), 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 1.5 million tonnes annually of which two thirds or more is fat-based. About 75 per cent of these fatty alcohols are used as alcohol sulfates, alcohol ethoxylates, or alcohol ethoxylate sulfates.

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 effected on acids, methyl esters or wax esters made *in situ* from acids and alcohols. The range of possible products from hydrogenolysis include esters, aldehydes, alkanes, ethers, and acetals in addition to the alcohols.

In the methyl ester route, acid-free esters are first made from the natural oils by methanolyis (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, at 250–300 bar and 210ºC. The volatile product is a mixture of hydrogen and methanol which can be separated and 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 200–230ºC and 200 bar.

Arguments have been presented for the acid route using some new technologies. These involve:

- conversion of oil to acids and fractionation of these, some of which may be sold as acids,
- preparation of methyl esters from acids using a resin bed as catalyst,
- reduction of esters to alcohols using a fixed bed catalyst (40 bar, 200–250ºC, chromium free catalyst).

These combined procedures can be used flexibly to produce acids, methyl esters and alcohols as required.

In the wax ester route, the starting materials are distilled or fractionated fatty acids and some long-chain alcohols already made. At an appropriate temperature, the acids and alcohols react without catalyst to produce wax esters. These and pure hydrogen are then passed to the fixed-bed hydrogenation reactor charged with catalyst. 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.

The present 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 240–250ºC is employed.

8.6.2 Ethoxylation and propoxylation of alcohols and esters

Substantial portions of the medium and long-chain alcohols are used only after conversion to ethoxylates or propoxylates. Ethoxylation of long-chain alcohols occurs at 135ºC under pressure for 30 minutes in a reaction with ethylene oxide usually catalysed by NaOH or KOH (~0.2%). The product is hydrophilic by virtue of one hydroxyl group and many ether links and is a mixture of compounds with up to 20 ethylene oxide units (see equation below).

When dodecanol is reacted with ethylene oxide (50% wt $\equiv$ 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 per cent. Compounds with fewer

ethylene oxide units can be made with proprietary catalysts such as $ZrSO_4(OR)_2$ or similar aluminium compounds. Under these conditions the products contain only 0–10 EO units with those having 4–6 EO units each around 20 per cent and those with 3 and 7 units around 10 per cent. These products, called narrow range ethoxylates, have good stability and skin mildness in liquid dishwashing products.

The ethoxylation of methyl and other esters, rather than alcohols, is an interesting development in this field since products with improved surfactant properties can be obtained from a less expensive starting material (ester rather than alcohol). Using an appropriate catalyst such as a composite aluminium and calcium metal oxide at 180ºC and 3 bar, the product has a narrower range of molecular weights with mainly 5–10 ethylene oxide units. 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 (see equation below).

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 below. 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.

$$ROH \rightarrow RO(CH_2CH_2O)_nH$$

$$RCOOMe \rightarrow RCO(OCH_2CH_2)_nOMe$$

$$\begin{array}{l} CH_2O(CH_2CH_2O)_xCOR \\ | \\ CHO(CH_2CH_2O)_yCOR \\ | \\ CH_2O(CH_2CH_2O)_zCOR \end{array}$$

Another development is the replacement of ethylene oxide, wholly or in part, by propylene oxide in the reaction both with alcohols and esters The branched-chain compounds which result have 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 aluminium complex as catalyst.

8.6.3 Sulfates and other alcohol esters

Both long-chain alcohols and their ethoxylated derivatives are widely used as sulfates resulting from reaction with sulphur trioxide or chlorosulfonic acid and generally used as sodium, ammonium, or monoethanolamine salts. Less

Table 8.2 Sulfates and other esters of long chain alcohols

$AEOSO_2OH$	sulfate
$AEOPO_3H_2$ or $(AEO)_2PO_2H$	phosphate
$AEOCOCH_2CH(OSO_3Na)COONa$	sulfosuccinate
$AEOCH_2COONa$	ethoxy carboxylates
$ROCOO(CH_2CH_2O)_nH$	carbonates
$RCOOCHCH(OH)CH_2OSO_2OH$	monoacylglycerol sulfate

AEO represents alcohol ethoxylate $RO(CH_2CH_2O)_{n-}$.
RO represents a fatty alcohol.

commonly, the alcohols are also used as phosphates after reaction with P_2O_5, as sulphosuccinates after reaction with maleic anhydride followed by sodium sulphite, and as ethoxy carboxylates after reaction with sodium chloro-acetate. They can also be made into alkyl carbonate ethoxylates by reaction of alcohol, poly(ethylene glycol) and dimethylcarbonate (Table 8.2).

8.6.4 Alkyl polyglycosides

The term alkyl polyglycoside is the name given to technical products made from starch (or other sources of glucose) and a fatty alcohol. These are sugar ethers in contrast to the sugar esters used in olestra (section 8.3.3). The alcohol is usually a mixture of C_8 and C_{10}, C_{12} and C_{14}, or C_{16} and C_{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 may be carried out in two stages. It is accompanied by extensive depolymerisation of the carbohydrate polymer so that the product is mainly, but not entirely, an alkyl glucoside. In the first step, butanol is used and in the second, the alcohol mixture (ROH) of desired chain length is used. The product is a mixture of compounds with (mainly) two different R groups and values of n lying between 1 and 5. The average value of n (degree of polymerisation) is usually 1.3–1.7. 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 8–14 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.

$$\text{starch} + \text{BuOH} \rightarrow [\text{glucose}]_n\text{OBu}$$
$$[\text{glucose}]_n\text{OBu} + \text{ROH} \rightarrow [\text{glucose}]_n\text{OR}$$

8.6.5 Guerbet alcohols and acids

Guerbet alcohols, first reported in 1899, are still of interest and are produced industrially on a limited scale. They are made by heating saturated alcohols

with KOH or potassium ethoxide at 230–300ºC. The product contains twice as many carbon atoms as the original alcohol and is a branched-chain monohydric alcohol that can be oxidised to the corresponding acid. Because the molecule is branched, it has a lower melting point than its straight-chain isomer and this increases its value as a component of lubricants (section 7.9). Guerbet alcohols are formed via aldehydes which dimerise under the alkaline reaction conditions. Guerbet derivatives are considered as potential lubricants. For example, di-Guerbet esters have been made by heating Guerbet alcohols with Guerbet acids at around 80ºC in the presence of 4-toluenesulphonic acid.

$$RCH_2CH_2OH \rightarrow [RCH_2CHO] \rightarrow [RCH_2CH_2CH(R)CHO] \rightarrow$$
$$RCH_2CH_2CH(R)CH_2OH \rightarrow RCH_2CH_2CH(R)COOH$$
Guerbet alcohol — Guerbet acid

8.7 Acetals/ketals and orthoesters

Surfactants with a weak bond built into the molecule are interesting because this feature leads to improved biodegradabilty and opens up other possible uses of these compounds such as drug delivery. The weak bonds can be broken 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).

Cyclic acetals/ketals result when aldehydes or ketones react with polyhydric alcohols such as glycerol, pentaerythryitol or glucose. The products are 1,3-dioxolanes (5-membered hetero ring) or 1,3-dioxanes (6-membered hetero ring) (Fig. 8.8). Any additional hydroxyl groups can be further functionalised. These compounds are made under anhydrous acidic conditions and are readily hydrolysed under aqueous acidic conditions.

Ortho-esters are made from ethyl orthoformate [$HC(OEt)_3$], alcohols, and monomethyl polyethylene glycol [$HO(C_2H_4O)_nMe$] in the presence of a little aluminium 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 largest percentage of products formed 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

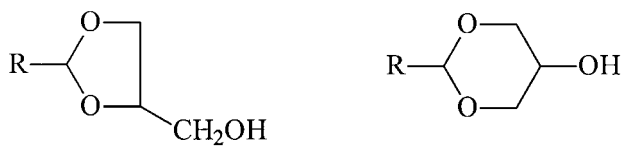
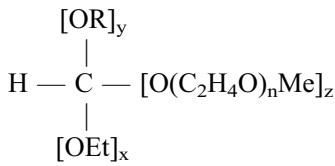


Figure 8.8 Acetals from RCHO and glycerol.

surface cleaners, textile treatment processing, etc. They are hydrolysed under mild acid or alkaline conditions and have good biodegradability.



Alkyl ethoxylates $[RO(EO)_nH]$ are viscous oils which are not always easy to handle but they react with carbon dioxide to form carbonates $[RO(EO)_nCO_2Na]$ which are solid and easily incorporated into granular detergents. In alkaline solution, the ethoxylates are quickly regenerated from the carbonates.

8.8 Nitrogen-containing compounds

Cationic surfactants (nitrogen containing compounds) 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. The major route to this kind of material involves the sequence:

fatty acid (RCOOH) $\rightarrow$ nitrile (RCN) $\rightarrow$ amines (RCH_2NH_2 etc).

The nitriles are produced from fatty acids (or esters) by reaction with ammonia and subsequent dehydration of the amide. Reaction occurs at around 300ºC in the presence of zinc oxide or other catalysts such as alumina, thoria, titanium oxide, or transition metal salts.

Hydrogenation of nitrile occurs with nickel or cobalt as catalyst. 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, can react 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.

$$RCH_2NH_2 + 2CH_2O \rightarrow RCH_2NMe_2$$

$$RCONMe_2 \rightarrow RCH_2NMe_2$$

$$RCH_2OH + Me_2NH \rightarrow RCH_2NMe_2$$

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 $[(HOCH_2CH_2)_2NMe_2X]$. They show better environmental characteristics than the quats themselves.

Tertiary amines can be oxidised with hydrogen peroxide to amine oxides and quaternised with alkyl halides to form quaternary ammonium salts (quats). Quaternising agents frequently used include methyl chloride, dimethyl sulfate or benzyl chloride:

$$R_3N^+O^- \qquad R_3N^+R'\ X^-$$

Still other nitrogen-containing surface-active compounds may be made from carboxylic acids, alcohols and amines. The alcohols and amines will add to acrylonitrile and, after catalytic hydrogenation, furnish ether amines and diamines thus:

$$ROH + CH_2{=}CHCN \rightarrow RO(CH_2)_2CN \rightarrow RO(CH_2)_3NH_2 \text{ (etheramine)}$$

$$RNH_2 + CH_2{=}CHCN \rightarrow RNH(CH_2)_2CN \rightarrow RNH(CH_2)_3NH_2 \text{ (diamine)}$$

The diamine can react again with acrylonitrile to give the triamine $RNH(CH_2)_3NH(CH_2)_3NH_2$.

Mono and diamines react with ethylene oxide as already described for the alcohols (section 8.6.2) to give ethyloxylated products such as:

$$RO(CH_2)_3NH_2 \rightarrow RO(CH_2)_3NH(CH_2CH_2O)_nH \text{ (ethoxylated etheramine)}$$

$$RNH(CH_2)_3NH_2 \rightarrow RNH(CH_2)_3NH(CH_2CH_2O)_nH \text{ (ethoxylated diamine)}$$

$$RNH_2 \rightarrow H(OCH_2CH_2)_nN(R)(CH_2CH_2O)_mH \text{ (ethoxylated amine)}$$

Diamidoamines, 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 (Fig. 8.9):

$$RCONH(CH_2)_2NH(CH_2)_2NHCOR \text{ (diamidoamine)}$$

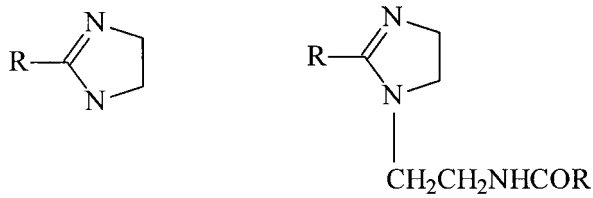


Figure 8.9 Imidazolines from RCOOH and diamine ($H_2N(CH_2)_2NH_2$) or triamine ($H_2N(CH_2)_2NH(CH_2)_2NH_2$).

8.9 Sulfonation and other reactions at the α-position

Carboxylic acids and their esters have an active methylene group in the α-position because the adjacent carbonyl function can stabilise either a radical or anion generated as an intermediate:

$$R\bar{C}HC(OR)=O \rightleftharpoons RCH=C(OR)-\bar{O}$$

This reaction should be confined to saturated acids and esters as competitive reactions occur at the allylic centres in unsaturated compounds. α-Substitution may be accompanied or followed by decarboxylation.

$$RCH_2COOH \rightarrow RCHXCOOH \rightarrow RCH_2X + CO_2$$

On an industrial scale, methyl esters are sulfonated by reaction with sulfur trioxide. The complex product resulting from this step is subsequently neutralised with sodium hydroxide to give a mixture of mono and di- sodium salts. The industrial product is usually dark coloured and has to be bleached. The surfactant properties vary with chain length and hence with the starting material which is generally a lauric oil or tallow. The sulfonates show good environmental properties.

The monosulfonate ($RCH(SO_3H)COOCH_3$) forms a mono sodium salt ($RCH(SO_3Na)COOCH_3$) and a disodium salt ($RCH(SO_3Na)COONa$). The disulphonate ($RCH(SO_3H)COOSO_2OCH_3$) forms the same disodium salt. Industrial products contain the mono and disodium salts in a ratio of around 4:1.

Saturated and monounsaturated acids form dianions ($R\bar{C}HC\bar{O}\bar{O}$) with lithium di-isopropylamide in hexamethylphosphoric acid. These react with a wide range of reagents to give products of the type RCHXCOOH. For example, X = D (D_2O), R′ (R′Br), CH_2OH (CH_2O), OOH (O_2, -78^oC), Br (Br_2), I (I_2). Decarboxylation accompanies the reaction with HCOOH and $PrONO_2$ to give RCH_2CHO and RCH_2NO_2 respectively.

8.10 Barton reaction

On a laboratory scale there is an interest in converting carboxylic acids (RCOOH) to *nor*-bromides (RBr) with one less carbon atom. These can be used as intermediates to prepare carboxyl labelled acids starting from the unlabelled acid. It is often easier to isolate acids from natural sources where these occur conveniently, than to carry out a full synthesis.

An old method of achieving this, by reaction of the silver salt with bromine (Hunsdiecker reaction), is not suitable for unsaturated acids because the bromine also reacts additively with double bonds. This has been superseded by the Barton reaction in which an acid chloride reacts with 2-mercaptopyridine-*N*-oxide in $BrCCl_3$ in the presence of 4-dimethyl-aminopyridine. This is exemplified in the synthesis of the [1-^{13}C] labelled forms of linoleic and linolenic acid.

$$RCOOH \rightarrow RBr \rightarrow R^*COOH$$

Bibliography and references

Akoh, C.C. (2002) Structured lipids. In: *Food Lipids, Chemistry, Nutrition, and Biotechnology* (eds C.C. Akoh & D.B. Min), Marcel Dekker, New York, pp. 877–908.

Auerbach, M.H., Kleimann, L.P. & Heydinger, J.A. (2001) Reduced-energy lipids. In: *Structured and Modified Lipids* (ed F. D. Gunstone), Marcel Dekker, New York, pp. 485–510.

Bornscheuer, U.T. (ed.) (2000) *Enzymes in Lipid Modification,* Wiley-VCH, Weinheim.

Bornscheuer, U.T., Adamczak, M. & Soumanou, M.M. (2003) Lipase-catalyzed synthesis of modified lipid. In: *Lipids for Functional Foods and Nutraceuticals* (ed F.D. Gunstone) The Oily Press, Bridgwater, pp. 149–182.

Gunstone, F.D. (2001) Chemical reactions of fatty acids with special reference to the carboxyl group, *European Journal of Lipid Science and Technology*, **103**, 307–314.

Hellberg, P-E. (2003) Cleavable surfactants – giving an unstable advantage, *Lipid Technology*, **15**, 101–105.

Kellens, M. (2000) Oil modification processes. In: *Edible Oil Processing*, (eds W. Hamm & R. J. Hamilton) Sheffield Academic Press, Sheffield, pp. 144–155.

Rousseau, D. & Marangoni, A.G. (2002) Chemical interesterification of food lipids: theory and practice. In: *Food Lipids, Chemistry, Nutrition, and Biotechnology I* (eds C.C. Akoh & D.B. Min), Marcel Dekker, New York, pp 301–333.

Rozendaal, A. & Macrae, A.R. (1997) Interesterification of oils and fats. In: *Lipid Technologies and Applications* (eds F.D. Gunstone & F.B. Padley), Marcel Dekker, New York, pp. 223–263.

Uphues, G. (1998) Chemistry of amphoteric surfactants, *Fett/Lipid*, **100**, 490–497.

Willis, W. & Marangoni, A.G. (2002) Enzymic interesterification. In: *Food Lipids, Chemistry, Nutrition, and Biotechnology I* (eds C.C. Akoh & D.B. Min), Marcel Dekker, New York, pp. 839–875.

Yankah, V.V. & Akoh, C.C. (2001) Zero-energy fat-like substances: Olestra. In: *Structured and Modified Lipids* (ed F.D. Gunstone), Marcel Dekker, New York, pp. 511–536.

9 Nutritional properties

9.1 Dietary fat – sources and composition

The human diet contains a wide range of different foods and drinks but the nature of their component parts is more restricted. The largest and most important component of all drinks is water, which is also an important part of virtually all food, reaching levels of 90 per cent and above in many fruits and vegetables. The human body can exist for a short period without food but it cannot survive long without water. Solid foods contain protein, carbohydrate and fat (lipid) as three macronutrients, along with a large number of important micronutrients. A varied diet of good quality consumed in appropriate amount will provide a healthy individual with all that is needed, with micronutrients accompanying the macronutrients.

It has been reported that a lean man of 70 kg is made up of water (42 kg, 60%), protein (12 kg, 17%), fat (12 kg, 17%) and a balance (4 kg, 6%) which includes glycogen and bone. The same source indicates that an obese man of 100 kg will contain 35 kg of fat, so that the 30 kg increase in weight is almost entirely additional fat (23 kg).

The energy levels of fat (38 kJ/g or 9 kcal/g), carbohydrate (17, 4), and protein (16, 3.8) are as indicated in parentheses. For a daily intake of 2000 kcals, 67 g of fat corresponds to 30 per cent of total energy. Levels of 35 per cent and 40 per cent of total energy correspond to daily fat intakes of 78 g and 89 g respectively. These weights relate to actual intake and do not allow for loss through incomplete absorption and consequent faecal loss. The caloric figure given above for fat (9 kcal/g) is an average value. Short-chain acids have lower energy values since they contain higher proportions of oxygen in their molecules and long-chain acids sometimes have lower energy values because of incomplete absorption. These concepts have been exploited in the development of fats with short and long chain fatty acids having energy values of around 5 kcal/g. Examples include Caprenin with C_8, C_{10} and C_{22} saturated acids and Benefat/Salatrim with $C_{2–4}$ and C_{18} acids.

The main use of the commercial oils and fats produced by the agricultural industry is as food for humans. This represents 80 per cent of the total (122.5 million tonnes in 2002/03), i.e. around 98 million tonnes, equivalent 19.7 kg/person/year or 54 g/person/day. This figure is obtained by dividing total

annual production by world population. It makes no allowance for fat that is wasted between production and the plate nor for invisible (not-counted) fat present in nuts, in phospholipids from animal and vegetable sources, in meat and in milk and dairy products other than butter. These average values vary between countries. For example, total disappearance per person in 2002/2003 (including that used as animal feed and in the oleochemical industry) is 50.9 kg for the United States, 50.5 kg for EU-15, 16.4 kg for China, and 11.5 kg for India, with even lower figures for several African countries. These average national consumption figures cover a wide range of levels between individuals. It is generally accepted that when an average value is quoted, most, but not all, individuals fall in the spread of 0.5–2.0 times the average value. Putting this another way, the lowest and highest 2.5 percentiles are very different from average values.

In 2002/2003 total production of animal fats (butter fat, lard, tallow and fish oil) together amounted to 18.6 million tonnes, equivalent to 5.6 per cent of world production of 17 major oils and fats. On the basis of some personal assumptions, it is estimated that 12.6 million tonnes of animal fats and 85.4 million tonnes of vegetable fats enter into the human food chain. This does not allow for the consumption of fat from sources outside the 17 selected oils and fats, such as that consumed when eating meat and dairy products other than butter. Consumption of animal fats may be markedly higher than those indicated here.

The fats we consume are of animal and vegetable origin. They are mainly triacylglycerols with minor amounts of phospholipids, glycolipids, free sterols and sterol esters and vitamins (section 1.6). Fats are the richest source of energy on a weight basis (see above) and excess of fat beyond that required for daily energy requirements is laid down as reserve depot fat, usually after some structural modification. Fat is laid down in anhydrous condition whereas carbohydrate is stored in limited amount and in hydrated form with even less energy (3 g water/1 g glycogen).

Fat (lipid) is present in virtually all the food we eat. It is the only material present in vegetable oils and animal fats, it is an important component of milk and milk-based products such as cream and cheese, eggs and meat, and is even present in leaves and green vegetables. Data exists for the amount and composition of fat in most foods. Table 9.1 is taken from UK data adapted from McCance & Widdowson by MAFF (1998) for 550 foods. European, Australian and US information is also available (Hands, 1996; USDA website).

The figures in Table 9.2 are taken from a survey of dietary intake of UK adults (19–64 years of age) in 2000/2001. The original document should be consulted for more detail. It is clear that a considerable amount of dietary fats comes from meat and meat products and from milk and milk products other than butter. These are sources of fat not included in statistics for commodity oils and fats (section 1.7). The cereal products will include baked goods made with butter or vegetable oil spreads.

Table 9.1 Levels of total fat and the major types of fatty acids (cited as g/100g of food) in selected foods selected from MAFF (1998)

Food	Fat content	Sat	Mono-unsat (*cis*)	Polyunsat n-6	Polyunsat n-3	*Trans* total
Cereals and cereal products						
Bread – white	1.9	0.40	0.25	0.62	0.04	0.00
Pasta – plain, cooked	1.5	0.28	0.28	0.34	0.02	0.01
Croissants	26.0	14.33	6.62	1.00	0.41	1.64
Digestive biscuits – plain	20.3	9.00	7.37	1.86	0.09	0.95
Madeira cake	15.1	8.43	3.28	1.34	0.27	0.71
Danish pastry	14.1	8.57	1.54	1.65	0.24	0.84
Fruit pies – double crust	14.0	5.36	5.09	1.42	0.26	1.20
Milk and milk products						
Whole milk	4.0	2.48	0.93	0.10	0.02	0.14
Semi-skimmed milk	1.7	1.07	0.39	0.05	0.01	0.07
Double cream	53.7	33.39	12.33	1.34	0.48	1.83
Soya milk	1.6	0.24	0.33	0.81	0.12	trace
Cheddar cheese	32.7	19.25	7.14	0.99	0.28	2.10
Cheesecake	12.3	.54	3.28	0.42	0.09	0.23
Eggs and egg products						
Hen eggs	11.2	3.15	4.31	1.61	0.08	0.12
Fats and oils						
Cooking fat	99.5	24.52	31.56	27.06	1.49	10.37
Butter	82.2	52.09	18.48	1.41	0.68	2.87
Fatspread 70% monounsaturated	70.0	9.44	31.63	11.73	3.79	10.45
Olive oil	99.9	14.3	73.00	7.50	0.70	trace
Palm oil	99.9	47.80	37.10	10.10	0.30	trace
Rapeseed oil	99.0	6.60	59.20	19.70	9.60	trace
Soybean oil	99.9	15.60	21.20	51.50	7.30	trace
Sunflower oil	99.9	12.00	20.50	63.20	0.10	trace
Meat and poultry						
Beef – lean, cooked	8.2	3.26	3.41	0.36	0.09	0.28
Lamb – lean, cooked	12.5	5.36	4.06	0.48	0.23	0.93
Pork – lean, cooked	6.7	2.31	2.56	1.02	0.12	0.03
Chicken – light meat, roasted	3.7	1.02	1.58	0.60	0.13	0.05
Beefburgers fried in veg oil	23.9	10.27	10.12	0.38	0.11	0.78
Beef sausage – grilled	19.5	7.69	8.35	1.26	0.15	0.38
Fish and fish products						
Cod – raw	0.7	0.13	0.08	0.02	0.26	0
Herring – raw	13.2	3.65	5.98	0.32	1.83	trace
Trout – rainbow, raw	5.2	1.10	1.85	0.41	1.32	0
Vegetables and vegetable dishes						
Potato chips – fast food chain	11.0	5.96	2.69	0.16	0.01	0.43
Vegetables – stir fry	4.1	0.77	2.17	0.30	0.23	0.01

(*continued*)

Table 9.1 (*continued*)

Food	Fat content	Sat	Mono-unsat (*cis*)	Polyunsat n-6	Polyunsat n-3	*Trans* total
Potato crisps – plain	34.2	14.04	13.51	*	*	*
Fruits, nuts and seeds						
Avocado	19.3	2.27	14.50	1.61	0.07	0
Peanuts	46.0	8.66	22.03	12.75	0.35	0
Sauces and dressings						
Mayonnaise	75.6	11.42	17.86	40.22	2.15	1.23
Salad cream	31.0	3.29	11.44	13.55	1.00	0.10

* Present but not in known amount.
Adapted with permission from MAFF (1998). Based on 550 results obtained between 1990 and 1997. The analyses are mean values measured on several samples. Full details of fatty acid composition are given in the book and some foods are detailed in the raw and cooked state. Some of the prepared foods may now have a different composition.
Similar information is available from American sources (Hands, 1996; USDA website).

9.2 Digestion, absorption and transport

Fat digestion begins in the mouth (lingual lipase) and continues in the stomach, but occurs mainly in the duodenum (small intestine). Unabsorbed material may be metabolised by bacteria in the colon but will also occur in the faeces. A problem associated with fat digestion, absorption and transport is that fat is insoluble in aqueous solutions such as blood, though the products of digestion are more hydrophilic and more easily dispersed. Lipids are therefore incorporated into lipoprotein complexes for transport through aqueous solutions (Table 9.3).

The duodenum is the major site of fat digestion but the stomach contributes by its churning action to create a coarse oil-in-water emulsion stabilised by phospholipids. Proteolytic digestion also releases lipids from food particles where they may be present as lipoprotein complexes. The fat emulsion entering the duodenum mixes with bile which acts as a powerful emulsifying agent and with pancreatic juice which contains lipase. Pancreatic lipase liberates fatty acids from the 1 and 3 positions of triacylglycerols. There is very little hydrolysis from the 2 position but acyl migration followed by hydrolysis may occur. Phospolipase A2 and a sterol ester hydrolase are also present to promote hydrolysis of phospholipids and sterol esters.

The rate of triacylglycerol hydrolysis depends on chain length. Short chain acids (C_8 and C_{10}) are hydrolysed faster and long-chain acids (C_{20} and C_{22}) are hydrolysed slower than the common C_{16} and C_{18} acids. Micelles are formed containing 2-monoacylglycerols, lysophospholipids, cholesterol and free acids,

Table 9.2 Amount and source of fat consumed in the UK in 2000/2001

Energy	Men 9.72 Mj (2313 kcal)		Women 6.87 Mj 1632 kcal)		Rec*	Meats*	Cereals*	Milk*	Fat*	Pots*
Fat	grams	en%	grams	en%						
Total	86.5	35.8	61.4	34.9	35	23	19	14	12	10
Saturated	32.5	13.4	23.3	13.2	11	22	18	24	11	7
Trans	2.91	1.2	2.04	1.2	2	21	26	16	18	6
Cis-monoene	29.1	12.1	20.2	11.5	13	27	17	10	11	12
n-3 polyunsat	2.27	1.0	1.71	1.0		17	17	4	7	17
n-6 polyunsat	12.9	5.4	9.49	5.3	6.5	18	20	3	14	13

* Rec = recommended, Meats = meat and meat products, Cereals = cereals and cereal products, Milk = milk and milk products, Fat = fat spreads, salad oils, frying oils, etc., Pots = potatoes and savoury snacks. Also included in the full tables are eggs and egg dishes, fish and fish dishes, vegetables (excluding potatoes), fruit and nuts, sugar, preserves, and confectionery, drinks and miscellaneous. These figures relate to men and women together. These figures relate to the National Diet and Nutrition Survey conducted with 1724 adults aged between 19 and 64. They are based on consumption over 7 days including food consumed outside the home between July 2000 and June 2001. Women who are pregnant or breastfeeding were excluded from the survey. Information taken from <www.nutrition.org.uk/ndnsadults> and <www.food.gov.uk/science/101717/ndnsdocuments/ndnsv2>.

Table 9.3 Human plasma lipoproteins

	Chylomicrons[a]	VLDL[a]	LDL[a]	HDL[a]
Protein	2	7	10	50
Triacylglycerol[b]	83	50	10	8
Phospholipid[b]	7	20	22	22
Cholesterol[b,c]	8	22	48	20
Density range	<0.95	0.98–1.006	1.019–1.063	1.063–1.210

[a] Major function: chylomicrons – transport of dietary fat, VLDL (very low density lipoproteins) – transport of endogenous fat, LDL (low density lipoproteins) – transport of cholesterol to peripheral tissues, HDL (high density lipoproteins) – reverse transport of cholesterol to liver.
[b] % particle mass.
[c] Free and esterified.
Adapted with permission from *The Report of the BNF Task Force,* p. 29.

Over 90 per cent of triacylglycerols are absorbed in this way but only about 50 per cent of the cholesterol esters are absorbed. Short and medium chain acids up to C_{10} are not incorporated into these micelles but are rapidly absorbed across the gut wall as free acids and find their way, bound to plasma albumin, via the portal vein, into the blood vessels supplying the liver where they are quickly metabolised. It is for this reason that medium-chain triacylglycerols are used therapeutically for patients unable to absorb long-chain acids and by athletes meeting large energy demands. The remaining metabolic products form mixed micelles with an outer core of amphiphilic materials.

The lipid hydrolysis products (monoacylglycerols, lysophospholipids, cholesterol, and fatty acids) pass into the enterocytes where lipids are resynthesised and assembled into the chylomicrons. Lipid digestion products are reconverted to triacylglycerols in the enterocyte by sequential esterification of 2-monoacylglycerols while appropriate enzymes promote re-formation of phospholipids and cholesterol esters. These products pass into circulation through the thoracic duct and are transported in the bloodstream to the liver (roughly 30%), fat deposits (about 30%) and the musculature and other organs (about 40%). The bloodstream also contains triacylglycerols and free acids (as albumin complexes) coming from the liver and the fat depots. During fasting, free acids from the fat depots circulate as albumin complexes and are removed either by peripheral tissue, where they are oxidised to provide energy, or by the liver, where they are oxidised or converted to triacylglycerols before being returned to the blood.

Dietary fat is transported as free acid to adipose tissue where it is converted to triacylglycerols. Endogenous fat, made mainly in the liver but also in other organs, is exported as VLDL into plasma. Cholesterol is carried to peripheral tissue in LDL and returned to the liver in HDL which acts as a scavenger for cholesterol.

9.3 Essential fatty acids

Some fatty acids required for animal health and wellbeing cannot be made by animals themselves and are therefore essential dietary requirements which must be obtained from plant sources. These acids are arranged in the families described in section 3.6 and two such families are of major importance.

One family consists of linoleic acid as the first or parent member along with its metabolites which are produced within a healthy animal so long as there is an adequate supply of linoleate (Scheme 4.14). Because of the structure of linoleic acid this family is described as n-6 or omega 6, the figure 6 indicating the position of the first double bond in the molecule with respect to the end methyl group. The most common metabolite in this family is arachidonic acid (20:4) which is the most important of three C_{20} molecules that act as precursors of the eicosanoids (section 7.3.5). Arachidonic acid in phospholipids (particularly phosphatidylinositols) is a vital constituent of cell membranes. The dietary levels at which these acids should be consumed is discussed in section 9.4.

The second important family of polyunsaturated fatty acids are those based on α-linolenic acid and recognised as n-3 or omega 3 acids (Scheme 4.14). The most important metabolites in this group are eicosapentaenoic acid (EPA, 20:5), docosapentaenoic acid (DPA, 22:5) and docosahexaenoic acid (DHA, 22:6).

The enzymes required for the metabolic changes of elongation and desaturation are the same for the n-6 and n-3 families and there is competition for access to the enzymes. While the conversion of linolenic acid to EPA and DHA is of paramount importance, it is not the major fate of the 18:3 acid in mass terms. Much of the linolenic acid is subject to β-oxidation to carbon dioxide and part of the intermediate acetate is converted to cholesterol and to saturated and monounsaturated acids. The value and purpose of this recycling process with polyunsaturated fatty acids, and particularly with linolenic acid, has been related to the need to sustain the optimal lipid composition of membranes, not only in terms of DHA, but also for levels of cholesterol and of saturated and monounsaturated acids. Much linolenic acid also finds it way into skin and fur.

Arachidonic acid is the precursor of series-2 prostaglandins and thromboxanes and series-4 leukotrienes, while eicosapentaenoic acid is the precursor of series-3 prostaglandins and thromboxanes and series-5 leukotrienes. These various eicosanoids have their own physiological properties, often working in opposite directions. Eicosanoids from arachidonic acid raise platelet activity and immune response while those from eicosapentaenoic acid have the opposite effect. Diets with too much linoleic acid will produce too much arachidonic acid and its metabolites and those with too little linolenic acid will produce too little EPA and its metabolites. To correct the balance it

may be necessary to increase the dietary intake of linolenic acid and, at the same time, to reduce the intake of linoleic acid which competes so strongly for the enzymes required for metabolic change.

Linoleic acid is readily available in many vegetable oils, including commodity oils such as soybean and sunflower (section 1.3) consumed in large quantities. Arachidonic acid is present in the human diet at much lower levels from animal products such as eggs and meat where the necessary metabolic changes have already been effected. Linolenic acid is less readily available in the diet of most people living in advanced societies. It is present at modest levels in soybean oil (~7%) and in rapeseed/canola oil (~10%) but these oils may have been subject to brush hydrogenation or to more extensive partial hydrogenation to reduce the level of linolenic acid (section 2.3.3). Additionally, seed breeders are striving to produce strains of these seeds with reduced levels of linolenic acid. Higher levels of this acid are present in speciality oils such as flaxseed oil (55–60%) and perilla oil (60–70%). EPA and/or DHA are present in oily fish (section 1.5.4) and are produced (at relatively high cost) by fermentation of microalgae and algae-like micro-organisms such as *Crypthecodinium cohnii* and *Schizochytrium sp.* By incorporating appropriate material into animal feeds it is possible to produce meat from non-ruminants and eggs with higher than normal ranges of these acids. For example, omega eggs with enhanced, but still low levels, of DHA are available in supermarkets.

The optimum balance of n-6 and n-3 acids in the human diet has become a contentious issue. When the humans were hunter-gatherers, eating lean meat, fish, green vegetables, fruit, nuts, and berries and exercising heavily and regularly, this ratio was probably between 1:1 and 3:1. Today in most advanced countries, and especially those such as the United States where diets are strongly based on soybean oil, the figure is around 10:1 (or higher). It is lower in countries such as Japan and the Mediterranean and Scandinavian countries where there is a higher consumption of fish. While many claim that the ratio should be at the lower end of a range between 5 and 10 to 1, there are others who argue for a ratio closer to 2:1 or even below that. These figures relate to adults, but they are different for babies, especially preterm infants and may be different again for the growing number of people of advanced years. There is a further question related to the efficiency of the conversion of linolenic acid to EPA and DHA. How much of the n-3 intake should be as linolenic acid from non-hydrogenated vegetable oils, and how much as EPA and DHA from marine sources? There are several problems concerning fish consumption. It is not always easily accessible, it is expensive, many people prefer not to eat it, others are unwilling to prepare fish dishes in the home, and it is not acceptable to most vegetarians. Also there are questions as to whether it is contaminated with undesirable material because of pollution of the oceans and finally whether there is enough to provide for everybody in a time of

diminishing stocks. It is for some of these reasons that plant geneticists are striving to develop plants that produce these long-chain polyunsaturated fatty acids in their seed oils. Proof of concept has been achieved in the laboratory and the green-house but it will take some years to produce such material on a commercial basis at an acceptable cost.

In the meantime, blends of vegetable oils are being marketed with what are claimed to be optimum ratios of polyunsaturated fatty acids. A Danish product ('Nutridan') is a blend of cold-pressed vegetable oils with a high content of linolenic acid, a n-6/n-3 ratio of less than one, and with added natural antioxidants from rosemary leaves to protect this highly-unsaturated oil from oxidative deterioration. It is claimed that this blend can be used in baked goods, to make spreads and mayonnaise, and can also be taken in capsule form (Shukla, 2003). A Canadian product is reported to contain coconut, olive and flaxseed oils.

Recent studies have suggested the importance of an appropriate balance between four categories of polyunsaturated fatty acids: n-6 and n-3 C_{18} acids and n-6 and n-3 $C_{20/22}$ acids. There is a growing consensus that in many diets, the consumption of n-3 acids, and particularly of EPA and DHA, is too low and that the consumption of linoleic acid is too high.

Table 9.4 contains information on these four groups of acids in a range of oils. Taken from an NIH website (National Institute of Health – USA), the values are expressed in units of mg/tablespoonful, presumably considered to be more user-friendly and to correspond with mg/5 mL of oil. It is clear from this selection that while linolenic acid is available in several vegetable sources

Table 9.4 Quantity (mg/tablespoonful) of n-6 and n-3 C_{18} and $C_{20/22}$ PUFA in some common food oils

Oil	18-carbon atoms		20/22-carbon atoms	
	n-6	n-3	n-6	n-3
Flaxseed	2240	7980	0	0
Salmon	210	525	92	4657
Sardine	274	592	239	3096
Cod liver	127	254	127	2557
Butter	288	185	0	0
Rape/canola	2842	1302	0	0
Soybean	6936	925	0	0
Soybean (ph)*	4746	354	0	0
Cocoa butter	381	14	0	0
Palm	1238	27	0	0
Corn	7888	95	0	0
Coconut	245	0	0	0
Sunflower	5413	27	0	0
Sunflower (HO)*	1952	0	0	0

* ph = partially hydrogenated, HO = high oleic.

Table 9.5 Comparison of average diets in Japan, Mediterranean countries, and the United States

Acid group	Japan	Mediterranean	United States
18:3 n-3 en%	0.90	0.50	1.00
18:2 n-6 en%	6.30	2.30	6.50
HUFA n-3 en%	1.30	0.35	0.03
HUFA n-6 en%	0.08	0.08	0.08
n-6/n-3 ratio	2.9	2.8	6.4
n-6 PUFA in tissues (%)	51	53	83

Source: NIH website.

the long-chain n-3 acids are only conveniently available from fish oils and that these also contain some long-chain n-6 acids.

The figures in Table 9.5 provide a comparison of four groups of polyunsaturated fatty acids in the average diets of Japan, Mediterranean countries and the United States. The n-6/n-3 ratio is much lower in Japan and the Mediterranean region than in the United States but this reduction is achieved in different ways. Compared to those in the United States, the Japanese consume virtually the same level of n-6 acids but reduce the n-6/n-3 ratio by consumption of higher levels of n-3 acids of all chain lengths. In contrast, dwellers in the Mediterranean region have an n-3 intake lower than in the United States but their intake of n-6 acids is also much lower. This is a consequence of their high consumption of olive oil in place of linoleic-rich vegetable oils. The values of n-6 PUFA in the tissues are calculated from dietary intake using equations developed by Lands (Lands *et al.*, 1992). They show how the level of polyunsaturated fatty acids in the tissues is related in a complex way to the dietary intake of all of the four types of the acids discussed in this paragraph.

Omega 3 acids have three important physiological functions:

1. As major components of biological membranes they are important in membrane structure and function. DHA is present in high concentrations in the retina, the brain and in sperm.
2. They can alter gene expression – down-regulating some enzymes and up-regulating others.
3. Finally EPA has an important role in the regulation of eicosanoids from arachidonic acid through competition for the metabolising enzymes. The eicosanoids from EPA and AA show different properties.

So how effectively is α-linolenic acid metabolised to DHA? In animal studies DHA is reported to be a more effective source of tissue DHA than α-linolenic acid by a factor of 7–20. Feeding α-linolenic acid leads to an increase in tissue α-linolenic acid, to small increases in EPA and DPA (22:5) in tissue, and to an even smaller increase in DHA. Results vary in different tissues and plasma levels, though easy to measure, are not always a good indication of tissue

levels. *In vivo* conversion of α-linolenic acid to EPA and DHA is a slow process and not as effective as direct consumption from fish lipids. Reducing the intake of linoleic acid assists conversion of α-linolenic acid to PUFA. Δ6-desaturation is rate-limiting in both the n-3 and n-6 series.

Why is there a need for enhanced intake of n-3 acids and of the long chain members in particular? Intakes of LC-PUFA are generally below recommended levels. For example, the average UK level of 215 mg/day is to be set against recommendations of 1.25 g/day by the British Nutrition Foundation and of 650 mg/day by a US scientist (Simopoulos). Average intakes for Australia and the United States at <100–200mg/day are also far short of the recommended levels. Linolenic acid is present in selected seed oils (unless these have been hydrogenated) and in green vegetables (up to 200 mg/100 g of fresh product). The long-chain members are present in fish and fish oils and, at lower levels, in meat, offal and eggs. Visible meat fat contains 0.5–1.0 per cent of these acids but there is only 0.1 per cent in lean meat. Novel sources of LC-PUFA have been developed from algae and microalgae and can be incorporated into infant formula at levels similar to those in breast milk. This is particularly important for pre-term infants (Knapp *et al*., 2003). Similar sources can be used as animal feed to produce eggs, chicken and pork meat enriched in DHA. There are however practical problems with these highly unsaturated materials linked to their susceptibility to oxidation. They must be supplied with appropriate antioxidants and/or they may be provided in encapsulated form with the capsule shell acting as a barrier to air/oxygen.

The biological importance of n-3 polyunsaturated fatty acids for human health is apparent in several ways. It is known that:

- blood pressure is reduced by DHA though EPA has a lesser effect,
- plasma cholesterol levels (total and LDL) and serum triacylglycerol levels are reduced by n-3 acids, especially those from marine sources,
- thrombosis risk is decreased by n-3 acids and particularly by long-chain polyunsaturated fatty acids from fish,
- there is strong support for the use of n-3 fatty acids in the secondary prevention of acute coronary syndromes (the mechanism by which n-3 marine acids protect against cardiac death is not known with certainty, but it may relate to their ability to prevent cellular damage during periods of ischemic stress),
- animal studies suggest n-3 acids reduce or protect against tumour development, though limited human studies are inconclusive,
- n-3 acids (especially EPA and DHA) provide benefits for sufferers with arthritis or Crohn's disease,
- neuropsychiatric disorders such as depression and schizophrenia may benefit from diets with n-3 acids and EPA is being examined intensively for this purpose.

9.4 Recommendations for dietary intake

Dietary requirements apply to healthy adults. Different recommendations may be appropriate for those who are not in good health, for babies and children under five years, for pregnant and lactating females and for older people. Dietary advice to the general population should always be part of a package that includes advice to stop smoking, to take more exercise, to maintain a healthy weight and to relieve stress. Frequently there is undue emphasis on fat consumption and the other factors are forgotten.

Dietary recommendations have been made in most developed countries. These tend to be fairly similar. This is not surprising for it takes a measure of courage (or stupidity) to differ from everyone else. It seems likely that few of those giving the advice have read the original research reports on which the advice is based. Many of these recommendations are based on the need to lower plasma cholesterol levels to reduce CHD. The following statements seek to present an overview of the general recommendations relating to fat. In summary they represent a call to limit total fat and saturated fat and to get the n-6/n-3 ratio right (when we can agree on what that should be).

Total fat intake ranges from 15–40 per cent energy across most of the world's population. Some have argued that the maximum should be 35 per cent as a practicable first step for those consuming excess of this figure. The prevalent view is that the maximum should be 30 energy per cent and that it may be desirable to be below this level.

Saturated acids are generally cited as 10 energy per cent maximum though one group recommends that this be below eight per cent. However, saturated acids represent a class and individual members have different nutritional properties. Acids with up to ten carbon atoms are metabolised in a different way from those with longer chains and need not be included for the purpose of this recommendation. This will have a marginal effect for dairy fats and for lauric oils. Also, it has been claimed that stearic acid has a smaller effect on plasma cholesterol levels, perhaps because it is so easily converted to oleic acid through the activity of the ubiquitous $\Delta 9$ desaturase. This leaves lauric (12:0), myristic (14:0), and palmitic (16:0) acids (section 9.7.2). Myristic acid has the largest influence on serum cholesterol but palmitic is the predominant dietary saturated acid (section 9.5).

It is recommended that levels of *trans* acids should not exceed 2 energy per cent (or 1 per cent in one proposal). There are three major dietary sources of acids with *trans* unsaturation: dairy fats and meats from ruminant animals, vegetable oils which have been subject to partial hydrogenation, and vegetable oils which have been heated to high temperatures during refining. In general, intakes of such acids have been reduced over the last 5–10 years, particularly in Europe, by changing the technology by which spreads are made. There is some risk that this is achieved at the cost of a small increase in levels of

saturated acids (sections 4.3.4 and 4.3.5). In the United States where fat consumption is based so heavily on soybean oil, much of it hydrogenated, ingestion of *trans* acids tends to be higher but there is growing pressure to reduce levels of *trans* acids here also.

Recommendations concerning polyunsaturated fatty acids relate to n-6 acids and n-3 acids and it may be necessary to have separate recommendations for the C_{18} members (linoleic and linolenic) and for the $C_{20/22}$ members. Dietary intake of n-6 PUFA will be mainly as linoleic acid and only to a small extent as arachidonic acid (20:4). It has been suggested that the n-6 acids should be around six energy per cent with 3–10 per cent representing a safe range but there is growing opinion that even these levels might be too high and arguments have been put forward for an intake of only two per cent and a maximum of three per cent. It has been calculated that with about 20–40 per cent of human body mass being fat and 15–20 per cent of body fat being linoleic acid, an adult American will have more than three kilograms of linoleic acid in his/her tissues. This large reservoir of n-6 acid may need three years to equilibrate with a change in dietary lipids.

For n-3 acids, separate recommendations are given for linolenic acid and for EPA/DHA. One authority suggests an intake of one energy per cent within a range of 0.5–2.5 per cent for linolenic acid and 0.5 per cent within a range of 0–2.0 per cent for EPA and DHA. Another group have expressed this slightly differently at 1.0 per cent for linolenic acid and 0.3 per cent for the higher acids with a minimum of 0.1 per cent for each of EPA and DHA.

The balance of dietary fat should consist of *cis* monounsaturated acids (almost entirely oleic acid) and this becomes particularly important when total fat intake exceeds 30 energy per cent. It is considered that oleic acid should represent 11–16 energy per cent of dietary intake and should not exceed this upper limit**.** Delplanque & Mendy (2003) recommend a ratio of oleic, linoleic and α-linolenic acid of (11–16):(4–6):1.

Because DHA is such an important component of the brain appropriate supplies of this acid are required by a developing foetus and will be supplied by the mother via the placental cord. This need continues after birth and should be met through human breast milk which in a well-nourished mother will contain adequate levels of DHA. Problems arise when the child is not breast-fed and, more significantly, in pre-term infants since brain development is particularly marked in the final trimester before birth. It is now recommended that during pregnancy and lactation dietary fat intake should be as for other adults except that the intake of DHA should be at a minimum level of 300mg/day. Infant formula for Western countries, expressed as percentage of fatty acids, should contain linoleic (10%), α-linolenic (1.50), arachidonic (0.50), docosahexaenoic (0.35) and eicosapentaenoic acid (<0.10). Slightly different levels are used in Japan with linoleic acid at 6–10 per cent and DHA at 0.6 per cent.

9.5 Cholesterol and phytosterols

Sterols are important minor components of most oils and fats (section 1.6.3). Those of plant origin contain a range of phytosterols while those coming from animal sources are rich in cholesterol. Typical levels of the latter for lard (0.4%), beef fat (0.1%), mutton tallow (0.2–0.3%) and butter (0.2–0.4%) are indicated in parenthesis. Eggs contain around 300 mg of cholesterol per egg. The sterol may be present as free sterol or associated with a fatty acid as sterol ester (section 1.6.3).

The human body contains around 100 g of cholesterol and requires about 1 g of new cholesterol each day. Total cholesterol represents around 0.2 per cent of body weight with one third in the brain and nervous tissue, one third in muscular tissue and the remainder in cell membranes. The daily requirement will be mainly of endogenous origin (600–1000 mg) with the balance from dietary sources (250–500 mg) coming mainly from eggs (~300 mg per egg) and animal fats. Only about 50 per cent is absorbed. Phytosterols interfere with the absorption of cholesterol and are being added to spreads to reduce cholesterol uptake (section 9.8.2). Cholesterol is transported in the blood as lipoproteins (Table 9.2)

Cholesterol is used for the production of bile salts (emulsifiers) in the liver, steroid hormones (e.g. sex hormones) in the adrenal glands, and of vitamin D in the skin. Elevated cholesterol levels in blood are recognised as one of several risk factors in cardiovascular disease. This level can be easily measured and is frequently used as an index of cardiovascular wellbeing. Levels vary between individuals. They are only slightly influenced by dietary intake of cholesterol and rather more by saturated fatty acids and by non-dietary influences. As discussed later (section 9.7.2) levels above around 230 mg/100 ml of blood (~6.0 mmol/L) are considered to be undesirable in terms of cardiovascular disease (section 9.7.2) but levels below 160–180 mg/100 ml may lead to risk of non-cardiovascular death.

The possible link between cholesterol oxidation products and coronary heart disease and other disease states makes it appropriate to discuss the source and formation of such compounds. Cholesterol (section 1.6.3) contains a cyclic double bond ($\Delta 5$) and two tertiary carbon atoms in its side chain (C_{20} and C_{25}), all sites where oxidation may occur (section 7.2.4.4). Some cholesterol oxides are produced as part of the normal metabolism of cholesterol to bile acids but at higher levels they affect human health by contributing to the development of atherosclerosis. When cholesterol oxides replace cholesterol in the cell membrane they alter its fluidity, permeability, stability, and other properties.

Oxidised animal-based foods represent a primary source of oxidised cholesterol. Such products are not present in fresh foods but are formed during handling prior to consumption, mainly through autoxidation. Cholesterol

esters are predominantly of linoleic acid while free cholesterol is associated with polyunsaturated fatty acids in phospholipids in cell membranes. In both cases, oxidation can be initiated in the polyunsaturated fatty acids and then involve the cholesterol molecule. This holds in the animal (before being prepared as food) and in the human, and in both, oxidation can be retarded by appropriate dietary antioxidants. Processing conditions should also be adapted to minimise oxidation, for example in the preparation of spray-dried eggs. Between 0.5 and one per cent of dietary cholesterol may be oxidised and the primary oxidation products include 7-α-hydroxy-, 7-β-hydroxy and 7-keto-cholesterol, cholesterol α- and β-epoxides, 3,5,6-trihydroxycholesterol and 20- and 25-hydroxycholesterol (Cuppett, 2003).

9.6 Conjugated linoleic acid (CLA)

C_{18} acids with two double bonds conjugated with each other were recognised as trace components of milk fat over 50 years ago and have engendered renewed interest in recent years following the recognition of their potential value in the treatment of cancer, obesity and diabetes.

CLA has been identified at low levels in milk fat (3–6 mg/g of total fat), butter fat (12–14), cheeses (2–20) and in lamb and beef meat (4–5). 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). It 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.

9*c*12*c*-18:2 → 9*c*11*t*-18:2 → 11*t*-18:1 → 9*c*11*t*-18:2 (see also Fig. 7.1)

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 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 separate fractions. This is best achieved using enzymes (such as that from the fungus *Geotrichum candidum*) which distinguish between these two isomers. The products of the isomerisation process are free acids which 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.

Several potential benefits have been claimed for CLA. Dietary supplementation has been shown to reduce the number and size of mammary tumours with the 9*c*11*t* isomer probably the more effective. CLA has also been used to alter body composition since it is claimed to reduce body fat, increase lean mass and improve feed efficiency and these effects are associated particularly with the 10*t*12*c* isomer. Positive results have been obtained with young animals and this is of interest to those concerned with meat production. Conclusions are less certain with humans.

It has also been claimed that in animals, CLA affects the immune function and has an effect on bone remodelling. In animal husbandry, attempts have been made to increase CLA levels and also to decrease fat production mainly by adjustment of dietary regimen. Increasing CLA levels in poultry meat and in eggs is seen as a potential method of increasing levels of CLA in the human diet.

9.7 Role of fats in health and disease

Modern medicine and modern standards of hygiene have freed the world, or at least many parts of it, from the killer diseases of previous centuries such as tuberculosis, smallpox and diphtheria and we inhabit a world where increasing numbers live healthier and longer lives. This has highlighted the diseases that remain and are killers not only of the old but of men and women in their prime, particularly coronary heart disease and cancer. We are increasingly aware that many diseases that remain, whether they are killers or not, are related in some part to life-style, of which diet, pollution of the environment, and level of physical activity are important factors. The following sections are related to the role of fat in some disease conditions. However it is important to realise that fat is only part of our diet and that diet is only part of the problem. It is inadvisable to focus on a single issue and ignore others. Fat has a very negative image at the present time and we need to correct that. Fat is an essential part of the diet and is linked to good health as well as to disease. It is important to optimise the quality and the quantity of fat consumed in relation to other aspects of lifestyle. This implies that we know what fats we should consume and in what quantity, that agriculture and the food industry can supply these and finally that we can persuade human populations to choose healthy and affordable diets. We must recognise that we are a long way from discovering the final truth in respect of dietary fat and that what is written in the following sections simply represents present views that may have to be modified in the light of new and further research. This is one of those areas of life in which ‘time makes ancient good uncouth’.

9.7.1 Obesity

Body mass index (BMI) is used increasingly as a measure of weight to height ratio and allows us to recognise five categories of body sizes. The body mass index is defined as weight (expressed in kg) divided by height squared (expressed in cm) and one classification is:

- Underweight <18.4
- Normal 18.5–24.9
- Overweight 25.0–29.9
- Obese 30.0–39.9
- Severely obese >40.0

A growing number of persons fall into the last three categories, probably as a consequence of imbalance over many years between increased caloric intake and decreased energy requirement resulting from more sedentary and less active lifestyles. An alternative indicator based on shape, relates waist circumference to height (Ashwell, website). In tackling this problem, attention is focused on fat because it is the most energy-dense of our nutrients. It is worth noting that the human race has developed thoughout its evolution in situations where lack of food was a common occurrence and surplus food, over anything but short periods of time, was virtually unknown. The human system did not need to be designed to deal with such a problem.

The problem is not new, even if it is becoming more widespread. Shakespeare has Henry tell Falstaff 'Leave gourmandising. Know that the grave doth gape thrice wider for thee than for other men.'

The problem of obesity is partly genetic (40–70%) and partly environmental (food intake and physical inactivity). Attention is often focused on long hours spent watching TV where inactivity is often accompanied by poor eating habits. In the United States nearly two thirds of the population is overweight or obese and almost 40 per cent are clinically obese. Concern is growing about the increase of obesity in children and adolescents.

In 1991, deaths associated with obesity in the United States (300 000) were second only to deaths associated with smoking (400 000) and it is likely that in the intervening years these numbers have become closer. Obesity is a potent risk factor for type-2 diabetes, hypertension and dyslipidemia. The average American seeking treatment for obesity weighs around 100 kg. However, it is worth noting that while fat intake in the United States fell on the basis of energy percentage from around 42 per cent in the 1960s to around 35 per cent in 1990, fat intake in terms of g/day fell between the 1960s and the 1980s then stabilised and began to increase in the mid-1990s.

In Europe, obesity figures are also on the increase and it is reported that 20 per cent of men and 25 per cent of women in the UK are obese, that these

levels have tripled in the last twenty years and that 9000 deaths a year are associated with this condition at a cost of £2.5 billion. Obesity is a major public health problem throughout Europe, especially among women in Southern and Eastern European countries. These countries are also among the highest for CVD. In Europe, the treatment of obesity-related diseases accounts for eight per cent of all medical costs.

There are factors other than dietary fat that are important in obesity and attention has been drawn to the beneficial role of dietary calcium in the partitioning of dietary energy, resulting in reduction in body fat and acceleration of weight loss and fat loss during periods of energy restriction. Dairy sources of calcium exert substantially greater effects than supplemental or fortified sources of calcium. This is considered to have important implications in the prevention of pediatric and adult obesity particularly in the light of the marginal calcium intakes exhibited by the majority of the population.

In discussing diet, obesity, and cardiovascular risk, Bonow & Eckel (2003) write:

> The recipe for effective weight loss is a combination of motivation, physical activity, and caloric restriction; maintenance of weight loss is a balance between caloric intake and physical activity with lifelong adherence. For society as a whole prevention of weight gain is the first step in curbing the increasing epidemic of overweight and obesity … physicians should recommend a healthy lifestyle that includes regular physical activity and a balanced diet.

For dietary fat they recommend: total fat 33 energy per cent, saturated acids 10 per cent, polyunsaturated fatty acids 6 per cent (and not exceeding 10%), *cis* monounsaturated acids 12 per cent, and *trans* unsaturated acids <2 per cent.

9.7.2 Coronary heart disease (CHD)

It has long been known that the blood system is important as a means of moving oxygen and nutrients from cell to cell and from organ to organ and as part of the system by which animals dispose of waste products. This system depends on a complex series of channels of wide-ranging diameter through which blood flows and through which gases and liquids pass from cell to blood stream and vice versa, and of a pump to force the necessary movement. All these have to last for the whole life and any fault with the pump (heart) or the channels (veins and capillaries) will cause difficulty.

Cardiovascular disease is a broad term embracing diseases of the blood vessels of the heart, brain (cerebrovascular disease, stroke) and the limbs (peripheral vascular disease). CVD is usually a culmination of atherosclerosis

(accumulation of material – plaque – in the walls of arteries of cells comprising connective tissue, lipids, calcium and debris resulting from cellular breakdown) and thrombosis

Coronary heart disease (CHD) is a major cause of death in the developed world with a peak age of death of 70–74 for men and 75–79 for women, but its too-common occurrence at an earlier stage in life is of greater concern. There are three stages in the development of CHD. Initial arterial injury leads to deposition of lipid and cell material (atherosclerosis) and to small blood clots (thromb) which contribute to the build up of fibrous plaque. Finally, instability of the plaque triggers formation of a major blood clot (thrombus) in the already-narrowed artery. This gives the potential for the blood (and oxygen) supply to the heart muscle to be blocked completely leading to myocardial infarction (heart attack). More simply, the three stages are: injury of coronary arteries, fibrous plaque formation and thrombosis leading to heart attack or stroke. The following have been recognised as risk factors: high blood pressure, high levels of plasma LDL (low density lipoprotein) cholesterol, low levels of plasma HDL (high density lipoprotein) cholesterol, high levels of plasma fibrinogen and low levels of plasma antioxidants. These risk factors are linked to a range of controllable and uncontrollable factors. The uncontrollable factors are family history, being male, advancing age, racial origin (Asians show higher rates of incidence than white Caucasians) and possibly low birth weight. Controllable factors include smoking, exercise (lack of), stress and diet. Serum cholesterol level should be below 230 mg/100 ml but low cholesterol levels (below ~160/180 mg/ml) are also undesirable.

The lipid hypothesis in respect of CHD is concerned with the relationship of blood cholesterol and saturated fatty acids (SFA) with CHD mortality. Diets with a high content of fat/SFA/cholesterol lead to high concentrations of total cholesterol in the blood and especially of LDL-cholesterol which results in a high morbidity and mortality from CHD. However, reducing the amount of fat/SAF/cholesterol in the diet reduces the concentration of cholesterol in the blood and especially in the LDL. This results in a lower risk of CHD and eventually a fall in morbidity and mortality. There is also, however, some concern how far reduced CHD mortality is linked to dietary changes and how far it is related to improved methods of treatment which reduce mortality levels.

This hypothesis is the basis of much dietary advice relating to fat consumption though it should be noted that there are those who dispute this proposal and have mounted strong arguments against it (Gurr, 1999). Once the link between cholesterol blood levels and fatty acid intake was accepted it became apparent that blood cholesterol levels could be raised or lowered by dietary changes in fatty acid composition and attempts were made to express these changes in the form of mathematical equations. These predictive

Table 9.6 Equations relating changes in plasma cholesterol levels (total and LDL) with changes in dietary fat intake

Keys *et al*. (1957)	
	$\Delta TC = 0.0621\Delta S - 0.0310\Delta P$
Pedersen *et al*. (2001)	
	$\Delta TC = 0.01\Delta(12{:}0) + 0.12\Delta(14{:}0) + 0.057\Delta(16{:}0) + 0.039\Delta(\textit{trans}F) + 0.031\Delta(\textit{trans}V) - 0.0044\Delta(18{:}1) - 0.017\Delta(18{:}2,18{:}3)$
	$\Delta LDL\text{-}C = 0.01\Delta(12{:}0) + 0.071\Delta(14{:}0) + 0.047\Delta(16{:}0) + 0.043\Delta(\textit{trans}F) + 0.025\Delta(\textit{trans}V) - 0.0044\Delta(18{:}1) - 0.017\Delta(18{:}2,18{:}3)$

S = saturated acids, P = polyunsaturated acid, F and V are *trans* acids present in partially hydrogenated fish and vegetable oils respectively. Results are expressed in mmol/L and can be converted to mg/dL by multiplying each coefficient by 38.67.

equations can be used to estimate changes in plasma cholesterol (both total and LDL cholesterol) following changes in fatty acid intake. They are related to changes in cholesterol levels and not to absolute values. The earliest of these equations (Keys *et al*., 1957) covered only saturated and polyunsaturated acids (expressed as percentage of energy), but later versions include *cis* monounsaturated acids, *trans* monounsaturated acids, and some even distinguish between individual fatty acids. The Keys equation and the latest by Pedersen *et al*. (2001) are given in Table 9.6. The Pedersen equation has been used by the Norwegian food industry to reformulate margarines with less of the cholesterol-raising fatty acids.

The Keys equation of 1957 shows that a rise in saturated acids leads to a rise in cholesterol levels, that polyunsaturated acids have the opposite effect and that monounsaturated acids were considered to be neutral. It was this conclusion that led to the development of spreads with reduced content of saturated acids and increased levels of linoleic acid, often based on sunflower oil. The coefficients indicate that the beneficial effect of polyunsaturated acid is only one half of the undesirable effect produced by raising the level of saturated acids. The more recent Pedersen equation of 2001 distinguishes three saturated acids and ignores those of shorter or longer chain length. Lauric acid has only a minor effect and myristic is greater than palmitic acid. *trans*-Acids also raise cholesterol levels with a different effect for those from fish oils and those from vegetable oils. Including five equations not detailed here the regression coefficients for saturated acids lie in the range +0.56 to +0.50 and for polyunsaturated acids in the range −0.15 to −0.31.

These equations relate to fatty acids present in the diet as glycerol esters and this requires another factor to be taken into account since there is good evidence that saturated fatty acids are more atherogenic in the 2-position. For example tallow and lard both contain about 24 per cent of palmitic acid but differ in that in lard almost all the palmitic acid is in the 2-position. Lard is much more atherogenic than tallow but after interesterification (randomisa-

tion) both fats have about eight per cent of palmitic in the 2-position and are equally atherogenic. This effect may be related to the fact that palmitic acid in the 2-position is absorbed more efficiently and corresponds to an increase in dietary saturated acid. Similar effects have been observed with synthetic triacylglycerols and with appropriate vegetable oils.

One study of CHD concludes that fats in the diet should not exceed 33 energy per cent with saturated acids (10%), polyunsaturated fatty acids (6 per cent and not exceeding 10%), monounsaturated fatty acids (12%) and *trans* acids (<2%) at the levels indicated. This is accompanied by advice to eat more fruit and vegetables, more starch foods, more oily fish and less full-fat dairy products, fatty meat and meat products, spreadable fats and high-fat bakery products, to choose low fat options where possible and to use less salt. Dietary advice to the general population should always be part of a package that includes advice to stop smoking, to take more exercise, to maintain a healthy weight and to relieve stress.

The evidence for a beneficial role for long-chain n-3 polyunsaturated fatty acids is becoming stronger, especially for secondary prevention. Intakes of 800–1000 mg/day are considered to be prudent for those at risk of a secondary attack. At the same time, high intake of linoleic acid should be discouraged because of its antagonistic effect on the incorporation of n-3 acids into membranes. The minimum that can be claimed is that 'consumption of long-chain n-3 acids may reduce the risk of CHD'. EPA-derived leukotrienes have less potent leukocyte activating effects than AA-derived leukotrienes and at least part of the anti-atherogenic mechanism of the n-3 acids is likely to be due to their effect on eicosanoid metabolism.

9.7.3 Diabetes

Diabetes mellitus is a chronic disease in which the metabolism of sugars (and of fats and proteins) is disturbed by lack of or by decreased activity of the hormone insulin, produced by the endocrine part of the pancreas. Its main characteristic is an increase in the level of blood sugar provoking acute symptoms such as thirst, frequent voiding and weight loss. The incidence of this disease is increasing all over the world and it is predicted that it will affect 210 million people by 2010. Diabetes is an independent risk factor for CVD.

Type 1 diabetes, representing only about 15 per cent of cases, is found particularly in children, adolescents and young adults. It results from auto-immune destruction of the insulin-secreting cells of the pancreas. Production of insulin declines and eventually ceases. However, most diabetic individuals (85%) have type 2 diabetes. Two dysfunctions are involved: decreased insulin secretion after a glucose challenge and a decrease in its activity on target organs (liver and muscles). This is called insulin resistance. Obesity is a major pre-disposing factor of this type of diabetes which is largely determined by

genetic factors. The metabolic consequences of this defect may not be apparent until the appearance of chronic complications.

One discussion on the nutritional management of this disease suggests that individuals with normal body weight and normal lipid levels should limit fat intake to less than 30 per cent total energy with saturated fatty acids restricted to 10 per cent, polyunsaturated acids to less than 10 per cent, and monounsaturated acids at 10–15 per cent. Those with elevated LDL levels should reduce saturated acids to seven per cent and cholesterol intake to less than 200 mg/day. However, n-3 polyunsaturated fatty acids should not be restricted. Aspects of nutritional management of this disease other than fats are not included here.

It is known that the various desaturases involved in the conversion of C_{18} polyunsaturated fatty acids to the important acids of longer chain length such as AA, EPA, and DHA are decreased in diabetic patients (section 4.5.4). As a consequence, the phospholipids in tissue lipids contain more saturated and monounsaturated acids and less LCPUFA, especially AA. This, in turn, affects membrane fluidity and eicosanoid production.

9.7.4 Inflammatory diseases

Inflammation is characterised by swelling, redness, pain and heat in localised areas of the body. These symptoms result from a series of interactions between cells of the target tissue, cells of the immune system, their products such as eicosanoids, cytokines, immunoglobulins and blood components.

The eicosanoids (prostaglandins, leukotrienes, hydroxy and hydroperoxy fatty acids and molecules such as PAF) are all regulators of inflammation affecting vascular permeability, vasodilation and vasoconstriction, platelet aggregation and deaggregation, further eicosanoid synthesis and serve as chemotactic agents. Dietary lipids modulate inflammation and influence the course of diseases such as arthritis, psoriasis, asthma, and inflammatory bowel disease mainly through changing the production of eicosanoids particularly by macrophages and neutrophils. These effects are the consequence of changing n-6/n-3 ratios.

Inflammation is a component of a range of acute and chronic human diseases characterised by the production of inflammatory cytokines, AA-derived eicosanoids, inflammatory mediators such as platelet activating factor, and adhesion molecules. Polyunsaturated fatty acids of the n-3 series act directly by inhibiting AA metabolism and indirectly by altering the expression of inflammatory genes. They are considered to be of therapeutic value for a variety of acute and chronic inflammatory conditions. But what is the appropriate balance between n-6 and n-3 acids? This may differ at different parts of the life cycle such as early development and aging and has yet to be determined.

9.7.5 Psychiatric disorders

In view of the importance of brain phospholipids and their component acids it is not surprising that the relation between dietary lipids and psychiatric disorders such as schizophrenia and depression have been investigated. Schizophrenic patients are known to have lower levels of polyunsaturated fatty acids (especially linoleic and arachidonic) in their brain phospholipids. This may be the consequence of increased phospholipid hydrolysis and/or decreased incorporation. The role of n-3 acids and especially EPA is under active consideration.

9.7.6 Cancer

The possibility of a link between cancer and dietary fat has received intensive study but no consensus has emerged. Such studies are complicated by the fact that cancers in different organs may react differently to dietary fats. Much attention has been given to the possible beneficial treatment of breast cancer with CLA (section 9.6) but there is still no final conclusion.

9.8 Functional foods

There is a growing realisation of the link between diet and health. This is important to the growing number of older people who want to live an active and healthy life as long as possible, to governments concerned about increasing health costs, and to the food industry oppressed by small margins on most conventional foods and looking for new products with higher profit margins. In the 30-year period 1995–2025, it is estimated that in the developed world the number of people over 80 will increase by 50 per cent and the number of those over 90 will double. All this is linked with concerns that the food chain should deliver safer and healthier foods and to changed eating habits where less food is prepared in the home kitchen and is either eaten out of the home or is prepared in the factory for home consumption. Under both these conditions there is some loss of control over the selection of food to be consumed. These trends are of interest to scientists and nutritionists seeking to improve the diet, to the food industry, to government and to the media. This increasing interest is especially marked in Japan where for several years there has been a system in place for recognition of ‘foods for specific health use’ or FOSHU with its own distinctive logo. Many other developed countries such as Finland have also shown considerable interest in this topic.

Functional foods contain bioactive molecules that promote health or reduce the risk of disease and are consumed as conventional foods at levels not very different from normal. A recent book (Gunstone, 2003) devoted to this topic

includes significant chapters on antioxidants (carotenoids, tocopherols, flavonoids, rosemary oil, rice bran oil and sesame oil), diacylglycerols, phytosterols, structured lipids (section 4.3.5), n-3 acids (section 9.3) and conjugated linoleic acid (section 9.6). Some of these have been covered in earlier sections of this chapter and others are discussed below.

9.8.1 Diacylglycerols

A cooking oil rich in diacylglycerols has been available in Japan since 1999. It is made by reaction of glycerol or 1-monoacylglycerol with fatty acids from natural edible oils using a 1,3-regiospecific lipase. The product is at least 80 per cent diacylglycerol of which around 70 per cent is the 1,3-isomer. This figure should be higher but some acyl migration occurs during the refining processes required to obtain the final product. Similar products are now being developed for the US market.

There is also evidence that phytosterols (sections 9.8.2) may be more effective in reducing blood cholesterol levels when taken in diacylglycerols rather than in triacylglycerols because of their greater solubility in the former. A diacylglycerol-rich oil with added phytosterols, available in Japan since 2001, is sold with permitted claims such as ‘it is less likely to become body fat’ and ‘it lowers blood cholesterol levels’ (section 9.8.2).

The different physiological effects of diacylglycerols and triacylglycerols, observed in both animals and humans, are not caused by differences in energy values (38.9 kJ/g and 39.6 kJ/g respectively) or of absorption of the fats (both 96.3%) but by their differing metabolic fates after absorption. 2-Monoacylglycerols resulting from normal triacylglycerol digestion are readily reconverted to triacylglycerols in epithelial cells but the 1-monoacylglycerols resulting from the diacylglycerol preparations are only poorly re-esterified.

In animal studies, the structural differences between diacylglycerols and triacylglycerols markedly affect their nutritional properties including body fat accumulation, serum lipid profile and the development of hyperinsulinemia and hyperleptinemia.

Clinical double-blind studies in humans have shown that it is possible to reduce the magnitude of postprandial lipemia by consuming diacylglycerols in place of triacylglycerols and therefore the former may be less atherogenic than the latter. Dietary diacylglycerol may therefore reduce the risk of coronary arteriosclerotic diseases by lessening the postprandial increase in the concentration of remnant-like lipoprotein particles.

In contrast to triacylglycerols, diacylglycerols suppress body weight and regional fat deposition, both visceral and hepatic. In a study of obese Americans (Watanabe & Matsuo, 2003), decreases in body weight were significantly higher in patients consuming diacylglycerols compared to those on triacylglycerols. It has also been suggested that the consumption of

Table 9.7 Average Western intakes, sources and metabolism of dietary sterols

Parameter	Cholesterol	Plant sterols	Plant stanols
Dietary intake (mg/day)	100–500	200–400	10–60
Dietary sources	egg yolk, butter, dairy products, meat, crustaceans	vegetable oils, corn, beans, grains	grains (wheat, rye, corn)
Endogenous synthesis (mg/day)	800–1200	not synthesised	not synthesised
Absorption (%)	40–60	5–15	0.1–2
Plasma concentration mg/dl	140–320	0.3–1.7	0.01
Excretion (%)	40–60	85–85	>98

Stanols are fully hydrogenated sterols
Source: Salo *et al*. (2003), p. 191.

diacylglycerol-rich oil might be useful for maintaining the quality of life of patients with diabetes.

9.8.2 Phytosterols

While animal fats contain cholesterol, plant lipids only very rarely contain this typical zoosterol but have a range of related products (phytosterols) which are mainly 4-desmethylsterols (Tables 9.7 and 9.8).

Phytosterols are not absorbed and interfere with the absorption of cholesterol thereby reducing blood cholesterol levels when consumed regularly as part of a healthy diet. Absorption of dietary cholesterol, normally at around 50 per cent, is reduced to 20 per cent in the presence of appropriate levels of phytosterols. To be effective, the phytosterols must be soluble in the emulsified fat phase in which they are ingested and this is best achieved when consumed as fatty acid esters. The minimum amount required to produce a

Table 9.8 Composition of phytosterols (% wt of total sterols) from wood and from vegetable oil

	Source	
Sterol	Wood	Vegetable oil
Sitosterol	72	45
Campesterol	8.2	26.8
Stigmaserol	0.3	19.3
Brassicasterol	0	1.6
Sitostanol	15.3	2.1
Campestanol	1.6	0.8
Other sterols	2.6	4.4

Source: Salo *et al*. (2003), p. 193.

significant effect is 0.8–1.0g/day but 2–3 g/day of sterol esters are required to obtain a clinically significant effect. The sterol esters are best ingested as part of a meal. In a trial group with mildly elevated cholesterol levels, average total cholesterol was reduced by 10 per cent after 15 months and average LDL cholesterol by 14 per cent.

There are two commercial sources of plant sterols. They may be obtained from tall oil (a by-product of the wood pulp industry, see section 1.3.17) where the potential supply is estimated to be 3000 tonnes/year. They can also be obtained as a by-product from the refining of soybean oil where the potential supply is said to be 5000–6000 tonnes/year. Further supplies could come from the refining of other vegetable oils. The composition of the sterol mixture from these two sources is given in Table 9.8. Phytosterol preparations are available mainly as fatty acid esters in margarine (since 1995) and also in yogurts, milk drinks, cheese spreads and snack bars.

Bibliography and references

Akoh, C.C. & Min, D.B. (eds) (2002) *Food Lipids – Chemistry, Nutrition, and Biotechnology*, 2nd edition, Marcel Dekker, New York. Contains several relevant chapters.

Ashwell <www.ashwell.uk.com/shape>.

BNF (1992) *Unsaturated Fatty Acids – Nutritional and Physiological Significance*, Chapman & Hall, London.

BNF Briefing paper (1999) n-3 Fatty acids and health. This contains useful information on: food sources of linolenic acid, and on daily intakes, CHD, cancer, inflammation, fetal and infant development, opportunities to increase intake, recommendations for intake levels.

Bonow, R.O. & Eckel, R.E. (2003) Diet, obesity, and cardiovascular risk, *New England Journal of Medicine*, **348**, 2057–2058.

F.W. Cain *et al*., United States Patent 6,534,110 (2003), Process for the preparation of materials with a high content of long chain polyunsaturated fatty acids

Carlson, S.E., Gibson, R.A. & Knapp, H.R. (eds) (2001) PUFA in maternal and child health, *Lipids*, **36**, 859–1076.

Ching Kuang Chow (ed.) (2000) *Fatty Acids in Foods and their Health Implications*, 2nd edition, Marcel Dekker, New York.

Christie, W.W. <www.lipid.co.uk>.

Cuppett, S.L. (2003) Cholesterol oxides: sources and health implications, *Lipid Technology Newsletter,* **9**, 9–13.

Delplanque, B. & Mendy, F. (2003) Dietary fatty acids: what are the limits for beneficial effects on blood lipid levels? *Lipid Technology Newsletter,* **9**, 77–81.

Fernie, C.E. (2003) Conjugated linoleic acid. In: *Lipids for Functional Foods and Neutraceuticals* (ed F.D. Gunstone), The Oily Press, Bridgwater, pp. 291–318.

Gunstone, F.D. (ed.) (2003) *Lipids for Functional Foods and Neutraceuticals,* The Oily Press, Bridgwater.

Gurr, M.I. (1999) *Lipids in Nutrition and Health – a Reappraisal*, The Oily Press, Bridgwater.

Hands, E.S. (1996) Lipid composition of selected foods. In: *Bailey's Industrial Oil and Fat Products*, 5th edition, (ed Y.H. Hui) Wiley, New York, pp. 441–505.

Keys, A., Andersen, J.T. & Grande, F. (1957) Prediction of serum-cholesterol responses of man to changes in the fats in the diet. *Lancet,* **2**, 959–996.

Knapp, H.K., Salem. Jr. N. & Cunnane, C. (2003) Dietary fats and health (ISSFAL Congress) *Lipids,* **38**, 299–496.

Kritchevsky, D. (2002) Fats and oils in human health. In: *Food Lipids – Chemistry, Nutrition,*

and Biotechnology, 2nd edition, Akoh, C. C. & Min, D. B. (eds), Marcel Dekker, New York, pp. 543–558.

Lands, W.E.M., Libelt, B., Morris, A., Kramer, N.C., Prewitt, T.E., Bowen, P., Schmeisser, D., Davidson, M.H. & Burns, J.H. (1992) Maintenance of lower proportions of (n-6) eicosanoid precursors in phospholipids of human plasma in response to added dietary (n-3) fatty acids, *Biochim. Biophys. Acta*, **1180**, 147–162.

Li, D., Bode, O., Drummond, H. & Sinclair, A.J. (2003) Omega-3 (*n*-3) fatty acids. In: *Lipids for Functional Foods and Neutraceuticals* (ed F.D. Gunstone), The Oily Press, Bridgwater, pp. 225–290.

MAFF (1998) Supplement to McCance & Widdowson's *The Composition of Foods*. Ministry of Agriculture, Fisheries and Food, and The Royal Society of Chemistry, London.

NIH <http://ods.od.nih.gov/eicosanoids/index/html>.

Pedersen, J.I., Muller. H. & Kirkhus, B. (2001) Serum cholesterol predictive equations with special emphasis on *trans* and saturated fatty acids. An analysis from designed controlled studies *Lipids*, **36**, 783–791.

Salo, P., Webster, I. & Hopia, A. (2003) Phytosterols. In: *Lipids for Functional Foods and Neutraceuticals* (ed F.D. Gunstone), The Oily Press, Bridgwater, pp. 1893–224.

Schmidt, E.B., Kristensen, S.D., De Caterina, R. & Endres, S. (eds) (2001) Prevention and treatment of vascular disease – a nutrition-based approach, *Lipids*, **36**, S1–S129.

Shukla, V.K.S. (2003) A designer oil for better health, *Inform*, **14**, 340–341.

Simopoulos, A.P., Leaf, A. & Salem, N. (2000) Workshop statement on the essentiality of and recommended dietary intakes for omega 6 and omega 3 fatty acids, Prostaglandins, Luekotrienes, *Essential Fatty Acids*, **63**, 119–121.

USDA nutrient database <www.nal.usda.gov/fnic/foodcomp>.

Watanabe, H. & Matsuo, N. (2003) Diacylglycerols. In: *Lipids for Functional Foods and Neutraceuticals* (ed F.D. Gunstone), The Oily Press, Bridgwater, pp. 113–148.

Williams, C.M. & Roche, H.M. (2001) Achieving optimal fatty acid intake through manufactured foods. In: *Structured and Modified Lipids* (ed. F.D. Gunstone), Marcel Dekker, New York, pp. 465–484.

10 Edible uses of oils and fats

10.1 Spreads: butter and ghee

It is expected that 122 million tonnes of 17 commodity oils and fats will be produced in 2002/2003 and that about 80 per cent of this will be used in food for humans. The resulting figure of almost 100 million tonnes is too high, by reason of loss and waste, but also too low because of other fat sources not included in statistical tables. Among these are nuts, dairy products other than butter and fat present in meat. This chapter describes the major food uses of oils and fats. Relevant information is also given in the previous chapter on the nutritional properties of these materials.

10.1.1 Butter

Butter from cow milk fat has been used as a spread and for baking and frying for many centuries. With the development of good quality margarine and other spreads, butter has become less popular. The disadvantages associated with butter are its high price compared to other spreads, its poor spreadability from the refrigerator, and its poor health profile resulting from its high fat content, its high level of saturated acids and of cholesterol, and the presence of *trans* unsaturated acids. Its advantages are its 'wholly-natural' profile and its superb flavour. The name butter is jealously guarded and it is not permissible to take anything away or to add anything to a product which is to be called butter; nevertheless ways of overcoming the disadvantages are being reported. For further information see Hettinga in Bailey (1996), Flack in Gunstone & Padley (1997) and Rajah in Rossell (1999).

Butter is a water-in-oil emulsion consisting of fat (80–82%) and an aqueous phase (18–20%) which contains salt and milk-solids-not-fat. The upper legal limit for water is 16 per cent. Butter is made from cow milk (3–4% fat) converted first to cream (30–45% fat) by centrifuging and then to butter by churning and kneading. During churning, there is a phase inversion from an oil-in-water to a water-in-oil emulsion. Details of annual production and disappearance are given in Table 10.1. Production peaked in the 1980s (6.0–6.4 million tonnes), fell to below 6 million tonnes in the late 1990s, but is rising again and is predicted to reach 7.8 million tonnes in the next 20 years.

Table 10.1 Production of butterfat and of margarine (million tonnes) between 1998 and 2002

	Butterfat					Margarine[a]				
	1998	1999	2000	2001	2002	1998	1999	2000	2001	2002
World	5.76	5.92	6.04	6.10	6.30	9.44	9.69	9.83	10.15	10.16
EU-15	1.59	1.62	1.62	1.58	1.64	2.44	2.23	2.12	2.06	2.04
Former Soviet Union	0.47	0.45	0.47	0.49	0.48	0.48	0.65	0/78	0.90	0.92
Central Europe	0.21	0.21	0.21	0.22	0.22	0.64	0.63	0.64	0.65	0.65
India	1.28	1.31	1.35	1.41	1.48	1.00	1.25	1.28	1.47	1.43
Pakistan	0.44	0.45	0.46	0.48	0.49	1.43	1.46	1.49	1.52	1.54
New Zealand	0.29	0.29	0.33	0.33	0.33					
United States	0.40	0.47	0.48	0.46	0.50	1.06	1.04	1.04	1.00	0.99
Turkey	0.08	0.09	0.09	0.10	0.10	0.65	0.67	0.68	0.69	0.70
Brazil	0.07	0.07	0.07	0.08	0.08	0.47	0.47	0.48	0.48	0.49

[a] Including vanaspati and vegetable ghee where appropriate.
Source: Oil World Annual, 2003, published by ISTA Mielke GmbH, Hamburg.

The biggest consumers are in the Indian subcontinent (India and Pakistan) and in EU-15 where France and Germany consume most.

Information on the composition of butter fat is given in section 1.5.1. 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} 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 fat's fatty acids is subject to change by modification of dietary intake. Further, since in ruminants (free) unsaturated acids 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 oil (soybean, linseed, rape/canola), but two other methods are now more commonly employed. In the first, calcium salts are used as a 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 contains appropriate proportions of n-9, n-6, and n-3 unsaturated acids and that it is over 75 per cent protected from metabolism in the rumen.

In times of over-supply there is an interest in extending the range of applications of milk fat by fractionation (section 2.3.2). However, the

triacylglycerol composition of milk fat is so complex (no individual triacylglycerol exceeds 5%) 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 some fractions that are harder and some 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. Anhydrous milk fat itself is used to make cakes. Mixed with the olein fraction it is used in cookies, biscuits and butter cream. Mixed with the stearin fraction it is used in fermented pastries and puff pastry. The olein fraction on its own is used in ice cream cones, waffles, butter sponges and in chocolate for ice cream bars.

In Europe, butters with differing fat levels (and therefore reduced caloric values) are designated as butter only when they contain 80–90 per cent fat, three quarter fat butter has 60–62 per cent fat, half fat butter has 39–41 per cent fat and dairy fat spreads have other fat levels. In the United States, 'light butter' must contain less than half of the normal level of fat and 'reduced butter' less than one quarter of the normal level.

Products are available in many countries that are blends of butter and vegetable oil – generally soybean oil. These cannot be called butter but are given an appropriate name that the consumer comes to think of as 'spreadable butter'. Spreadable butters developed in New Zealand are made by fractionation of butter followed by recombination of appropriate fractions.

10.1.2 Ghee

In India, milk fat is consumed partly as butter but also as ghee though consumption of the latter is declining and is now probably below one quarter of the combined total. At the same time, demand in other countries is growing, probably reflecting the migration of people from the subcontinent of India. Ghee is a concentrate of butter fat with over 99 per cent milk fat and less than 0.2 per cent moisture. It has a shelf-life of 6–8 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 per cent. In other procedures, the aqueous fraction is allowed to separate and some of it is run off before residual moisture is removed by heating. Ghee has a cooked caramellised flavour varying slightly with the method of preparation (Achaya in Gunstone & Padley, 1997; Rajah in Rossell, 1999) The vegetable oil-based alternative to ghee is called vanaspati (section 10.2.2).

10.2 Spreads: margarine and vanaspati

10.2.1 Margarine

Margarine has been produced for over 100 years. During the 1860s large sections of the European population migrated from country to town and changed from rural to urban occupations. At the same time, there was a rapid increase in population in Europe and a general recession in agriculture leading to a shortage of butter, especially for the growing urban population. As a consequence, the price rose beyond the reach of many poor people. The situation in France was so bad that the government of the day offered a prize for the best proposal for a butter substitute which would be cheaper and would also keep better.

The prize was won by the French chemist, Hippolyte Mège Mouriès who patented his product in France and in Britain in 1869. His process required the softer component from fractionated tallow, skimmed milk and macerated cows' udder. The product was described as mixed glycerol esters of oleic and margaric acids and was therefore called oleo-margarine. Margaric acid was thought to be heptadecanoic acid (17:0) though it was actually a eutectic mixture of palmitic (16:0) and stearic (18:0) acids.

For a long time, margarine was considered as a cheap and inferior substitute for butter. In several countries regulations were passed that prohibited the addition of colouring matter so that white margarine would compare even less favourably with the more familiar yellow butter. Now the situation is different. These impediments have disappeared and margarine is widely accepted as having several advantages over butter. It is a more flexible product which can be varied for different markets and modified to meet new nutritional demands such as desirable levels of cholesterol, phytosterols, saturated or *trans* acids, and fat content, as well as the statutory levels of certain vitamins. Further information is available from Flack in Gunstone & Padley (1997) and from Rajah in Rossell (1999).

Table margarine is made from appropriate oils and fats (soybean, rape/canola, cottonseed, palm, palmkernel, coconut) which may have been fractionated, blended, hydrogenated in varying degrees and/or interesterified. Fish oil (hydrogenated or not) may also be included. Other ingredients include surface-active agents, proteins, salt, and water along with preservatives, flavours and vitamins.

Margarine production involves three basic steps: emulsification of the oil and the aqueous phases, crystallisation of the fat phase and plasticification of the crystallised emulsion. Water-in-oil emulsions are cooled in scraped-wall heat exchangers during which time fat crystallisation is initiated, a process known as nucleation, and during which the emulsion drop size is reduced. There follows a maturing stage in working units during which crystallisation

approaches equilibrium, though crystallisation may continue even after the product has been packed. The lipid in margarine is part solid (fat) and part liquid (oil) and the proportion of these two varies with temperature. The solid/liquid ratio at different temperatures is of paramount importance in relation to the physical nature of the product.

Individual crystals are between one tenth and several micrometres (μm) in size and form clusters or aggregates of 10–30 μm. One gram of fat phase may contain up to 10^{12} individual crystals. The aqueous phase is present in droplets, generally 2–4 μm in diameter, stabilised by a coating of fat crystals.

It is desirable that margarine taken from the refrigerator at 4ºC should spread easily. For this the proportion of solids should be 30–40 per cent at this temperature and should not exceed the higher value. For the sample to 'stand up' at room temperature (and not collapse to an oily liquid) it should still have 10–20 per cent solids at 10ºC. Finally, so that it melts completely in the mouth and does not have a waxy mouth feel, the solid content should be less than three per cent at 35ºC. These important parameters can be attained with many different fat blends. Formulations have to be changed slightly to make the product suitable for use in hot climates.

Fats usually crystallise in two different forms known as β' and β (section 6.1.2). Of these the β form is thermodynamically more stable and will therefore be formed in many fats and fat blends. But sometimes the fat remains in the β' form. For margarines and other spreads the β' form is preferred since the crystals are smaller, are able to entrap more liquid to give firm products with good texture and mouth feel and impart a high gloss to the product. β Crystals, on the other hand, start small but tend to agglomerate and can trap less liquid. It is therefore desirable to choose a blend of oils which crystallise in the β' form.

Margarines and shortenings made from rapeseed/canola, sunflower and soybean oil after partial hydrogenation tend to develop β crystals. This can be inhibited or prevented by the incorporation of some cottonseed oil, hydrogenated palm oil, palm olein, tallow, modified lard, or hydrogenated fish oil: all of which stabilise crystals in the β' form. The canola, sunflower, and soybean oils all have high levels of C_{18} acids, while the remaining oils have appreciable levels of C_{16} acids (or other chain length) along with the C_{18} acids and thus, contain more triacylglycerols with acids of mixed chain length.

There are many formulations for making margarines, and different recipes are used around the world depending on the oils most readily available in any particular locality. Practically all of them contained partially hydrogenated oils and therefore, had appreciable levels of *trans* acids. Attempts are now being made to reduce the levels of such acids on nutritional grounds. Considerable progress has been made in European formulations and the average content of *trans* acids has fallen in recent years. Many preparations approach zero *trans*. New legislation in the United States will make it

necessary to indicate the content of *trans* acids and this will probably have an influence on formulations. However, it must be realised that it is not possible to make spreads without a proportion of solid triacylglycerols which must contain saturated and/or *trans* monoene acids. If the level of *trans* acids is to fall, then there must be some rise in the content of saturated acids. Nor is it sufficient merely to blend hardstock (material with a high proportion of solid triacylglycerols) with oils containing 'healthy' mono and polyunsaturated acids. The blended oils may have to be interesterified to get the appropriate distribution of fatty acids in the triacylglycerols.

It is not possible to list all the formulations used to make margarines and the following examples are merely indicative (in the following blends hydrogenated means partially hydrogenated):

- Blends of hydrogenated soybean oils with or without unhydrogenated soybean oil,
- Blends of canola oil, hydrogenated canola oil and hydrogenated palm oil or palm stearin,
- Blends of various hydrogenated cottonseed oils,
- Blends of edible tallow with vegetable oils (soybean, coconut),
- Blends of palm oil with hydrogenated palm oil and a liquid oil (rapeseed, sunflower, soybean, cottonseed, olive),
- Palm oil (60%), palmkernel oil (30%) and palm stearin (10%),
- Palm stearin (45%), palmkernel oil (40%) and liquid oil (15%).

For hot climates a harder formulation is required as in the last two examples from Malaysia.

Table 10.2 gives details of the fatty acid composition of spreading fats and Table 10.1 provides information on production levels of margarine in the period 1998 to 2002.

Margarine is expected to have a shelf-life of about 12 weeks. With good ingredients and absence of pro-oxidants (copper), oxidative deterioration is not likely to be a problem but care must be taken to avoid microbiological contamination in the aqueous phase. This is avoided by hygienic practices during manufacture, the addition of some salt (8–10 per cent in the aqueous

Table 10.2 Approximate fatty acid composition of spreading fats

Fat	Saturated	Monoene*	Polyene
Butter	63–70	28–31	1–3
Stick (packet) veg oils only	18–21	45–66	14–35
Stick (packet) veg and animal fats	29–40	46–52	9–19
Soft (tub)	17–19	35–52	29–48

* US stick margarines contain 17–36 per cent *trans* acids and soft margarines contain 10–18 per cent *trans* acids, but products with lower levels of *trans* acids are now being produced.

phase, corresponding to a little over 1 per cent in the margarine), control of pH of any cultured milk that may be used, and careful attention to droplet size in the emulsion.

Margarines are now available with added phytosterols which, it is claimed, are able to reduce blood cholesterol levels (section 9.3.2). The phytosterols are obtained from tall oil and added as hydrogenated sterol esters or from soybean oil and added as unsaturated sterol esters to margarines at around the eight per cent level. Margarine is a suitable vehicle for phytosterol addition because it is a food used widely and regularly but unlikely to be over-consumed. Intake of phytosterols is normally 200–400 mg/day, though higher for vegetarians, and the intake of 1.6–3.3 g/day, recommended by those offering this special margarine, is markedly higher. Normally about 50 per cent of ingested cholesterol is absorbed but with an adequate supply of phytosterols, which are absorbed only at the five per cent level, absorption of cholesterol falls to about 20 per cent (see Salo *et al*. in Gunstone, 2003).

Spreads with reduced levels of fat (down to 40 per cent or less) are popular with consumers (as an alternative to restricting the amount of normal spread consumed). As a consequence there is much less margarine with the statutory level of at least 80 per cent of fat and the word 'spread' has largely replaced the word 'margarine'. Spreads contain more water than full fat spreads and require emulsifiers (monoacylglycerols or polyglycerol esters, section 10.10). It is also usual to add thickeners such as gelatin, sodium alginate, pectin, and carrageenan to the aqueous phase.

Industrial margarines are used mainly for bakery products and are discussed in section 10.3.

10.2.2 Vanaspati

It has been estimated that production of vanaspati in 1998 was 4.7 million tonnes (mainly in Pakistan 1.4, India 1.0, Iran 0.5, Egypt 0.4 and Turkey 0.4). Vanaspati can be considered as vegetable ghee. It is used mainly for frying and for the preparation of sauces, sweets, and desserts. Traditionally vanaspati was a blend of hydrogenated seed oils (cottonseed, groundnut, soybean, rape/canola and palm) but increasingly palm oil has become a significant component. The product should melt between 31 and 41ºC though generally it is close to 37ºC in India and 36±2ºC in Pakistan. A wide range of oils is used including soybean, rapeseed, sunflower, cottonseed, palm olein and palm oil. Because the method of production involves hydrogenation vanaspati contains high levels of acids with *trans* unsaturation (over 50 per cent in India and about 27 per cent in Pakistan). However, with increasing use of palm oil in vanaspati the need for hydogenation is reduced with a consequent fall in the level of *trans* acids. Figures around three per cent have been reported in Pakistan (Achaya in Gunstone & Padley, 1997; Rajah in Rossell, 1999).

10.3 Baking fats, doughs and shortenings

The use of oils and fats in baking processes ranks along with frying and spreads as a major food use of these materials. The products range through breads, layered doughs, cakes, biscuits (cookies) and biscuit fillings, pie crusts, short pastry and puff pastry. The fats used to produce this wide range of baked goods vary in their properties and particularly in their melting behaviour and their plasticity. It is possible to attain these properties with different blends of oils and preferred mixtures vary in different regions of the world. In addition to the desired physical properties, it is necessary to meet two further requirements. One is oxidative stability related to the shelf-life of the baked goods. The other is the necessity to respond to current nutritional demands. A good baked item will be tasty, have good texture, have a reasonable shelf-life in terms of rancidity and palatability and texture and it will be a healthy food. Sometimes the pressures for appropriate physical properties and for nutritional requirements work in opposite directions and a compromise has to be made. As already discussed with the spreads, a plastic fat containing solid and liquid components must have some solid triacylglycerols which implies a certain level of saturated acids or of acids with *trans* unsaturation despite the nutritional concerns associated with these compounds.

Fats used to make doughs are almost entirely plastic fats, i.e. mixtures of solid and liquid components which appear solid at certain temperatures and which deform when pressure is applied. Fats exert their influence by interaction with flour and (sometimes) sugar that are the other major components of a baked product.

Baking fats include butter or margarine which are greater than 80 per cent fat along with an aqueous phase and shortenings with 100 per cent fat. The latter are described as shortenings as they give pastry the crispness and flakiness which is suitable for its edible purpose. Industrial margarine has the fat/water ratio required of margarine, but it will differ from margarine spread in that it will have fat components which produce the physical properties required by its specific end-use. Changes in the composition of fat in margarine spread designed to increase their nutritional value have not always carried through to the baking fats, which are often richer in saturated fatty acids and/or acids with *trans* unsaturation. But there seems little doubt that the appropriate changes will come. Baked goods contain what is described as ‘hidden’ fat and it is easy to forget the fats they contain when delicious pastries, cakes and biscuits are being eaten.

The prime function of fat in a cake is to assist in aeration and to modify the texture of the product. The first stage in making a cake is to produce a batter containing a fine dispersion of air bubbles largely stabilised by fat crystals. During baking, the fat melts and the water-in-oil emulsion inverts, with the air

being trapped in the aqueous phase. As baking continues, the starch is hydrated and gelatinised, the protein starts to coagulate, and the air cells expand through the presence of steam and carbon dioxide (produced from baking powder).

In short pastry, aeration is only of secondary importance. The fat needs to be fairly firm and should be distributed throughout the dough as a thin film. Lard, beef tallow olein and hardened vegetable oils may be employed. Sometimes butter or margarine is used.

In puff pastry (pie crust, Danish pastries, croissants) fat acts as a barrier, separating layers of dough from one another. Liberation of gas or steam during baking produces a layer structure. This requires a fat of higher-melting point than normal (~42ºC) with a higher solid-fat content achieved through an appropriate degree of hydrogenation.

Small amounts of fat (2–5%) are added to bread dough.

Further information is available in articles by Metxroth and by O'Brien in Bailey (1996) and by Podmore in Rossell (1999).

10.4 Frying oils and fats

The use of oils and fats as a frying medium in both shallow and deep-frying mode is an important component in the whole picture of food applications. Pre-fried and fried foods are now a significant component of our dietary intake and around 20 million tonnes of oils and fats are used in this way. This represents a significant share of the 90 million tonnes used for dietary purposes though not all the frying fat is consumed.

Frying is usually carried out at a temperature of 165–185ºC and is an efficient method of heat transfer that allows quick cooking and adds flavour to fried food. Some oil is absorbed by the fried food. In shallow pan frying surplus oil is cleaned away at the end of the frying operation. In deep fat frying residual oil is reused until such time as it has to be discarded because of its poor quality. This low-grade material may be added to animal feed as an energy source or it may be converted to methyl esters and used as bio-diesel (section 11.6).

Frying is carried out on a domestic scale, in restaurants and fast food outlets on a batch scale using 4–20 kg of oil, and under industrial conditions in continuous mode with one tonne or more of oil to produce fried products for retail outlets.

Popular fried foods include French fries, chicken, fish, meat, potato crisps, tortilla chips, extrusion snacks, doughnuts, nuts and noodles. During frying, oil is transferred to the food so that fried foods contain additional fat at a level of 10–40 per cent. Fat from the food is also transferred to the frying oil so that however carefully oil quality is controlled at the beginning, the oil soon

becomes contaminated, for example, with fish oil or with animal fat depending on the food being fried.

Under the conditions of frying, a number of changes occur in the oil. These include hydrolysis producing free acids and partial glycerol esters, oxidation producing flavour notes some of which are considered to be desirable and others not so, and thermal changes leading to polymeric products and acyl groups with *trans* unsaturation and with five and six-membered ring systems. Volatile products will be quickly lost under the conditions of steam distillation which exist during frying, hence the characteristic smell of frying operations, but compounds of higher molecular weight remain in the frying oil. With continued use, the oil begins to smoke, to foam and to become more viscous. Oil absorbed by the fried food has to be replaced by fresh oil and turnover and replacement of fat are important factors in a good quality frying operation. This ensures that low quality oil is not being used. Under the best frying conditions, the major health concern may not be the small amount of artefacts but rather the increased level of fat that is being consumed.

A good frying oil will have high oxidative stability, a high smoke point (low level of free fatty acid) and show minimum colour darkening. The oil may be chosen because it gives a distinctive flavour to the fried food. This applies with corn oil, olive oil, groundnut oil and tallow. Alternatively, a refined blend may be used (cottonseed, groundnut, soybean, palm olein) which may have been subject to partial hydrogenation. A good frying oil will be low in saturated and in *trans* unsaturated acids for nutritional reasons and low in polyunsaturated fatty acids to increase oxidative stability. Consequently, it will be high in *cis* monounsaturated acids. In France and Belgium, it is forbidden by law to use frying oils with more than two per cent of linolenic acid. Special oils used for frying include olestra in the United States and 'Good Fry' in Europe. The latter is a high-oleic sunflower oil with up to six per cent of sesame oil and/or ricebran oil, both of which contain powerful antioxidants. Many high-oleic oils are being developed through conventional seed breeding or through genetic modification and all these will be considered as potential frying oils.

Further details are given in books and articles by Perkins & Erikson (1996); Mounts in Gunstone & Padley (1997); Rossell in Rossell (1999); Boskou & Elmadfa (1999); Gertz *et al*. (2001).

10.5 Salad oils, mayonnaise and salad cream and French dressings

Salad oils used for mayonnaise and salad cream should be oxidatively stable and be free of solids even when stored in a refrigerator at about 4ºC. Several vegetable oils may be used. Those containing linolenic acid (soybean oil, rape/canola oil) are usually lightly hydrogenated to decrease the level of linolenic

acid and so enhance oxidative stability. All oils are generally winterised to remove high-melting glycerol esters that would crystallise and the waxes sometimes present in solvent-extracted oil. The latter lead to a haze in the oil when it is cooled. Salad oils must pass a 'cold test' which requires that the oil remain clear for 5.5 hours at refrigeration temperature. After appropriate treatment soybean, rapeseed/canola, corn and sunflower oils are suitable to produce mayonnaise.

Mayonnaise is an oil-in-water emulsion containing between 65 per cent (legal minimum) and 80 per cent of oil. The aqueous phase contains vinegar, citric acid and egg yolk. This last contains lecithin which serves as an emulsifying agent. Lemon and/or lime-juice, salt, syrups, seasonings, spices and antioxidants are optional constituents. These components may be mixed together at temperatures not exceeding 5ºC (cold process) or at temperatures around 70ºC (hot process). A typical mayonnaise contains vegetable oil (75–80% by weight), vinegar (9.4–10.8%), egg yolk (7.0–9.0%), and small amounts of sugar, salt, mustard and pepper. 'Light' mayonnaise contains about 30–40 per cent of oil and in low-calorie dressings the level is below 10 per cent. Salad creams are similar but contain much less oil (30–40%) along with cooked starch materials, emulsifiers, and gums to provide stability and thickness. They are cheaper than mayonnaises.

French dressings are temporary emulsions of oil, vinegar or lemon juice and seasonings. Because the emulsions are not stable, the dressings are usually shaken immediately before use. A non-separating product can be made by addition of egg yolk or other emulsifying agents. Production levels in the United States during the period 1993–1998 were reported to be 610–626 tonnes, comprising salad dressings and mayonnaise (410–440 tonnes) and pourable dressings (180–200 tonnes).

Further information is available in chapters by Mounts in Gunstone & Padley (1997); Podmore & Aikens in Rossell (1999).

10.6 Chocolate and confectionery fats

Chocolate is an important fat-containing food based mainly but not always entirely on cocoa butter. Confectionery fats are materials with similar physical/functional properties to cocoa butter. Legal definitions of chocolate limit the amount of fat other than cocoa butter which may be used. The incorporation of milk fat into chocolate, the limited use of other fats, and the complete replacement of cocoa butter are discussed later in this section (see also Beckett, 2000; Timms, 2003).

It has proved difficult to find the annual production of cocoa butter but one estimate gives figures of cocoa beans (2.9 million tonnes), cocoa butter (1 million tonnes) and chocolate (5 million tonnes). Production figures for cocoa

butter are not included in the statistics generally cited for oil and fat production.

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 (~150ºC), 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. 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.). Cocoa powder is the residue after extraction and still contains 10–24 per cent of fat. 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 is a solid fat melting at 32–35ºC. It is in high demand because its characteristic melting behaviour gives it properties which are significant in chocolate. At ambient temperature it is hard and brittle giving chocolate its characteristic snap but it also has a steep melting curve that allows for complete melting at mouth temperature. This gives a cooling sensation and a smooth creamy texture. Typical figures for Ghanaian cocoa butter taken from Table 10.3 show that the content of solid falls from 45 to around one per cent between 30 and 35ºC. The hardness of cocoa butter is related to its solid fat content at 20 and 25ºC. This melting behaviour is related in turn to the chemical composition of cocoa butter. The fat is rich in palmitic (24–30%), stearic (30–36%), and oleic acids (32–39%) 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). Cocoa butter has a high content of saturated acids which raises health concerns, but it has been argued that much of this is the non-cholesterolemic stearic acid. Cocoa butter is also a rich source of flavonoids which are considered to be powerful antioxidants.

Cocoa is grown mainly in West Africa, South East Asia and in South and Central America. The composition of cocoa butter from these different sources varies slightly as shown in Table 10.3 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 in the melting profile. The content of the important SOS triacylglycerols varies between 87.5 per cent in Malaysian and 71.9 per cent in Brazilian cocoa butter with the African samples midway between these. There is, however, some evidence that the cocoa butters of different geographical origin are becoming more alike.

Table 10.3 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 ºC	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) – tempering 40 hours at 26ºC				
20ºC (%)	76.0	75.1	62.6	82.6
25ºC (%)	69.6	66.7	53.3	77.1
30ºC (%)	45.0	42.8	23.3	57.7
35ºC (%)	1.1	0.0	1.0	2.6

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

The crystal structure of cocoa butter has been studied intensively because of its importance in understanding the nature of chocolate (section 6.1.2). The solid fat has been identified in six crystalline forms designated I–VI. Some crystals show double chain length (D) and some triple chain length (T). The six forms have the following melting points (ºC) 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 moulding characteristics and has a stable gloss and favourable snap at room temperature. Procedures to promote this form are necessary and its change to form VI must be inhibited. Form V is usually obtained as a result of extensive tempering, i.e. 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 is termed 'bloom'. It is promoted by fluctuations in temperature during storage and by migration of liquid oils from nut centres. Though this is a harmless change, it is considered undesirable because it may be mistaken for microbiological contamination. Bloom can be inhibited by addition of a little 2-oleo 1,3-dibehenin (BOB) to the cocoa butter. This phenomenon is discussed in more detail by Padley in Gunstone & Padley (1997), by Smith in Gunstone (2001) and by Timms (2003).

The simplest plain chocolate contains sugar and cocoa liquor with cocoa butter the only fat present. A typical plain chocolate contains cocoa mass (~40%, which still has some cocoa butter), sugar (~48%), added cocoa butter (~12%), and small amounts of lecithin and other materials. The total fat content of this mix is around 31 per cent. In some European countries it is permissible to replace some of the cocoa butter with up to five per cent of another fat with similar fatty acid and triacylglycerol composition taken from a prescribed list of tropical fats (see Talbot in Rossell (1999) and Timms (2003)) This represents about 15 per cent of the fat phase. The permitted tropical fats come from palm, illipe, shea, sal, kokum and mango and may be used in a fractionated form (see Table 1.4).

Milk chocolate contains between 3.5 and 9 per cent of milk fat and white chocolate is based on sugar, cocoa liquor and cocoa butter. If the latter is not entirely refined then it will retain some of the flavour normally associated with chocolate. Chocolate normally contains up to 0.4 per cent of lecithin, from soybeans, sunflower, or rape. This aids the processing of the chocolate by reducing the viscosity of molten chocolate. Polyglycerol ricinoleate is sometimes added to optimise viscosity.

Cocoa butter alternatives (CBA) is a general name covering cocoa butter equivalents (CBE), cocoa butter improvers (CBI), cocoa butter replacers (CBR) and cocoa butter substitutes (CBS) (Smith in Gunstone 2001). Cocoa butter equivalents have the same general chemical composition and hence the same physical properties as cocoa butter and include the tropical oils or 'hard butters' described above. These can be blended to give mixtures of POP, POSt, and StOSt very similar to cocoa butter and fully miscible with it. The level at which cocoa butter can be replaced by a CBE is limited only on a legal basis and not on a functional basis. CBEs must be: compatible with cocoa butter by virtue of their similar fatty acid and triacylglycerol composition, have a melting range equivalent to that of cocoa butter, yield the β-polymorph when processed and tempered in the same way as cocoa butter and give a product that is at least as good as cocoa butter in respect of bloom. The market for CBEs in those European countries where their use in chocolate is permitted is estimated to be 20 000–25 000 metric tonnes, but it could rise to three times this level if all EU countries accepted their use as legal.

Cocoa butter replacers (CBR) are usually based on vegetable oils (soybean, cottonseed, palm) that have been fractionated and partially hydrogenated. They may contain *trans* unsaturated acids at levels up to 60 per cent and have a different triacylglycerol composition from cocoa butter. They do not require tempering but should be compatible with cocoa butter.

Cocoa butter substitutes (CBS) are usually based on lauric fats. They share some of the physical properties of cocoa butter but have a different composition. Coatings based on CBS fats do not require to be tempered but are used in the molten state for coating. They give a superior gloss and have very sharp melting characteristics. Further information is given by Wainwright in Bailey (1996) and by Birkett in Rossell (1999).

The Chocolate Directive 2000/36/EC was adopted by all member states in August 2003. This piece of EU legislation is not easy to understand and those who make chocolate are still arguing about the final details. Put simply, if not completely correctly, it indicates that chocolate which normally contains at least 25 per cent of cocoa butter may also contain up to five per cent (in addition or as part replacement) of other vegetable oils taken from a prescribed list (section 1.4.1).

10.7 Ice cream

The annual production of ice cream in the United States is reported to be about 54 million hectolitres (i.e. 5400 million litres) suggesting that the global figure is at least twice this level. This quantity of ice cream will contain around 0.8–1.0 million tonnes of fat which will be mainly from milk fat but may include a range of vegetable fats such as sunflower, groundnut, palm, palmkernel and coconut.

Ice cream contains water (60–70%) and total solids (30–40%) with the latter including fat (5–12%), milk solids other than fat (10–12%), sucrose (12–14%), glucose solids (2–4%), emulsifier (0.2–0.5%) and stabiliser (0.1–0.3%). Legal requirements for fat vary from country to country as does the possibility of replacing some or all of the dairy fat with vegetable fat.

Fat in ice cream contributes to structure. It stabilises the aerated foam, improves melting resistance, imparts creaminess and contributes to taste. Its most important properties are its melting characteristics, solid to liquid ratio at various temperatures, and its taste profile. Production of ice cream occurs through nine stages: selection and weighing of ingredients, mixing of these in an appropriate sequence at 20–35ºC, pasteurisation (70–75ºC) or sterilisation (95ºC), homogenisation at 75ºC, cooling to <5ºC, ageing at 5ºC for at least 4 hours, freezing at −5 to −10ºC, hardening at −25 to −35ºC and storage at −18 to −20ºC. Further information is available in chapters by Goff in Gunstone & Padley (1997); and Flack in Rossell (1999).

10.8 Incorporation of vegetable oils into dairy products

Vegetable oils may be incorporated into food products as a replacement for dairy fat. This happens when local supplies are inadequate, as in some tropical countries where the climate is not suitable for large scale dairy farming and also for consumers concerned about the saturated acids and cholesterol present in milk fat. In addition, it is possible to produce milk fat replacements in a more convenient form as, for example, in long-life cream. The possible use of vegetable fat in ice cream has already been discussed in section 10.7.

So-called 'filled milk' is made from skim milk powder reconstituted with an appropriate vegetable oil. This should be free of linolenic acid, have only a low content of linoleic acid, and contain antioxidant so that it is oxidatively stable. Palm oil, palmkernel oil and coconut oil are most frequently used and these may be partially hydrogenated to provide further stability against oxidation.

Non-dairy coffee whiteners, available in powder or liquid form, generally contain 35–45 per cent fat which is usually partially hydrogenated palmkernel oil.

Cheeses have been developed based on vegetable fat rather than dairy fat. Several formulations have been described incorporating soybean oil, with or without hydrogenation, palm oil, rapeseed oil, lauric oils, and high-oleic sunflower oil. Attempts have been made to incorporate into these products, some of the short-chain acids which are characteristic of milk fat and give cheese some of its characteristic flavour.

Non-dairy whipping creams, made with hardened palmkernel oil and coconut oil (each about 17%), are convenient because they have a long shelf-life at ambient temperature. They are popular in Britain. First produced for the bakery and catering market with high overrun and good shape retention, they are now supplied to the retail market for domestic use. Pouring creams containing about nine per cent of each of the two lauric oils are also available. Both creams also contain buttermilk powder (7%), guar gum (0.10–0.15%), emulsifying agent (0.30–0.35%), β-carotene (0.25%) and water.

10.9 Edible coatings and spray processing

Foods are sometimes coated with thin layers of edible material to extend shelf-life by minimising moisture loss, to provide gloss for aesthetic reasons, and to reduce the complexity and cost of packaging. The thin layers may be carbohydrate, protein, lipid, or some combination of these. The lipids most commonly used are waxes (candelilla, carnauba, or rice bran), appropriate triacylglycerols or acetylated monoacylglycerols. The latter are able to produce flexible films at temperatures below those appropriate for the waxes

even though they are poorer moisture barriers. The foods most frequently coated are citrus fruits (oranges and lemons), deciduous fruits (apples), vegetables (cucumbers, tomatoes, potatoes), candies and confectioneries, nuts, raisins, cheeses and starch-based products (cereals, doughnuts and ice cream cones and wafers).

Vegetable oils used to coat food products must be liquid at room temperature and must have high oxidative stability. They serve as a moisture barrier, a flavour carrier, a lubricant or release agent, as an anti-dust or anti-cake agent and as a gloss enhancer. They are used at low levels and are sprayed on to large exposed surfaces of products during roasting, frying, or handling. Traditionally they are made from commodity oils like soybean or cottonseed. These are cheap but require elaborate processing (partial hydrogenation and fractionation) to develop the required physical state and chemical stability. New high-oleic oils may also be used. These are more costly but they bring added value in terms of their superior nutritional properties resulting from lower *trans* acids and lower saturated acids and in the reduced need for processing. Lauric oils such as coconut oil, palmkernel oil, or palmkernel olein are used to spray cracker type biscuits to provide an attractive appearance, maintain crispness by acting as a barrier to moisture, and improve eating quality (see Shellhammer & Krochta in Gunstone & Padley, 1997).

10.10 Emulsifying agents

Fatty acids and their derivatives are amphiphilic. This means that their molecules have hydrophilic (lipophobic) and lipophilic (hydrophobic) regions. If these are appropriately balanced, then the molecules can exist in a physically stable form between aqueous and fatty substances. They can therefore be used to stabilise both oil-in-water and water-in-oil emulsions and are important components of many of the fat-based products described in the earlier sections of this chapter. Applications of emulsifiers in foods include film coatings, stabilising and destabilising emulsions, modification of fat crystallisation, dough strengthening, crumb softening and texturisation of starch-based foods. Krog (in Gunstone & Padley, 1997) estimates that production of food emusifiers is about 250 000 metric tonnes of which about 75 per cent are monoacylglycerols or compounds derived from these.

Monoacylglycerols are most often made by glycerolysis of natural triacylglycerol mixtures in the presence of an alkaline catalyst (180–230ºC, 1 hour). Fat and glycerol (30 per cent by weight) will give a mixture of monoacylglycerols (around 58 per cent, mainly the 1-isomer), diacylglycerols (about 36%) and triacylglycerols (about 6%). This mixture can be used in this form or it can be subjected to high-vacuum thin-film molecular distillation to

give a monoacylglycerol product (around 95 per cent and at least 90 per cent of the 1-mono ester) with only low levels of diacylglycerols, triacylglycerols, and free acids (section 8.3.3). Attempts are being made to develop enzyme-catalysed glycerolysis reactions occurring under milder reaction conditions. The oils most commonly used include lard, tallow, soybean, cottonseed, sunflower, palm and palmkernel oil – all in hydrogenated or non-hydrogenated form. Glycerol monostearate (GMS) is a commonly used product of this type.

Specific properties of a monoacylglycerol may be improved by acylation of one of the free hydroxyl groups by reaction with acid (lactic, citric) or acid anhydride (acetic, succinic, diacetyltartaric). For the most part these have the structures shown:

$CH_3(CH_2)_nCOOCHCH(OH)CHOCOR$ where R is:

CH_3 (acetate),
$CH(OH)COOH$ (lactate),
CH_2CH_2COOH (succinate),
$CHOAcCHOAcCOOH$ (diacetyltartrate) and
$CH_2C(OH,COOH)CH_2COOH$ (citrate).

Propylene glycol ($CH_3CHOHCH_2OH$) also reacts with fatty acids to give mixtures of mono (about 55%, mainly 1-acyl) and diacyl esters (about 45%). A 90 per cent monoacyl fraction can be obtained by molecular distillation.

Other emulsifying agents include the partial esters of polyglycerols (a polyether with 2–10 glycerol units but mainly 2–4 units), sorbitan and its polyethylene oxide derivatives, 6-monoacyl sucrose and stearoyl lactate, usually as the sodium or calcium salt (Stauffer in Bailey, 1996; Krog in Gunstone & Padley, 1997).

Bibliography and references

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

Boskou, D. & Elmadfa, I. (eds) (1999) *Frying of Food – oxidation, nutrient and non-nutrient antioxidants, biologically active compounds and high temperatures,* Technomic, Lancaster, Penn.

Gertz, C. *et al*. (2001) Symposium on deep fat frying – optimal operation, *European Journal of Lipid Science and Technology*, **102**, 505–594.

Gunstone, F.D. (ed.) (2001) *Structured and Modified Lipids*, Marcel Dekker, New York.

Gunstone, F.D. (ed.) (2003) *Lipids for Functional Foods and Neutraceuticals*, The Oily Press, Bridgwater.

Gunstone, F.D. & Padley, F.B. (eds) (1997) *Lipid Technologies and Applications*, Marcel Dekker, New York.

Hettinga, D. (1996) Butter fat. In: *Bailey's Industrial Oil and Fat Products,* Volume 3, (ed Y.H. Hui) pp. 1–64, John Wiley, New York.

Hui, Y.H. (ed.) (1996) *Bailey's Industrial Oil and Fat Products,* 5th edition, John Wiley, New York.

Perkins, E.G. & Erickson, E.D. (eds) (1996) *Deep Frying: Chemistry, Nutrition, and Practical*

Applications, AOCS Press, Champaign, Illinois.
Rossell, B. (ed.) (1999) *LFRA Oils and Fats Handbook, Volume 1, Vegetable Oils and Fats*, Leatherhead Food RA, Leatherhead.
Timms, R.E. (2003) *Confectionery Fats Handbook: Properties, Production, and Application*. The Oily Press, Bridgwater.

11 Non-edible uses of oils and fats

11.1 Introduction

Before the general availability of mineral oil and its fractions, oils and fats of vegetable and animal origin were widely used as illuminants and as lubricants. In biblical times, lamps were based on wicks burning, probably in olive oil, and there is evidence that the same oil was used as a lubricant when man-handling large stones for the building of the pyramids. It has also been reported that the axles of ancient chariots were greased with a mixture of animal fat and lime, with calcium soap as a probable by-product.

Eventually, oils and fats used were replaced for those purposes by products derived from mineral oil and its fractions. Lamps used kerosene, and lubricants were based on mineral oils enhanced by a range of additives. Today, there is a limited return to oils and fats as alternatives to mineral oil-based products. The driving forces for this change are mainly environmental. Oils and fats are a renewable resource, liberating carbon dioxide trapped from the atmosphere only months or a few years earlier, in contrast to fossil fuels that cannot be replaced on a human time-scale. Also fat-based products are more easily bio-degraded so that these compounds remain in the environment for only a short time. These are important issues, but some common sense is called for. Since the annual production of oil and gas is about 30 times greater than that of oils and fats and the majority of the latter must necessarily be consumed as food and feed, the impact of environmentally-friendly products based on oils and fats can only have a marginal effect on the total use and consumption of mineral oil products. For example, biodiesel cannot replace the demand for conventional diesel fuel and will diminish it only marginally (section 11.6).

Production and consumption data are available for 17 commodity oils (section 1.7). About 14 per cent of total production, representing 15–17 million tonnes, is used by the oleochemical industry and this chapter is devoted to the major non-edible uses of oils and fats. These are based mainly on the chemistry of fatty acids, sometimes in respect of the carboxyl group (Chapter 8) and sometimes in respect of olefinic centres (Chapter 7). Further information on some of the topics covered here may be found in these chapters.

Surface-active compounds are the most important oleochemicals in volume terms. These are usually based on saturated or monounsaturated fatty acids which fall into three groups depending on their chain length. Compounds with 12 and 14 carbon atoms per molecule are obtained from the two lauric oils – coconut and palmkernel – and are in competition with identical products of the petrochemical industry. Those with 16 and 18 carbon atoms come mainly from tallow or palm stearin. C_{20} acids are available from fish oils and erucic acid (22:1) is derived mainly from high-erucic acid rapeseed oil or from Crambe oil. Other oleochemical uses exploit the high unsaturation of oils such as linseed and soybean oil, while castor oil is a source of several important chemicals. Linseed and castor are classed as industrial oils, significant proportions of tallow, coconut, and palmkernel oils are used to produce oleochemicals, and some use is also made of soybean oil, rapeseed oil, palm oil, and fish oil. These materials may be used as glycerol esters or as free acids, alkyl esters, alcohols, or amines derived from natural triacylglycerols. Another important oleochemical feedstock is tall 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. Further information is given in section 1.3.17.

11.2 Basic oleochemicals

The basic oleochemicals are acids, methyl (or other alkyl) esters, alcohols, amines (and other *N*-compounds), and glycerol. Traditionally, these have been produced mainly in North America, Western Europe and Japan from local or imported oils and fats. This is now changing and countries in South East Asia, particularly Malaysia, have become major producers of basic oleochemicals using the increasingly large supplies of indigenous raw material available in that region of the world. This is shown in Table 11.1 containing projected figures for 2000, 2005 and 2010. Over the ten-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.

The materials used in the oleochemical industry and the processes employed in this industry are set out in Table 11.2 and Fig. 11.1 shows the relationship between the oils and fats and the basic oleochemicals.

11.2.1 Acids

Fat hydrolysis to give fatty acids and glycerol has been described in section 8.2. The fatty acids are used in large quantities to make soaps, esters and amines and have many minor applications also.

Table 11.1 Estimates for 2000, 2005 and 2010 of basic oleochemicals by region and by chemical type (million tonnes)

	2000	2005	2010
World total	5.76	6.69	7.75
West 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%)
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: The sum of the oleochemical types exceeds the world total because of some double counting.

11.2.2 Esters

Esters can be made by esterification from acids and alcohols or from existing esters, including triacylglycerols, by reaction with alcohols (alcoholysis), acids (acidolysis), or other esters (interesterification). On an industrial scale, ester production is most commonly undertaken by methanolysis of triacylglycerols (natural oils and fats) in the presence of an appropriate catalyst which may be acidic, alkaline or enzymic. Large-scale methanolysis is used to make methyl esters for use as biofuel, as solvent, or as an intermediate in the

Table 11.2 The oleochemical industry

Raw materials	tall oil, tallow, coconut oil, palm oil, palmkernel oil, soybean oil, sunflower oil, canola oil
Unit operations to produce basic oleochemicals	splitting, distillation, fractionation, hydrogenation, methylation, deionisation, hydrophilisation
Basic oleochemicals	fatty acids, methyl esters, fatty alcohols, fatty amines, glycerol
Operations to produce derivatives of basic oleochemicals	amidation, chlorination, dimerisation, epoxidation, ethoxylation, quaternisation, sulfation, sulfonation, transesterification, saponification
Oleochemical derivatives	amides, dimer and trimer acids, epoxidised oils and esters, ethoxylates, sulfonates, sulfates, esters, soaps, and salts
End-use markets	building auxiliaries, candles, cleaning agents, cosmetics, detergents, fire extinguishing agents, flotation, food emulsifiers, insecticides, leather, lubricants, paints, paper, pesticides, pharmaceuticals, plastics, rubber, soaps, textiles, tyres

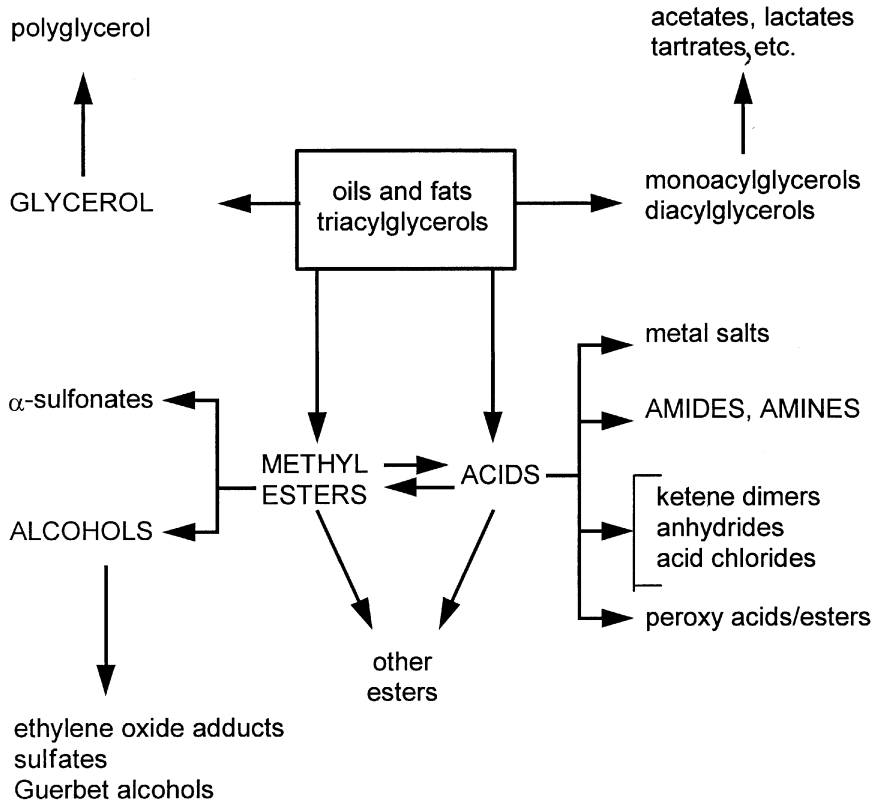


Figure 11.1 Inter-relationships between triacylglycerols and their derivatives. Basic oleochemicals are acids, methyl esters, alcohols, amines and glycerol and are in capitals.

production of alcohols. They can also be hydrolysed to acids. More details concerning these procedures are contained in section 8.3.

In one procedure, low grade oils with up to 30 per cent of free acids (crude palm oil) are reacted with methanol at 80±5ºC in the presence of a solid acid catalyst such as a sulfonated ion exchange resin. This converts the free acids to methyl esters and is followed by methanolysis of the glycerol esters using an alkaline catalyst, such as sodium hydroxide, at 70±5ºC. 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 greater than 98 per cent using sodium hydroxide as a catalyst. Under optimum conditions the reaction requires 6–8 minutes and may take place during passage through the reaction plant. See also section 8.3 and Choo *et al.* in Gunstone & Padley (1997).

11.2.3 Alcohols

Long-chain alcohols are important oleochemicals produced on a commercial scale by hydrogenolysis of acids, methyl esters or triacylglycerols as described in section 8.6.1 and in Gunstone (2001).

11.2.4 Fatty amines

Fatty amines, produced at a level of around 500 000 tonnes per annum, are the starting point for several types of nitrogen-containing compounds used as surfactants (section 8.7). Acids are converted to nitriles – probably via amides – by reaction with ammonia at 280–360ºC in the presence of zinc oxide, manganese acetate, bauxite, or cobalt salts as the catalyst. The nitriles are then reduced to primary or secondary amines by hydrogenation in the presence of ammonia and a nickel or cobalt catalyst (120–180ºC, 20–40 bar for primary amines and higher temperatures and lower pressures for secondary amines). These are converted to tertiary amines ($RNMe_2$, R_2NMe) by catalytic reaction with formaldehyde and to quaternary ammonium compounds (quats) by further reaction with alkylating agents such as methyl chloride or sulfate or benzyl chloride. The quats may have one, two, or three long alkyl chains. Tertiary amines can also be made directly from long-chain alcohols and dimethylamine in the presence of thallium sulfate at 360ºC. Amine oxides are made from tertiary amines and hydrogen peroxide. These various types of nitrogen-containing compounds are cationic surfactants.

$$RCOOH \rightarrow RCONH_2 \rightarrow RCN \rightarrow RCH_2NH_2 \rightarrow [RCH_2]_2NH$$

11.3 Surfactants

The most important property of the fatty acids and their derivatives for oleochemical purposes is their *amphiphilic* nature. This means that part of the molecule (the alkyl chain) is lipophilic (hydrophobic) and part (the head group) is hydrophilic (lipophobic). Because of this the molecule can exist comfortably at an oil water interface and can reduce the surface tension at such interfaces. This property is fundamental in all living systems and also in many food and other man-made systems where aqueous and fatty phases must co-exist. It confers surface activity (hence such compounds are called surfactants) which allows appropriate compounds to act as emulsifying agents, detergents, lubricants, etc. The simplest and oldest examples are soaps such as sodium palmitate and oleate. The alkyl chain is lipophilic and the carboxylate group is hydrophilic. The detergent properties of such compounds have been exploited for a long time.

The balance between the hydrophilic and lipophilic properties – known as the HL balance – can be varied by modification of each of the two regions of the molecule and especially by change in the nature of the head group. The alkyl chain can vary in length and degree of unsaturation. The head group can show greater variation and includes carboxylates, sulfonates and sulfates, partial esters of glycerol (or other polyhydric alcohols such as propanediol, trimethylolpropane, pentaerythitol and carbohydrates), alcohols, their sulfates and ethylene oxide derivatives, and amines, other nitrogen-containing compounds, and derivatives of amino acids. The final products may be acids, alcohols, amides, esters, or ethers.

Propanediol	$CH_3CH(OH)CH_2OH$
Trimethylolpropane (TMP)	$CH_3CH_2C(CH_2OH)_3$
Pentaerythritol (PE)	$C(CH_2OH)_4$

Although the production of surfactants is now a mature business new developments are continually described (section 11.3.4). It has been claimed that 90 per cent of oleochemical products are used as surface-active materials of some kind. This includes many personal-care items and cosmetic components, as well as lubricants and traditional surfactants.

A detergent is a formulation containing many components responsible for washing and cleansing. The surfactant is the component within the detergent which has the major influence on the properties of an aqueous solution in relation to wetting, foaming, dispersing solids, emulsifying oil and removing dirt. The major surfactants fall into four categories depending on the nature of the head group. These are:

1. Anionic surfactants, e.g. carboxylates, sulfates, sulfonates
2. Nonionic surfactants, e.g. ethoxylates of alcohols, acids, amines, amides
3. Cationic surfactants, e.g. amines, quats, imidazolines
4. Zwitterionic/amphoteric surfactants, e.g. betaines.

Of these, anionic surfactants are used in greatest amount, but the nonionics are significant and growing more quickly than the anionics. Cationic and amphoteric surfactants are used only at lower levels. Figures estimated for the year 2000 are given in Table 11.3. These do not include 7 million tonnes of soap, which is also an anionic surfactant. The major outlets for these compounds are given in Table 11.4. These are dominated by household products followed by materials with other significant uses.

Surfactant properties include those listed in Table 11.5. Sometimes these operate in opposite directions and suitable materials or mixtures must be selected for a particular purpose. The major applications of surfactants are listed Table 11.6. These compounds have been widely used in North America, Western Europe and Japan but demand is spreading through all developed and developing countries.

Table 11.3 Estimated consumption and value of surfactants by type and by region for 2000

	Western Europe		North America		Japan	
	Million tonnes	US$ million	Million tonnes	US$ million	Million tonnes	US$ million
Anionic	1.32	(1.30)	1.95	(1.45)	0.49	(1.28)
Nonionic	0.97	(1.06)	1.04	(1.43)	0.56	(1.82)
Cationic	0.25	(0.32)	0.27	(0.57)	0.10	(0.25)
Amphoteric	0.07	(0.07)	0.09	(0.24)	0.04	(0.13)
Total	2.61	(2.75)	3.34	(3.69)	1.19	(3.48)

Table 11.4 Consumption and value of surfactants by application in Western Europe, North America, and Japan for 2000

	Western Europe		North America		Japan	
	Million tonnes	US$ million	Million tonnes	US$ million	Million tonnes	US$ million
Household products	1407	(1440)	1320	(1440)	321	(940)
Cosmetics and toiletries	122	(230)	192	(250)	133	(401)
Cleaning products	215	(210)	281	(310)	97	(233)
Textiles	160	(175)	310	(320)	121	(273)
Plastics and paints	303	(320)	371	(410)	111	(217)
Mining and petroleum	163	(130)	599	(620)	60	(127)
Agrochemicals	85	(161)	124	(205)	39	(75)
Other	153	(87)	147	(95)	306	(1004)
Total	2608	(2753)	3344	(3690)	1188	(3478)

Table 11.5 Properties of surfactants

emulsification	defoaming	detergency
de-emulsification	water-repelling	sanitising
wetting	dispersing	lubricity
foaming	solubilising	emolliency

Table 11.6 Major applications of surfactants

Consumer products	Industrial products/industries	
Detergents	Detergents and cleaners	Food industry[b]
Dishwashing agents	Textiles and fibres	Pulp and paper, inks
Cleaning agents	Mining and ore flotation	Agrochemicals
Personal care products[a]	Petroleum production	Leather and skin
Cosmetics	Engine lubricants	Metal working
Pharmaceuticals	Paints, lacquers, plastics	Cement and concrete

[a] Soaps, shampoos, bubble baths, toothpaste, shaving cream, shower gels, etc.
[b] additives, cleaners and biocides.

11.3.1 Anionic surfactants

Anionic surfactants are manufactured and used in greater volume than other types of surfactants. Soap belongs to this category and is still the surfactant in greatest use. However, other anionic surfactants with superior properties, especially in hard water, have been developed. These are mainly:

- fatty alcohol sulfates [$ROSO_3H$]
- fatty alcohol ether sulfates [$R(OCH_2CH_2)_nOSO_3H$]
- α-sulfonated esters [$RCH(SO_3H)COOMe$].

The lipophilic alkyl chain is the most expensive component of the surfactant.

11.3.1.1 Production from carboxylic acids

Though still made by alkaline hydrolysis of natural fats (saponification), the sodium or potassium salts of fatty acids (soaps) are also made by neutralisation of fatty acid with alkali. The acids are made by splitting, followed by purification by distillation or by hydrophilisation (section 2.3.2). Other anionic surfactants, made from acids or their derivatives, include the following:

- Sarcosinates and taurates (amides) and isethionates (esters) made from $CH_3CH(NH_2)CH_2COOH$, $CH_3CH(NH_2)CH_2CH_2SO_3H$ and $HOCH_2CH_2SO_3H$ respectively by acylation with acid chlorides and used as their sodium salts.
- Methyl esters react with SO_3 to give α-sulfonates which are used mainly as a mixture of mono- [$RCH(SO_3Na)COOMe$] and disodium salts [$RCH(SO_3Na)COONa$].
- Sulphuric acid reacts with hydroxy compounds such as castor oil or mono- and di-acylglycerols to form sulfates [$RCH(OSO_3H)R'$] or with olefinic compounds to form sulfonates [$RCH_2CH(SO_3H)R'$].

11.3.1.2 Production from alcohols

Both long-chain alcohols and their ethoxylated derivatives are widely used as sulfates formed by reaction with sulfur trioxide or chlorosulfonic acid and generally used as sodium, ammonium, or monoethanolamine salts. Less commonly, the alcohols are also used as phosphates after reaction with phosphorus pentoxide, as sulfosuccinates after reaction with maleic anhydride followed by sodium sulfite, and as ethoxy carboxylates after reaction with sodium chloroacetate. They can also be made into alkyl carbonate ethoxylates by reaction of alcohol, poly[ethylene glycol] and dimethylcarbonate.

$AEOSO_2OH$	sulfate
$RCOOCHCH(OH)CH_2OSO_2OH$	monoacylglycerol sulfate

$AEOPO_3H_2$ or $(AEO)_2PO_2H$	phosphate
$AEOCOCH_2CH(OSO_3Na)COONa$	sulfosuccinate
$AEOCH_2COONa$	ethoxy carboxylates
$ROCOO(CH_2CH_2O)_nH$	carbonates

AEO represents alcohol ethoxylate $RO(CH_2CH_2O)_{n-}$, RO fatty alcohol, and RCOO an acyl group,

Anionic surfactants, whether produced by the oleochemical or petrochemical industries, are active components in detergents used in personal care products and for washing clothes and hard surfaces (dishes, walls, floor, etc). The level of surfactant is usually in the range 5–20 per cent and the balance includes, as appropriate, phosphate, zeolite, bleaching agent, optical brightener, fragrance and water (see Porter in Gunstone & Padley (1997); and Roberts in Gunstone & Hamilton (2001)).

11.3.2 Nonionic surfactants

Nonionic surfactants are uncharged molecules with a head group which is polar by virtue of its several hydroxy and/or ether groups. The hydrophobic group is a long alkyl chain of petrochemical or oleochemical origin. The hydroxy groups come from a range of appropriate head groups such as glycerol, polyglycerol or low molecular weight carbohydrates and the ether groups by reaction of OH groups (in acids or alcohols) or of NH groups (in amines or amides) with ethylene oxide or propylene oxide. Some typical structures are given:

$R(OCH_2CH_2)_nOH$	polyethylene oxide (EO) derivative of the alcohol ROH
$HO(CH_2CH_2O)_q N(R)(OCH_2CH_2)_qOH$	bis-polyethylene oxide (EO) derivative of the amine RNH_2

Nonionic surfactants are used in a wide range of industries. Ethylene oxide itself is a product of the petrochemical industry and is a hazardous chemical with undesirable environmental properties. Attempts are being made to develop alternative products. Both acyl and alkyl derivatives of sugars are of growing interest since the acyl or alkyl chains and the carbohydrate all come from renewable resources (section 11.3.2c). See Bognolo in Gunstone & Padley (1997), Hill *et al.* (1997).

11.3.3 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 as detailed in section 8.5.2.

11.3.4 Alkyl polyglycosides

The term alkyl polyglycoside is the name given to technical products made from starch (or other sources of glucose) and a fatty alcohol. The latter is usually a mixture of C_8 and C_{10} alcohols, C_{12} and C_{14} alcohols, or C_{16} and C_{18} alcohols derived from appropriate fatty acid sources. It follows that all the substrates are renewable resources. The reaction between starch and alcohol is usually catalysed by acids such as sulfuric or 4-toluenesulfonic and may be carried out in two stages. Reaction is accompanied by extensive depolymerisation of the carbohydrate polymer so that the product is mainly, but not entirely, an alkyl glucoside. In the first step, butanol reacts with depolymerised starch and in the second step, reaction occurs with the mixed alcohols (ROH) of desired chain length. The product is a mixture of compounds with (mainly) two different R groups and values of *n* lying between 1 and 5. The average value of *n* (degree of polymerisation) is usually 1.3–1.7. 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 8–14 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.

$$\text{starch} + \text{butanol} \rightarrow [\text{glucose}]_n\text{OBu}$$
$$[\text{glucose}]_n\text{OBu} + \text{ROH} \rightarrow [\text{glucose}]_n\text{OR}$$

11.3.5 Cationic surfactants: cyanides, amines and other nitrogen-containing compounds

Cationic surfactants are nitrogen-containing compounds with one or more alkyl chains of fatty origin. Most often these come from tallow, rape, palm, fish and coconut oil or from tall oil fatty acids. Some typical examples are given in Table 11.7.

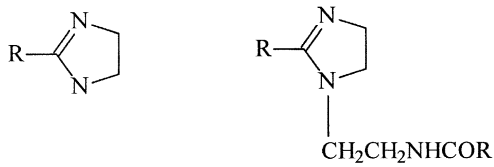
Cationic surfactants (nitrogen containing-compounds) show high substantivity (strong adherence) to natural surfaces and find extensive use in fabric softening, hair conditioning, corrosion inhibition, mineral flotation and as bactericides. About 40 per cent of cationic surfactants are used as fabric softeners. Adsorption of a cationic surfactant onto fabric surfaces improves handling, eases ironing, imparts antistatic properties, and helps perfume retention. The remainder is used for organo-clays, in mineral flotation, road construction and as biocides.

Amides ($RCONH_2$), derived from oleic acid and more especially from erucic acid (22:1), are used mainly as anti-stick and anti-block additives for polyethylene film in which they are incorporated at a 0.1–0.5 per cent level. This is a permitted food-packaging material. They are also used as water repellents for textiles, mould release agents and in rubber goods and printing

Table 11.7 Major nitrogen-containing cationic surfactants

Products	Structure	Reactants
Amines	RNH_2, R_2NH, R_3N	Nitriles (section 11.3.4)
Quaternary salts (quats)	$[R_2NMe_2]^+X^-$	Tertiary amines (section 11.3.4)
Amido amines	$RCONH(CH_2)_3NMe_2$	Acid and polyamines
Imidazolines	(see below)	Acid and polyamines
Esteramine	$RCOOCH_2CH_2NMe_2$	Acids and ethanolamine derivatives
Etheramines	$RO(CH_2)_3NH_2$	Alcohols and acrylonitrile
Amine oxides	$[R_2NMe_2]^+O^-$	Tertiary amines (section 11.3.4)

Imidazolines from RCOOH and diamine ($H_2N(CH_2)_2NH_2$) or triamine ($H_2N(CH_2)_2NH(CH_2)_2NH_2$)



inks. It has been reported that 22 000 tonnes of high erucic oil are used to furnish about 7000 tonnes of erucamide.

The major routes to amines are described in section 8.8.

11.3.6 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 as, for example, in the molecule shown which contains two quaternary groups linked through a tetramethylene spacer.

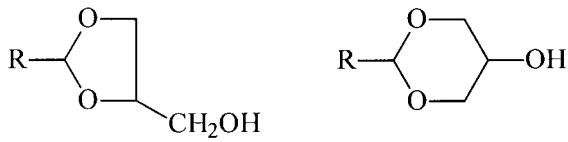
$$RN^+(Me)_2(CH_2)_4N^+(Me_2)R$$

Such molecules are capable of wide variation in terms of the hydrophobic, hydrophilic and spacer groups. Gemini surfactants have some remarkable properties. In particular, compared with conventional surfactants they have lower critical micelle concentrations and reduce surface tension by about two orders of magnitude. Only a small number of these have so far been marketed but there is a lot of research activity in this field.

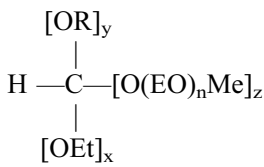
Surfactants with a weak bond built into the molecule are of interest because this leads to improved biodegradabilty and opens up other possible uses of these compounds, such as drug delivery. The weak bonds can be broken by enzymes, by reactions occurring at sewage plants, or by chemical or physical

processes involving acid, alkali, ozone, heat or UV light. Most often these compounds are acetals/ketals or ortho esters.

Cyclic acetals/ketals result when aldehydes or ketones react with polyhydric alcohols such as glycerol, pentaerythryitol, or glucose. The products are 1,3-dioxolanes (5-membered hetero ring) or 1,3-dioxanes (6-membered hetero ring). These compounds are made under anhydrous acidic conditions and are readily hydrolysed under aqueous acidic conditions. Remaining hydroxyl groups can be further functionalised:



Ortho esters are made from ethyl orthoformate ($HC(OEt)_3$), alcohols, and monomethyl polyethylene glycol ($HO(EO)_nMe$) in the presence of a little aluminium chloride. The product is a mixture of many compounds having the structures shown below in which x, y and z each 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.



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:

$R_2N^+Me_2\ X^-$ (quat) $\qquad (RCOOCH_2CH_2)_2N^+Me_2\ X^-$ (ester quat)

The ester quats are stable to acids but are easily hydrolysed by alkali to soap [RCOONa] and the compound $[(HOCH_2CH_2)_2N^+Me_2\ X^-]$. They show better ecological characteristics than the quats themselves.

Alkyl ethoxylates $[RO(EO)_nH]$ are viscous oils which are not always easy to handle but they react with carbon dioxide to form solid carbonates $[RO(EO)_nCO_2Na]$ which are easily incorporated into granular detergents. In alkaline solution, the ethoxylates are quickly regenerated from the carbonates.

11.4 Personal care products

The European Cosmetics Directive describes personal care products as

> any substance or preparation intended to be placed in contact with the various external parts of the body (epidermis, hair system, nails, lips, and external genital organs) or with the teeth or mucous membranes of the oral cavity with a view exclusively or mainly to cleansing them, perfuming them, changing their appearance, and for correcting body odours and/or protecting them and keeping them in good condition.

Personal care products include skincare products, fragrances, materials for hair care, personal hygiene, oral hygiene, make-up and shaving products.

Lipids are important components of many such preparations acting as emollients, stabilisers for emulsions of oil and aqueous solutions, solvents for lipophilic ingredients and as a source of essential fatty acids and of minor components present in unsaponifiable material. They include a wide range of vegetable oils and of acids, esters and alcohols derived from these, waxes, phospholipids, glycolipids and sphingolipids. Lipids are important components of the skin. Various kinds of surface-active compounds are used including anionic, cationic, non-ionic and amphoteric materials. Alkyl esters such as isopropyl myristate and salts of fatty acids (zinc, magnesium, sodium, aluminium) also find wide use.

Materials used in cosmetics must be of uniform high quality and after standard refining procedures they may be further refined by chromatography. Such ‘super-refined’ materials have less colour and odour than normally refined oils and fats and therefore require less pigment and less fragrance, two very expensive ingredients in cosmetic products. Lipids used in cosmetics must be non-irritant – especially to the eyes – chemically stable to hydrolysis, have very low oral and percutaneous toxicity, must be free of microbial contamination and be oxidatively stable.

11.5 Lubricants

Today’s lubricants are a mixture of base fluid (~90%) and a range of other materials added to improve performance. The base fluid is most often mineral oil. This was not always so and until 100–150 years ago, lubricants were based largely on oils and fats of vegetable and animal origin depending on the amphiphilic nature of these molecules. For example, the trade name ‘Castrol’ is based on castor oil. This is an excellent lubricant but not a long-lasting one. The major force for change in current practice is the poor biodegradability of mineral oil and the problems that this causes when oil is spilled or has to be disposed of. However, the replacement of mineral oil by fatty oil is not

straightforward and it has to be remembered it can only be partial because of the growing demand for lubricating oil and the limited availability of fatty oil for non-food use.

The demand for lubricants in Europe was around 4.25 million tonnes in 1999 of which bio-lubricants amount to only 101 000 tonnes. It is estimated that by 2010 this will have doubled without regulation but have increased to around 1.3 million tonnes (30% of the 2010 market) with appropriate supportive legislation.

Erhan & Perez (2002) report that oil-based lubricants in US total around 10 million tonnes with 70 per cent motor oils and 10 per cent as hydraulic fluids. Vegetable oil base stocks are used mainly for hydraulic fluids with a smaller use in two-stroke engines, chain saw bar oils, railway oils, outboard engines and as drilling oils. It is desirable to use oils low in both polyunsaturated acids that promote oxidation and saturated acids which affect cold properties through crystallisation.

There are two types of ester lubricants which can replace the mineral oils. They may be natural glycerol esters, carefully selected for the purpose or they may be esters manufactured from selected polyhydric alcohols and carboxylic acids that may or may not be derived from oils and fats. The latter include:

- Esters of polybasic acids such as succinic, adipic, azelaic, sebacic, mellitic (benzene-1,2,4-tricarboxylic acid) and dimer acids with short and medium-chain alcohols (C_8–C_{13}) which may be branched-chain.
- Esters of polyhydric alcohols such as trimethylolpropane and pentaerythitol with short-, medium-, or long-chain acids. These polyhydric alcohols have no hydrogen on the carbon atom β to the ester function and this prevents a high-temperature decomposition that would otherwise limit the usefulness of these compounds.

These esters are 4–15 times more expensive than mineral oils and are therefore only used in special circumstances such as in aviation engines operating under a wide range of temperatures and in compressors which must undergo long continuous running with minimum maintenance.

There is increasing interest in using appropriate vegetable oils as alternatives to both the conventional mineral oils and the high-priced esters described above. Vegetable oils have both advantages and disadvantages: their advantages are essentially environmental – they are biodegradable, non-toxic, non-carcinogenic and are a renewable resource; their disadvantages relate to their limited viscosity range and to their lower oxidative and hydrolytic stability which in turn relate to unsaturated centres and ester functions present in these molecules.

Vegetable oils make the best lubricants when they have high levels of oleic acid and low levels of both saturated and polyunsaturated fatty acids. This holds for rapeseed oil with a working temperature range of -40 to $+110$ºC

and high-oleic sunflower oil with a working temperature range of −30 to +130ºC, and for the many other high-oleic oils being developed. Saturated acids should be as low as possible because of the effect that they have on the cold properties of the oil. Polyunsaturated fatty acids are undesirable because they reduce oxidative stability. As with mineral oils it is necessary to include a range of additives such as pour point depressants, extreme pressure and anti-wear compounds, antioxidants and viscosity index improvers. These too should be biodegradable. Vegetable oils can also be used as cutting fluids with machine tools with a consequent improvement in the working conditions of the operatives.

The many uses of lubricating oils can be divided into two categories: total loss and long-term use. In the total loss group, the oil is used once and then 'lost'. This is true for chain saws where the oil ends up on the forest floor, two stroke engines, external greasing of wheels, cables, flanges, open gears, etc. and for mould oils used in the building industry to assist removal of temporary cladding. The use of lubricants in mobile and stationary engines, compressors, and turbines are examples of long-term use.

Ester lubricants are now being applied in the following areas: automotive applications, aviation applications, refrigeration lubricants, compressor lubricants, two-stroke engines, hydraulic fluids, greases, quenching oils, demoulding agents and lubricants in the food industry. Ester lubricants including vegetable oils are especially valuable where particular properties, such as low toxicity or easy biodegradabilty are called for or when there is a marked risk of accidental loss.

In comparing lubricants of different types the important properties to be assessed include:

- kinematic viscosity,
- cold properties (cloud point, pour point, and cold filter plugging point),
- fire resistance properties (flash point and fire point),
- chemical properties (acid value, saponification value, iodine value and hydroxyl value along with newer chromatographic and spectroscopic properties),
- performance properties (Noack test, oxidation stability and hydrolytic stability)

See also Bondioli in Gunstone & Hamilton (2001) for more information.

11.6 Biodiesel

The term biodiesel usually describes the methyl esters of a readily-available natural oil or fat prepared for use as automotive fuel. The choice of starting material is not critical and soybean oil (in the United States), rapeseed oil (in

Europe), palm oil (in Malaysia) and waste fat (in Japan and other countries) have all been employed. The methyl esters can be used in partial or complete replacement of conventional diesel fuel without modification of the engine and without noticeable diminution of efficiency. The pressure to produce and use biodiesel is largely environmental but the relative availability of mineral oil and fatty oil means that the impact can be only marginal. Using yield and production data, it is possible to calculate that the amount of land required to produce one tonne of biodiesel is 2.67 hectares for soybean, one hectare for rape seed, and 0.14 hectare for palm. In Europe there is a second reason for producing biodiesel in that farmers are required not to use all their land for food crops. They may however use the 'set aside' land to grow crops for non-food purposes such as oil seed rape for biodiesel production. However with alterations in the Common Agricultural Policy this is set to change. Until now, biodiesel has been used mainly in admixture with diesel fuel by captive fleets such as taxis and buses mainly in inner urban areas where atmospheric conditions are poorest. But this may change. Already there are some countries where biodiesel can be purchased at normal petrol stations and this is set to increase. Environmental concerns for which biodiesel offers potential benefits centre around air pollution (a local and immediate problem) and global warming (a world-wide and long-term problem).

Methyl esters are preferred because the technology for producing these on a large scale already exists (section 8.3.3). There is some interest in ethyl esters since these can be made with ethanol. This is made by fermentation of renewable raw materials whereas methanol is a product of the petrochemical industry and is based on non-renewable resources. However, ethyl esters are more difficult to store as they tend to be hygroscopic. Isopropyl esters have certain advantages over methyl esters, especially at low temperatures in adverse winter conditions, because of their lower solidification point. Positive claims have also been made for amides ($RCONEt_2$) and tertiary amines (RCH_2NMe_2). The methyl esters can be used as heating oil, as lubricants, and as an environmentally acceptable solvent (e.g. for cleaning oil spills) in addition to their role as biofuel and as a source of fatty alcohols (section 8.5.1).

There are four ways of obtaining diesel fuel from vegetable oils, all designed to reduce the high kinematic viscosity of triacylglycerol oils: transesterification, dilution, microemulsification and co-solvent blending, and pyrolysis but the first is the one most commonly employed at present.

Advantages of biodiesel:

- Careful studies have shown that the energy recovered from biofuel and other products exceeds that of all the necessary inputs by a factor of 2.5–4.
- Biodiesel is a non-toxic product. It is 98 per cent biodegradable in 21 days compared with 50 per cent for normal diesel. It performs satisfactorily in standard environmental tests with fish, bacteria and plants.

- When used in an engine and compared with mineral fuel, biodiesel produces less sulfur, smoke, pollutants, unburnt fuel and polycyclic aromatic hydrocarbons though carbon monoxide, benzene, nitrogen oxides and aldehydes may be slightly higher.
- Carbon dioxide liberated when biodiesel is used represents carbon dioxide trapped through photosynthesis during the growing of the plant. Also, the esters come from a renewable resource and do not deplete non-renewable resources.

Disadvantages of biodiesel:

- There is an argument that the global demand for food is so great that oils and fats should not be used unnecessarily for non-food purposes. Nevertheless despite calorie-shortage in some parts of the world there are local surpluses of oils and fats and biodiesel is one way of reducing these.
- Some advocates of biodiesel, however, have failed to grasp certain important facts related to numbers and areas. For example the European Parliament has set targets for the growing use of biodiesel up to 2010. In 2003 European farmers grow oilseed crops on about 5.6 million hectares (mainly rapeseed but also some sunflower and soybean) but to provide the projected 7 million tonnes of biodiesel would require 6.4 million hectares and lead to consequent distortion of several markets such as the present use of rapeseed oil for food and other non-food purposes and would result in a large oversupply of glycerol.
- A major problem with biodiesel is its cost. A true comparison of the real cost of biodiesel and conventional diesel of mineral origin is very difficult because so many factors have to be taken into account. Using common accounting procedures, biodiesel is only economic if governments are willing to reduce the levels of taxation normally applied to automotive fuel. Assessment of relative cost is not made any easier by frequent fluctuations in the price of both mineral and vegetable oils. The demand for biodiesel may be assisted by government legislation requiring that a certain amount of non-mineral fuels must be used. The reasons for this would be partly environmental, partly to assist farmers, and partly to reduce the need for imported mineral oil for strategic reasons.
- As already indicated, production levels of biodiesel, even under the most favourable circumstances, can only have a small impact on the market for conventional diesel fuel.

In 2000 it was reported that commercial production capacity for biodiesel (methyl esters) was about 1.1 million tonnes, with over 90 per cent of this in Europe and particularly in Germany (500 000 tonnes), and France (400 000 tonnes). But with additional plants under construction in Germany (based on rape seed oil), United States (soybean oil), Malaysia (palm oil) and several

other countries, it is expected that biodiesel will be available more extensively. Standard specifications and analytical methods are detailed by Knothe & Dunn in their recent review (2001) of biofuels.

11.7 Glycerol

When fats are subjected to hydrolysis or methanolysis glycerol is liberated as a by-product at a level equivalent to about 10 per cent of the weight of fat and world production is about 930 000 tonnes. Supply of glycerol will rise with increasing demand for acids and esters and the rise could be significant if there is a large increase in the use of biofuels (methyl esters). When lipids are eaten, glycerol is 'lost' being completely metabolised but if the demand for olestra grows this will change as this zero-calorie fat is made from sucrose and fatty acids with glycerol being recovered at the hydrolysis stage.

Glycerol is obtained mainly in the production of soap (~180 000 tonnes of glycerol), free acids (~350 000 tonnes), and esters (for biodiesel and alcohol production) 270 000. Synthetic glycerol prepared from propene is estimated at only ~80 000 tonnes. Since glycerol recovery and sale is an important element in the economic viability of biofuel production a fall in the price of glycerol due to increased supply is a matter of concern and attempts are being made to find new uses for glycerol and for compounds which can be obtained from it. Among these are glycidol (2,3-epoxypropan-1-ol), glycerol carbonate, and polyglycerol. The last is a mixture of hydroxy ethers (mainly linear but also branched and cyclic) with up to 7 glycerol units but mainly 2–4.

$$HOCH_2CHOHCH_2O[CH_2CHOHCH_2O]_nCH_2CHOHCH_2OH$$

a linear polyglycerol

Glycerol is available in several grades. Refined material is at least 96 per cent pure and generally greater than 99.5 per cent. Its value lies in its physical properties: it is hygroscopic, colourless, odourless, viscous, has a sweet taste, is low-boiling, non-toxic, emollient, a good solvent and water-soluble. Among the major uses are oral care products, food and food emulsifiers, tobacco products, polyurethanes, prescription drugs and over-the-counter medicines, cosmetics, etc.

11.8 Dibasic acids

Short and medium chain dibasic acids serve as monomers in the production of polymeric esters and amides. The most important are in the range C_6 to C_{13} and are produced by the petrochemical industry (C_6 and C_{12}) or by the oleochemical industry (C_9, C_{10} and C_{13}).

Ozonolysis of monoene fatty acids furnishes monobasic and dibasic acids of which the latter are more valuable. Oleic acid will yield nonanoic and azelaic acids (both C_9) and erucic acid gives nonanoic and brassylic acid (C_{13}). The first of these is an important industrial process, the second has yet to be exploited. Ozonolysis of oleic acid is carried out in a solution of nonanoic acid at 25–45ºC and the first-formed product is heated to 100ºC in the presence of a manganese salt.

Industrial ozonolysis presents some difficulties and alternative strategies are being examined though there is no evidence that any of them have been used on a commercial scale. One method employs oxygen in the presence of aldeydes as an oxidising agent and proceeds via an epoxide. A second is oxidation with sodium hypochlorite in a three-stage process involving emulsification of oleic acid in water, non-catalytic oxidation with sodium hypochlorite and alkali and cleavage of the diol with sodium hypochlorite and ruthenium chloride as catalyst.

Yet another route to dibasic acids involves metathesis twice and does not require oxidation (section 7.7). The metathesis catalyst is B_2O_3-Re_2O_7 and Al_2O_3/SiO_2 activated with $SnBu_4$ and high yields are reported. As an example, methyl oleate and ethylene give methyl 9-decenoate which in a second metathesis reaction gives the diester of 9-octadecendioate. This can be converted to the C_{18} dibasic acid. The C_{20} and C_{26} dibasic acids can be made in a similar manner from 10-undecenoic acid and from erucic acid (Δ13–22:1) respectively.

Sebacic acid (decanedioic acid) is obtained from castor oil or ricinoleic acid by reaction with sodium hydroxide at 180–270ºC (section 11.9). It is used with 1,6-diaminohexane to make a 6,10-nylon required for toothbrushes, textile products, technical fibres and belting.

In addition to their use in the production of polyamides and polyesters, di-esters of the dibasic acids with appropriate straight and branched chain acids are used as plasticisers in PVC and as synthetic lubricants (section 11.5).

11.9 Dimers, isostearic acid, estolides, Guerbet alcohols and acids

Dimer acids are also dibasic acids and are produced on a commercial scale by heating unsaturated acids for 4–8 hours at around 230ºC with montmorillonite clay or some alternative cationic catalyst. Distillation gives a monomer, a dimer containing some trimer and a more extensively polymerised residue. The reaction occurs with both monoene and diene acids and tall oil, containing both these acids in similar amounts, is used widely for this purpose. The distilled oligomer is about 80 per cent dimer and 20 per cent trimer. Oleic acid gives mainly acyclic and monocyclic dimers while linoleic acid produces mainly mono- and bi-cyclic compounds. They have not been fully identified but they are C_{36} dibasic acids and are used mainly as polyamides.

The dimer acids react with ethylene diamine and other diamines to give non-reactive polyamides which have excellent adhesive properties and are used as hot-melt adhesives in shoe making and book binding. Molecules with more than two amine groups (such as diethylene triamine with the formula $H_2N(CH_2)_2NH(CH_2)_2NH_2$) react with dibasic acids to give polyamides with a free NH_2 group which can act as a curing agent for epoxy resins. Imidazolines of the dimer acids act as corrosion inhibitors. Short-path distilled dimers (95–99% pure) are used in polymer applications. It was estimated in 1995 that 100 000 tonnes of acids were converted to dimer acids in the United States and 35 000 tonnes were similarly treated in Western Europe.

The monomeric acid remaining after dimerisation is a complex mixture of saturated and unsaturated acids with and without chain-branching. After hydrogenation and crystallisation, a concentrate of branched-chain saturated acids can be recovered. This acid (isostearic) is low-melting by virtue of its branched-chain structure and is used in lubricants and cosmetics, usually in esterified form.

Dimerisation is usually carried out in the presence of only 1–2 per cent of water. With more water (9–10%) estolides are formed (section 7.9).

Guerbet alcohols which are dimeric but only monofunctional were first reported in 1899 and are now produced industrially on a limited scale. They are described in section 8.6.5.

11.10 Oleochemicals from castor oil

Castor oil differs from other commodity oils and fats in that it contains high levels of ricinoleic acid (12-hydroxyoleic acid). Castor oil or ricinoleic acid is a useful starting point for several useful chemicals. These are summarised below and further details are reported by Caupin in Gunstone & Padley (1997).

- Sulfation converts the hydroxyl group to a sulfate 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.
- Dehydration of castor oil and of castor acids gives products enriched in diene acids, some of them with conjugated unsaturation. These products are valuable alternatives to drying oils such as tung oil.
- Hydrogenated castor oil and hydrogenated castor acids, with higher melting points than the non hydrogenated 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 widely 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 180–200ºC with a 1:1 caustic/castor ratio the major products are 2-octanone and 10-hydroxydecanoic acid. At 250–275ºC and a 2:1 ratio the products are 2-octanol and sebacic (decanedioic) acid. The dibasic acid, when reacted appropriately, gives a nylon (polyamide) and efficient lubricants (esters).
- Splitting ricinoleic acid with steam gives C_7 and C_{11} products. This splitting process has been improved greatly by the development of a continuous steam-cracking process. Heptanal is used in perfumes and 10-undecenoic acid shows antifungal properties. It can also be converted via 11-amino-undecanoic acid to a polyamide (Rilsan).

11.11 Surface coatings and inks

Surface coatings are applied to a range of surfaces (wood, paper, metal, plastic) to protect (against moisture, oxygen, sunlight, radiation and pollutants such as sulfur dioxide), to decorate, or to disguise. About half the annual production of paint is used for 'architectural purposes' i.e. for internal and external use in buildings. The remainder is used for coating cars, machines, domestic appliances, road markings, etc.

Paints may contain up to four components:

1. A binder (also described as resin or vehicle) to provide adhesion and cohesion. This component contains any vegetable oil that may be present in the paint.
2. A pigment to provide colour and opacity. Paints without pigments are varnishes.
3. Solvents (thinners) such as turpentine, white spirit, or other hydrocarbon required to aid manufacture and application. Attempts are now being made to reduce the amount of these volatile organic compounds (VOC) and where possible to replace them with water or with non-volatile material.
4. Additives such as thickeners or wetting agents.

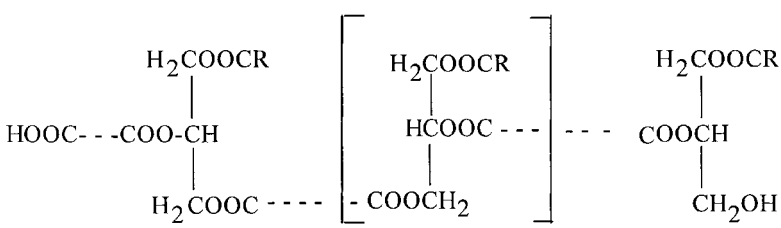
Most surface coatings today are alkyd resins (polyesters) containing significant amounts of fatty acids. An alkyd resin is made from the following components:

- A polybasic acid, generally phthalic acid (benzene-1,2-dicarboxylic acid) used in the form of its anhydride but sometimes isophthalic acid (benzene-1,3-dicarboxylic acid) or trimellitic acid (benzene-1,2,4-tricarboxylic acid).
- A polyhydric alcohol such as glycerol or pentaerythritol [$C(CH_2OH)_4$] with glycerol being frequently supplied in the form of a monoacylglycerol.
- Fatty acids, mainly unsaturated and supplied as free acids, triacylglycerols, or most often as monoacylglycerols derived from appropriate vegetable oils. The fatty materials are usually from a drying oil like linseed oil or semi-drying oils like soybean and dehydrated castor oil. Other oils sometimes used include castor, tung, coconut, safflower, sunflower and tall oil. Alkyds usually contain 30–70 per cent of fatty acids and total usage of vegetable oils in surface coatings is probably about one million tonnes each year. It is desirable that the vegetable oil serving as 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 conjugation of the polyunsaturated system with a proprietary catalyst to speed drying.

After application as a liquid, the paint must harden to a solid film though evaporation of the volatile thinner, reaction of the binder with atmospheric oxygen, and sometimes by interaction with other components present such as polyols and isocyanates. The drying or hardening process can be accelerated by the addition of dryers such as cobalt salts of short chain acids which promote oxidation, by ultraviolet light, or by pre-treatment of the oil to make it more easily oxidised/polymerised. The drying process continues after the film has hardened leading, eventually, to flaking.

Although alkyds with linolenic acid dry faster than those with linoleic acid the former also leads to more extensive oxidation with the undesirable result that the paint film becomes yellow in an ammonia atmosphere.

In a simple example, vegetable oil is heated with glycerol to give monoacyl glycerol and then with phthalic anhydride. This produces the intermediate formulated below which reacts further at its carboxyl group with another polyhydric molecule leading to compounds of high molecular weight. Finally these undergo oxidative polymerisation of the unsaturated acyl groups and this process called ‘drying’ (see Bentley in Gunstone & Hamilton, 2001).



Other components may be added to alkyds to produce some particular effect as in the following examples:

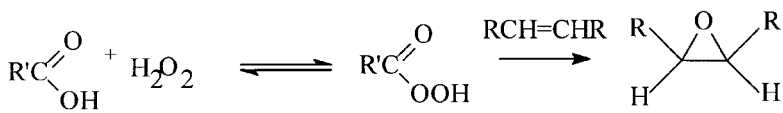
- Urethane alkyds have part of their structure incorporating a di-isocyanate such as tolylene di-isocyanate. This effectively replaces part of the phthalic anhydride. Urethane alkyds are tougher and improve mechanical properties of paints.
- Addition of a few per cent of a polyamide resin based on dimer fatty acids (section 11.8.1) gives thixotropic alkyds suitable for decorative paints.
- Alkyds with linseed or dehydrated castor acids may be reacted with styrene or vinyl toluene to give quicker drying properties.
- Silicone modifications give improved durability desired for exterior maintenance finishes.

Vegetable oil-based inks incorporating materials from soybean, linseed, rape, palm and others have several benefits over the more traditional petroleum-based products. These include superiority in rub resistance, production of a deeper black, provide outstanding colour, quicker adjustment to change in colour and easier removal when recycling paper using methyl esters as cleaning solvent. In addition they have the usual range of environmental advantages in that they are biodegradable (apart from the pigment), low in VOC and PAH, easier to clean from the press, and are based on a renewable resource. Such inks are being widely used for newsprint.

11.12 Epoxides, hydroxy acids, polyurethanes

Epoxidation converts olefins to epoxides (three-membered cyclic ethers) which have industrial uses in their own right, but are also reactive molecules easily converted into other useful products. Some epoxy acids such as vernolic (12,13-epoxyoleic acid) occur naturally (section 7.4.1) but the most widely used epoxy oils are produced from polyunsaturated oils such as linseed and soybean. Epoxidised palm oil is also made and used in Malaysia. The annual production of epoxidised soybean oil is around 100 000 tonnes.

To produce material on this scale, soybean oil is reacted with formic acid and hydrogen peroxide or acetic acid, sulfuric acid, and hydrogen peroxide at around 60ºC for 10–15 hours. Reaction occurs via the peroxy acid thus:



Sulphuric acid catalyses the equilibrium process by which peroxy acetic acid is formed. The final product will usually have lost all its unsaturation, but the

epoxide value is a little lower than expected because the epoxides are reactive compounds easily subject to ring opening, especially under the acidic reaction conditions

Epoxidised soybean oil, epoxidised linseed oil and epoxidised octyl oleate are used mainly as plasticisers and stabilisers in PVC. They add flexibility to the polymer and scavenge any hydrogen chloride that is liberated by heat or light. Epoxidised palm oil with its lower oxirane value is less effective in this respect, but is a satisfactory plasticizer (see Gunstone in Gunstone & Padley, 1997).

Enzymic oxidation with hydrogen peroxide and the lipase Novozyme 435^{R} has been reported. For epoxidation of oils it is necessary to add some free acid (5%). This is oxidised to peroxy acid and this effects epoxidation which can be carried partially or completely. This is an interesting process but there is no evidence that it has been used commercially.

Epoxy compounds, especially in acidic media, react readily with nucleophiles to give interesting and potentially useful compounds. For example alcohols give hydroxy ethers and amines give hydroxy amines. Polyfunctional nucleopohiles such as diols can provide polymeric products. The hydroxy compounds can be used to produce polyurethanes.

$$\text{Epoxide} + R'OH \rightarrow RCH(OH)CH(OR')R \text{ (hydroxy ether)}$$

Venturello's catalyst $Q_3PO_4[W(O)(O_2)_2]_4$, where Q is a phase transfer catalyst, is made from tungsten powder, hydrogen peroxide, phosphoric acid and a phase transfer catalyst. It is used in conjunction with H_2O_2 to convert olefins to epoxides. The catalyst dissolves in the aqueous phase and in the fatty phase. Reaction occurs without a solvent if the mixture is properly emulsified at 100ºC for about one hour or more slowly at room temperature (days). The catalyst can be recovered and recycled. To produce polyol the epoxide must be ring opened, for example with 50 per cent phosphoric acid at around 100ºC. If the oil is to be completely reacted then the two steps can be combined in a one pot reaction. Reaction with a disocyanate will give a polyurethane for a market estimated at 7 million tonnes in 2000 and rising by 1 million tonnes each year. The degree of hydroxylation can be varied to give different types of PU products. Castor oil (with two or three OH groups in each tag molecule) can also be used to make PU.

Bibliography and references

Erhan, S.Z. & Perez, J.M. (eds) (2002) *Biobased Industrial Fluids and Lubricants*, AOCS Press, Champaign, Illinois.

Gunstone, F.D. & Padley, F.B. (eds) (1997) *Lipid Technologies and Applications*, Marcel Dekker, New York.

Gunstone, F.D. (2001) Chemical reactions of fatty acids with special reference to the carboxyl

group, *European Journal of Lipid Science and Technology*, **103**, 307–314.
Gunstone, F.D. & Hamilton, R.J. (eds) (2001) *Oleochemical Manufacture and Applications*, Sheffield Academic Press, Sheffield.
Hill, K., Rybinski, W. von & Stoll, G. (1997) *Alkylpolyglycosides: Technology, Properties, and Applications*, VCH, Weinheim.
Hui, Y.H. (ed.) (1996) *Bailey's Industrial Oil and Fat Products*, 5th edition, five volumes, John Wiley, New York.

Index

THE CHEMISTRY OF OILS AND FATS

Sources, Composition, Properties and Uses

Frank D. Gunstone

The three major macronutrients are proteins, carbohydrates, and lipids (oils and fats). This book is devoted to lipids, which are an important part of life for all of us. What are these materials in molecular terms? Where do they come from? What happens to them between the harvesting of crops and the appearance of the oils and fats in different products in the supermarket? How does nature produce these molecules and can we act on nature to modify the materials to increase their beneficial properties? How important are the minor products present in the fats that we consume? Since oils and fats vary, how can we analyse them? What are their physical, chemical and nutritional properties? How do the fats that we consume affect our health and well-being in both quantitative and qualitative terms? What are their major food and non-food uses?

This book provides a broad source of reference on oils and fats chemistry for graduates entering the food and oleochemical industries, postgraduate researchers and nutritionists. It offers a point of entry to the detailed literature.

The Author
Frank D. Gunstone is Professor Emeritus, University of St Andrews and Honorary Research Professor, Scottish Crop Research Institute, Dundee, UK

Blackwell Publishing
ISBN 1-4051-1626-9

B2373

USA / Canada: CRC Press

Front panel photograph courtesy of Canola Image Library, Canola Council of Canada