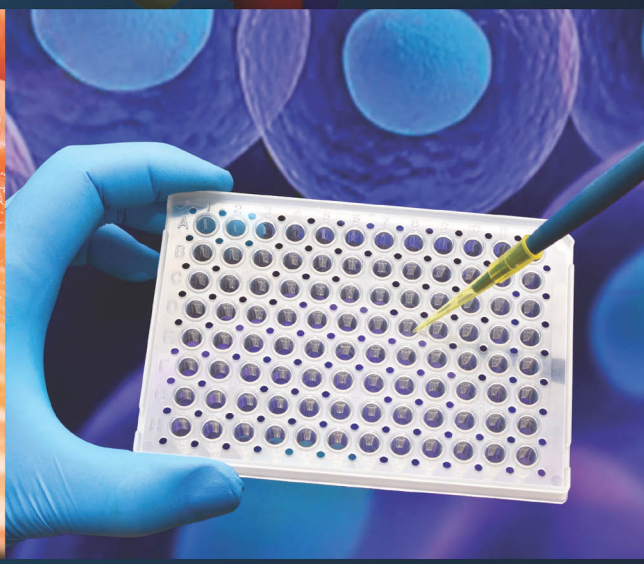
FOURTH EDITION

FOOD LIPIDS

Chemistry, Nutrition, and Biotechnology



Casimir C. Akoh

CRC Press
Taylor & Francis Group

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Preface

The first edition of *Food Lipids* was published in 1998 and the second edition in 2002 by Marcel Dekker, Inc. Taylor & Francis Group, LLC, acquired Marcel Dekker and the rights to publish the third and subsequent editions. I firmly believe that this book has provided those involved in lipid research and instruction with a valuable resource for materials and information. On behalf of my late former coeditor, Professor David B. Min, I thank all those who bought the previous editions and hope that you will find the fourth edition equally or more interesting and helpful. Before describing the content of the current edition, I would like to pay tribute to Professor Min, who worked very hard with me in seeing to the successful recruitment of potential authors and the publication of the previous editions. I miss him dearly and dedicate this fourth edition to his memory and his contributions to our knowledge on lipids, especially, lipid oxidation.

Taylor & Francis Group and I felt the need to update the information in the third edition and include more recent topics of interest to the readers and users of this text/reference book. We continue to believe, based on the sales of the previous editions and the comments of instructors and those who purchased and used the book, that this textbook is and will continue to be suitable for teaching food lipids, nutritional aspects of lipids, and lipid chemistry/biotechnology courses to food science and nutrition majors. The aim of the first, second, third, and fourth editions remains unchanged: to provide a modern, easy-to-read textbook for students and instructors and a reference book for professionals and practitioners with an interest in lipids. The book is also suitable for upper-level undergraduate, graduate, and postgraduate instruction. Scientists and professionals, who have left the university and are engaged in research and development in the industry, government, regulatory, or academics will find this book a useful reference. In this edition, I have deleted some chapters and renamed some chapters and/or had new authors rewrite some of the old chapters with updated references and added many new chapters to reflect current knowledge and interests. In other words, the new edition represents a substantial change from the previous editions. Section I increased from seven to nine chapters and Section II from three to seven, Section III decreased from eight to five, Section IV still has nine chapters but some are new, and Section V increased from five to seven. A great effort was made to recruit and select contributors who are internationally recognized experts. I thank the new authors and the prior authors who updated their chapters for their exceptional attention to detail and timely submission of their manuscripts.

Overall, there are 37 chapters in the fourth edition, and the text has been updated with new and available information. Again, some chapters were removed and new ones added. The new additions or modifications are Chapters 2, 6, 8 through 11, 13 through 17, 20, 22 through 24, 26, 28 through 32, 36, and 37. It is almost impossible to cover all aspects of lipids. I feel that with the added chapters, the book covered most topics that are of interest to our readers. The book is divided into five main sections: chemistry and properties, processing and food applications, oxidation and antioxidants, nutrition, and biotechnology and biochemistry.

I remain grateful to the readers and users of the previous editions and sincerely hope that the much improved and updated fourth edition will meet your satisfaction. Comments on this edition are welcomed. Based on the comments of readers and reviewers of the past editions, I hope that the current edition is an improvement with new chapters and new ways of studying and utilizing lipids to benefit our profession and consumers. I apologize in advance for any errors and urge you to contact me or the publisher if you find mistakes or have suggestions to improve the readability and comprehension of this text.

Special thanks go to our readers and to the editorial staff at Taylor & Francis Group, LLC, for their expertise, suggestions, and completing the publication on time.

Casimir C. Akoh

Editor

Casimir C. Akoh, a certified food scientist (2013), is a distinguished research professor of food science and technology and an adjunct professor of foods and nutrition at the University of Georgia, Athens. He is coeditor of the book *Carbohydrates as Fat Substitutes* (Marcel Dekker, Inc.); coeditor of *Healthful Lipids* (AOCS Press); editor of *Handbook of Functional Lipids* (CRC Press); coeditor of *Food Lipids* (first, second, and third editions); coeditor of *Palm Oil: Production, Processing, Characterization, and Uses* (AOCS Press); author or coauthor of more than 254 referenced SCI publications and more than 40 book chapters; and holder of three U.S. patents. He has made over 275 presentations and given more than 160 invited presentations at national and international conferences.

He is a fellow/WABAB Academician of the International Society of Biocatalysis and Agricultural Biotechnology (2015) and a fellow of the Institute of Food Technologists (2005), American Oil Chemists' Society (2006), the American Chemical Society (2006), a member of the International Society of Biocatalysis and Agricultural Biotechnology, and the Phi Tau Sigma Honorary Society (Honor Society for Food Science). He is currently an associate editor of the *Journal of the American Oil Chemists' Society* and the *Journal of Food Science*, serves on the editorial boards of the *European Journal of Lipid Research and Technology,* the *Journal of Biocatalysis and Agricultural Biotechnology,* and the *Annual Reviews of Food Science and Technology.*

He is a highly cited scientist in agricultural science and has received numerous national and international professional awards for his work on lipids, including the 1996 International Life Sciences Institute of North America Future Leader Award, the 1998 IFT Samuel Cate Prescott Award, 1999 Creative Research Medal Award, 2000 Gamma Sigma Delta Distinguished Senior Faculty Research Award, the 2003 D.W. Brooks Award, the 2004 AOCS Stephen S. Chang Award, the 2004 Distinguished Research Professor, the 2008 IFT Stephen S. Chang Award, the 2008 IFT Research and Development Award, the 2009 AOCS Biotechnology Division Lifetime Achievement Award, the 2012 IFT Nicholas Appert Award, the 2012 AOCS Supelco/Nicholas Pelick Research Award, and the 2015 University of Nigeria, Nsukka, Alumni Achievement Award.

He received his PhD (1988) in food science from Washington State University, Pullman. He holds MS and BS in biochemistry from Washington State University and the University of Nigeria, Nsukka, respectively.

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Section I

Chemistry and Properties

1 Nomenclature and Classification of Lipids

Sean Francis O'Keefe and Paul J. Sarnoski

CONTENTS

I. DEFINITIONS OF LIPIDS

No exact definition of lipids exists. Christie [1] defines lipids as "a wide variety of natural products including fatty acids and their derivatives, steroids, terpenes, carotenoids, and bile acids, which have in common a ready solubility in organic solvents such as diethyl ether, hexane, benzene, chloroform, or methanol."

Kates [2] says that lipids are "those substances which are (a) insoluble in water; (b) soluble in organic solvents such as chloroform, ether or benzene; (c) contain long-chain hydrocarbon groups in their molecules; and (d) are present in or derived from living organisms."

Gurr and James [3] point out that a standard definition describes lipids as "a chemically heterogeneous group of substances, having in common the property of insolubility in water, but solubility in nonpolar solvents such as chloroform, hydrocarbons or alcohols."

Despite its common usage, definitions based on solubility have obvious problems. Some compounds that are considered as lipids, such as C1–C4 very short-chain fatty acids (VSCFAs), are completely miscible with water and insoluble in nonpolar solvents. Some researchers have accepted this solubility definition strictly and exclude C1–C3 fatty acids from the definition of lipids, keeping C4 (butyric acid) only because of its presence in dairy fats. Additionally, some compounds that are considered as lipids, such as some *trans* fatty acids (those not derived from bacterial hydrogenation), are not derived directly from living organisms. The development of synthetic acaloric and reduced calorie lipids such as the sucrose polyester olestra (trade name Olean®) complicates the issue because these lipids may fit into solubility-based definitions but are not derived from living organisms, may be acaloric, and may contain esters of VSCFAs.

The traditional definition of total fat of foods used by the U.S. Food and Drug Administration (FDA) has been "the sum of the components with lipid characteristics that are extracted by Association of Official Analytical Chemists (AOAC) methods or by reliable and appropriate procedures." The FDA has changed from a solubility-based definition to "total lipid fatty acids expressed as triglycerides" [4], with the intent to measure caloric fatty acids. Solubility and size of fatty acids affect their caloric values. This is important for products that take advantage of the calorie-based definition, such as Benefat/Salatrim, so these products would be examined on a case-by-case basis. Food products containing sucrose polyesters would require special methodology to calculate caloric fatty acids. Foods containing vinegar (~4.5% acetic acid) present a problem because they will be considered to have 4.5% fat unless the definition is modified to exclude water-soluble fatty acids or the caloric weighting for acetic acid is lowered [4].

Despite the problems with accepted definitions, a more precise working definition is difficult, given the complexity and heterogeneity of lipids. This chapter introduces the main lipid structures and their nomenclature.

II. LIPID CLASSIFICATIONS

Classification of lipids is possible based on their physical properties at room temperature (oils are liquid and fats are solid), polarity (polar and neutral lipids), essentiality for humans (essential and nonessential fatty acids), or structure (simple or complex). Neutral lipids include fatty acids, alcohols, glycerides, and sterols, whereas polar lipids include glycerophospholipids and glyceroglycolipids. The separation into polarity classes is rather arbitrary, as some short-chain fatty acids are very polar. A classification based on structure is, therefore, preferable.

Based on structure, lipids can be classified as derived, simple, or complex. Derived lipids include free fatty acids and alcohols, which are the building blocks for the simple and complex lipids. Simple lipids, composed of fatty acids and alcohol components, include acylglycerols, ether acylglycerols, sterols, and their esters and wax esters. In general terms, simple lipids can be hydrolyzed to two different components, usually an alcohol and an acid. Complex lipids include glycerophospholipids (phospholipids), glyceroglycolipids (glycolipids), and sphingolipids. These structures yield three or more different compounds on hydrolysis.

The fatty acids constitute the obvious starting point in lipid structures. However, a short review of standard nomenclature is appropriate. Over the years, a large number of different nomenclature systems have been proposed [5]. The resulting confusion has led to a need for nomenclature standardization. The International Union of Pure and Applied Chemists (IUPAC) and International Union of Biochemistry (IUB) collaborative efforts have resulted in comprehensive nomenclature

standards [6], and the nomenclature for lipids has been reported [7–9]. Only the main aspects of the standardized IUPAC nomenclature relating to lipid structures will be presented; greater detail is available elsewhere [7–9].

Standard rules for nomenclature must take into consideration the difficulty in maintaining strict adherence to structure-based nomenclature and elimination of common terminology [5]. For example, the compound known as vitamin K_1 can be described as 2-methyl-3-phytyl-1,4-naphthoquinone. Vitamin K_1 and many other trivial names have been included into standardized nomenclature to avoid confusion arising from long chemical names. Standard nomenclature rules will be discussed in separate sections relating to various lipid compounds.

Fatty acid terminology is complicated by the existence of several different nomenclature systems. The IUPAC nomenclature, common (trivial) names, and shorthand (*n*- or ω) terminology will be discussed. As a lipid class, the fatty acids are often called free fatty acids (FFAs) or nonesterified fatty acids (NEFAs). IUPAC has recommended that fatty acids as a class be called fatty acids and the terms FFA and NEFA eliminated [6].

A. Standard IUPAC Nomenclature of Fatty Acids

In standard IUPAC terminology [6], the fatty acid is named after the parent hydrocarbon. Table 1.1 lists common hydrocarbon names. For example, an 18-carbon carboxylic acid is called octadecanoic acid, derived from octadecane, the 18-carbon aliphatic hydrocarbon. The name octadecanecarboxylic acid may also be used, but it is more cumbersome and less common. Table 1.2 summarizes the rules for hydrocarbon nomenclature.

Double bonds are designated using the Δ configuration, which represents the distance from the carboxyl carbon, naming the carboxyl carbon number 1. A double bond between the 9th and 10th carbons from the carboxylic acid group is a Δ9 bond. The hydrocarbon name changes to indicate the presence of the double bond; an 18-carbon fatty acid with one double bond is called octadecenoic acid, one with two double bonds is named octadecadienoic acid, etc. The double-bond

TABLE 1.1
Systematic Names of Hydrocarbons

Carbon Number	Name	Carbon Number	Name
1	Methane	19	Nonadecane
2	Ethane	20	Eicosane
3	Propane	21	Henicosane
4	Butane	22	Docosane
5	Pentane	23	Tricosane
6	Hexane	24	Tetracosane
7	Heptane	25	Pentacosane
8	Octane	26	Hexacosane
9	Nonane	27	Heptacosane
10	Decane	28	Octacosane
11	Hendecane	29	Nonacosane
12	Dodecane	30	Triacontane
13	Tridecane	40	Tetracontane
14	Tetradecane	50	Pentacontane
15	Pentadecane	60	Hexacontane
16	Hexadecane	70	Heptacontane
17	Heptadecane	80	Octacontane
18	Octadecane		

TABLE 1.2
IUPAC Rules for Hydrocarbon Nomenclature

1. Saturated unbranched acyclic hydrocarbons are named with a numerical prefix and the termination "ane." The first four in this series use trivial prefix names (methane, ethane, propane, and butane), whereas the rest use prefixes that represent the number of carbon atoms.
2. Saturated branched acyclic hydrocarbons are named by prefixing the side chain designation to the name of the longest chain present in the structure.
3. The longest chain is numbered to give the lowest number possible to the side chains, irrespective of the substituents.
4. If more than two side chains are present, they can be cited either in alphabetical order or in order of increasing complexity.
5. If two or more side chains are present in equivalent positions, the one assigned the lowest number is cited first in the name. Order can be based on alphabetical order or complexity.
6. Unsaturated unbranched acyclic hydrocarbons with one double bond have the "ane" replaced with "ene." If there is more than one double bond, the "ane" is replaced with "diene," "triene," "tetraene," etc. The chain is numbered to give the lowest possible number to the double bonds.

Source: IUPAC, *Nomenclature of Organic Chemistry, Sections A, B, C, D, E, F, and H*, Pergamon Press, London, U.K., 1979, p. 182.

positions are designated with numbers before the fatty acid name (Δ9-octadecenoic acid or simply 9-octadecenoic acid). The Δ is assumed and often not placed explicitly in structures.

Double-bond geometry is designated with the *cis–trans* or *E*/*Z* nomenclature systems [6]. The *cis*/*trans* terms are used to describe the positions of atoms or groups connected to doubly bonded atoms. They can also be used to indicate relative positions in ring structures. Atoms/groups are *cis* or *trans* if they lie on same (*cis*) or opposite (*trans*) sides of a reference plane in the molecule. Some examples are shown in Figure 1.1. The prefixes *cis* and *trans* can be abbreviated as *c* and *t* in structural formulas.

The *cis*/*trans* configuration rules are not applicable to double bonds that are terminal in a structure or to double bonds that join rings to chains. For these conditions, a sequence preference ordering must be conducted. Since *cis*/*trans* nomenclature is applicable only in some cases, a new nomenclature system was introduced by the Chemical Abstracts Service (CAS) and subsequently adopted by IUPAC (the *E*/*Z* nomenclature). This system was developed as a more applicable system to describe isomers by using sequence ordering rules, as is done using the *R*/*S* system (rules to decide which ligand has priority). The sequence rule–preferred atom/group attached to one of a pair of doubly bonded carbon atoms is compared with the sequence rule–preferred atom/group of the other of the doubly bonded carbon atoms. If the preferred atom/groups are on the same side of the reference plane, it is the *Z* configuration. If they are on the opposite sides of the plane, it is the *E* configuration. Table 1.3 summarizes some of the rules for sequence preference [10]. Although *cis* and *Z* (or *trans* and *E*) do not always refer to the same configurations, for most fatty acids *E* and *trans* are equivalent, as are *Z* and *cis*.

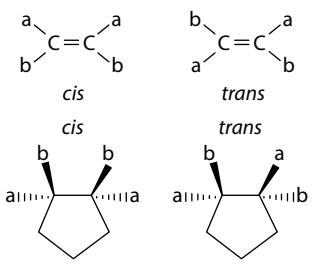


FIGURE 1.1 Examples of *cis*/*trans* nomenclature.

TABLE 1.3
Summary of Sequence Priority Rules for *E/Z* Nomenclature

1. Higher atomic number precedes lower.
2. For isotopes, higher atomic mass precedes lower.
3. If the atoms attached to one of the double-bonded carbons are the same, proceed outward concurrently until a point of difference is reached considering atomic mass and atomic number.
4. Double bonds are treated as if each bonded atom is duplicated.

Source: Streitwieser Jr., A. and Heathcock, C.H., *Introduction to Organic Chemistry*, Macmillan, New York, 1976, p. 111.

B. Common (Trivial) Nomenclature of Fatty Acids

Common names have been introduced throughout the years and, for certain fatty acids, are a great deal more common than standard (IUPAC) terminology. For example, oleic acid is much more common than *cis*-9-octadecenoic acid. Common names for saturated and unsaturated fatty acids are illustrated in Tables 1.4 and 1.5. Many of the common names originate from the first identified

TABLE 1.4
Systematic, Common, and Shorthand Names of Saturated Fatty Acids

Systematic Name	Common Name	Shorthand
Methanoic	Formic	1:0
Ethanoic	Acetic	2:0
Propanoic	Propionic	3:0
Butanoic	Butyric	4:0
Pentanoic	Valeric	5:0
Hexanoic	Caproic	6:0
Heptanoic	Enanthic	7:0
Octanoic	Caprylic	8:0
Nonanoic	Pelargonic	9:0
Decanoic	Capric	10:0
Undecanoic	—	11:0
Dodecanoic	Lauric	12:0
Tridecanoic	—	13:0
Tetradecanoic	Myristic	14:0
Pentadecanoic	—	15:0
Hexadecanoic	Palmitic	16:0
Heptadecanoic	Margaric	17:0
Octadecanoic	Stearic	18:0
Nonadecanoic	—	19:0
Eicosanoic	Arachidic	20:0
Docosanoic	Behenic	22:0
Tetracosanoic	Lignoceric	24:0
Hexacosanoic	Cerotic	26:0
Octacosanoic	Montanic	28:0
Tricontanoic	Melissic	30:0
Dotriacontanoic	Lacceroic	32:0

TABLE 1.5
Systematic, Common, and Shorthand Names of Unsaturated Fatty Acids

Systematic Name	Common Name	Shorthand
c-9-Dodecenoic	Lauroleic	12:1ω3
c-5-Tetradecenoic	Physeteric	14:1ω9
c-9-Tetradecenoic	Myristoleic	14:1ω5
c-9-Hexadecenoic	Palmitoleic	16:1ω7
c-7,*c*-10,*c*-13-Hexadecatrienoic	—	16:3ω3
c-4,*c*-7,*c*-10,*c*-13-Hexadecatetraenoic	—	16:4ω3
c-9-Octadecenoic	Oleic	18:1ω9
c-11-Octadecenoic	*cis*-Vaccenic (Asclepic)	18:1ω7
t-11-Octadecenoic	Vaccenic	[a]
t-9-Octadecenoic	Elaidic	[a]
c-9,*c*-12-Octadecadienoic	Linoleic	18:2ω6
c-9-*t*-11-Octadecadienoic acid	Rumenic[b]	[a]
c-9,*c*-12,*c*-15-Octadecatrienoic	Linolenic	18:3ω3
c-6,*c*-9,*c*-12-Octadecatrienoic	γ-Linolenic	18:3ω6
c-6,*c*-9,*c*-12,*c*-15-Octadecatetraenoic	Stearidonic	18:4ω3
c-11-Eicosenoic	Gondoic	20:1ω9
c-9-Eicosenoic	Gadoleic	20:1ω11
c-8,*c*-11,*c*-14-Eicosatrienoic	Dihomo-γ-linolenic	20:3ω6
c-5,*c*-8,*c*-11-Eicosatrienoic	Mead's	20:3ω9
c-5,*c*-8,*c*-11,*c*-14-Eicosatetraenoic	Arachidonic	20:4ω6
c-5,*c*-8,*c*-11,*c*-14,c-17-Eicosapentaenoic	Eicosapentaenoic	20:5ω3
c-13-Docosenoic	Erucic	22:1ω9
c-11-Docosenoic	Cetoleic	22:1ω11
c-7,*c*-10,*c*-13,*c*-16,*c*-19-Docosapentaenoic	DPA, Clupanodonic	22:5ω3
c-4,*c*-7,*c*-10,*c*-13,*c*-16,*c*-19-Docosahexaenoic	DHA, Cervonic	22:6ω3
c-15-Tetracosenoic	Nervonic (Selacholeic)	24:1ω9

[a] Shorthand nomenclature cannot be used to name *trans* fatty acids.
[b] One of the conjugated linoleic acid (CLA) isomers.

botanical or zoological origins for those fatty acids. Myristic acid is found in seed oils from the Myristicaceae family. Mistakes have been memorialized into fatty acid common names; margaric acid (heptadecanoic acid) was once incorrectly thought to be present in margarine. Some of the common names can pose memorization difficulties, such as the following combinations: caproic, caprylic, and capric; arachidic and arachidonic; linoleic, linolenic, γ-linolenic, and dihomo-γ-linolenic. Even more complicated is the naming of EPA, or eicosapentaenoic acid, usually meant to refer to *c*-5,*c*-8,*c*-11,*c*-14,*c*-17-eicosapentaenoic acid, a fatty acid found in fish oils. However, a different isomer *c*-2,*c*-5,*c*-8,*c*-11,*c*-14-eicosapentaenoic acid is also found in nature. Both can be referred to as eicosapentaenoic acids using standard nomenclature. Nevertheless, in common nomenclature, EPA refers to the *c*-5,*c*-8,*c*-11,*c*-14,*c*-17 isomer. Docosahexaenoic acid (DHA) refers to all-*cis* 4,7,10,13,16,19-docosahexaenoic acid.

C. Shorthand (ω, *n*-) Nomenclature of Fatty Acids

Shorthand (*n*- or ω) identifications of fatty acids are found in common usage. The shorthand designation is the carbon number in the fatty acid chain followed by a colon, then the number of double bonds and the position of the double bond closest to the methyl end of the fatty acid molecule.

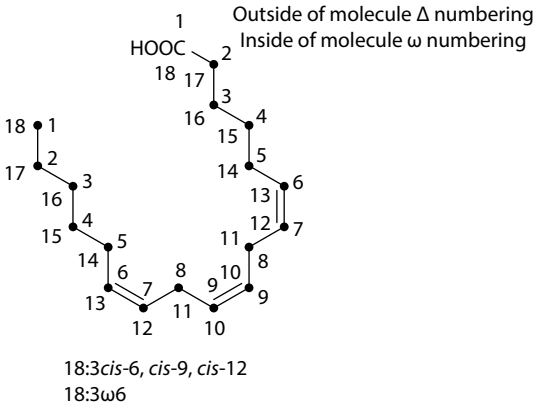


FIGURE 1.2 IUPAC Δ and common ω numbering systems.

The methyl group is number 1 (the last character in the Greek alphabet is ω, hence the end). In shorthand notation, the unsaturated fatty acids are assumed to have *cis* bonding and, if the fatty acid is polyunsaturated, double bonds are in the methylene-interrupted positions (Figure 1.2). In this example, CH_2 (methylene) groups at Δ8 and Δ11 interrupt what would otherwise be a conjugated double-bond system.

Shorthand terminology cannot be used for fatty acids with *trans* or acetylene bonds, for those with additional functional groups (branched, hydroxy, etc.), or for double-bond systems (≥2 double bonds) that are not methylene interrupted (isolated or conjugated). Despite the limitations, shorthand terminology is very popular because of its simplicity and because most of the fatty acids of nutritional importance can be named using this system. Sometimes, ω is replaced by *n*- (18:2*n*-6 instead of 18:2ω6). Although there have been recommendations to eliminate ω and use *n*- exclusively [6], both *n*- and ω are commonly used in the literature and are equivalent.

Shorthand designations for polyunsaturated fatty acids (PUFAs) are sometimes reported without the ω term (18:3). However, this notation is ambiguous, since 18:3 could represent 18:3ω1, 18:3ω3, 18:3ω6, or 18:3ω9 fatty acids, which are completely different in their origins and nutritional significance. Two or more fatty acids with the same carbon and double-bond numbers are possible in many common oils. Therefore, the ω terminology should always be used with the ω term specified.

III. LIPID CLASSES

A. Fatty Acids

1. Saturated Fatty Acids

The saturated fatty acids begin with methanoic (formic) acid. Methanoic, ethanoic, and propanoic acids are uncommon in natural fats and are often omitted from definitions of lipids. However, they are found nonesterified in many food products. Omitting these fatty acids because of their water solubility would make the case for also eliminating butyric acid, which would be difficult given its importance in dairy fats. The simplest solution is to accept the very short-chain carboxylic acids as fatty acids while acknowledging the rarity in natural fats of these water-soluble compounds. The systematic, common, and shorthand designations of some saturated fatty acids are given in Table 1.4.

2. Unsaturated Fatty Acids

By far, the most common monounsaturated fatty acid is oleic acid (18:1ω9), although more than 100 monounsaturated fatty acids have been identified in nature. The most common double-bond

position for monoenes is Δ9. However, certain families of plants have been shown to accumulate what would be considered unusual fatty acid patterns. For example, *Eranthis* seed oil contains Δ5 monoenes and nonmethylene-interrupted PUFAs containing Δ5 bonds [11]. Erucic acid (22:1ω9) is found at high levels (40%–50%) in Cruciferae such as rapeseed and mustard seed. Canola is a rapeseed oil that is low in erucic acid (<2% 22:1ω9) and low in glucosinolates.

PUFAs are best described in terms of families because of the metabolism that allows interconversion within, but not among, families of PUFA. The essentiality of ω6 and ω3 fatty acids has been known since the late 1920s. Signs of ω6 fatty acid deficiency include decreased growth, increased epidermal water loss, impaired wound healing, and impaired reproduction [12,13]. Early studies did not provide clear evidence that ω3 fatty acids are essential. However, since the 1970s, evidence has accumulated illustrating the essentiality of the ω3 PUFA.

Not all PUFAs are essential fatty acids (EFAs). Plants are able to synthesize *de novo* and interconvert ω3 and ω6 fatty acid families via desaturases with specificity in the Δ12 and Δ15 positions. Animals have Δ5, Δ6, and Δ9 desaturase enzymes and are unable to synthesize the ω3 and ω6 PUFAs *de novo*. However, extensive elongation and desaturation of EFA occurs (primarily in the liver). The elongation and desaturation of 18:2ω6 is illustrated in Figure 1.3. The most common of the ω6 fatty acids in our diets is 18:2ω6. Often considered the parent of the ω6 family, 18:2ω6 is first desaturated to 18:3ω6. The rate of this first desaturation is thought to be limiting in premature infants, in the elderly, and under certain disease states. Thus, a great deal of interest has been placed in the few oils that contain 18:3ω6, γ-linolenic acid (GLA). Relatively rich sources of GLA include black currant, evening primrose, and borage oils. GLA is elongated to 20:3ω6, dihomo-γ-linolenic acid (DHGLA). DHGLA is the precursor molecule to the 1-series prostaglandins. DHGLA is further desaturated to 20:4ω6, precursor to the 2-series prostaglandins. Further elongation and desaturation to 22:4ω6 and 22:5ω6 can occur, although the exact function of these fatty acids remains obscure. Relatively high levels of these fatty acids are found in caviar from wild but not cultured sturgeon.

Figure 1.4 illustrates analogous elongation and desaturation of 18:3ω3. The elongation of 20:5ω3 to 22:5ω3 was thought for many years to be via Δ4 desaturase. The inexplicable difficulty in identifying and isolating the putative Δ4 desaturase led to the conclusion that it did not exist, and the pathway from 20:5ω3 to 22:6ω3 was elucidated as a double elongation, desaturation, and β-oxidation.

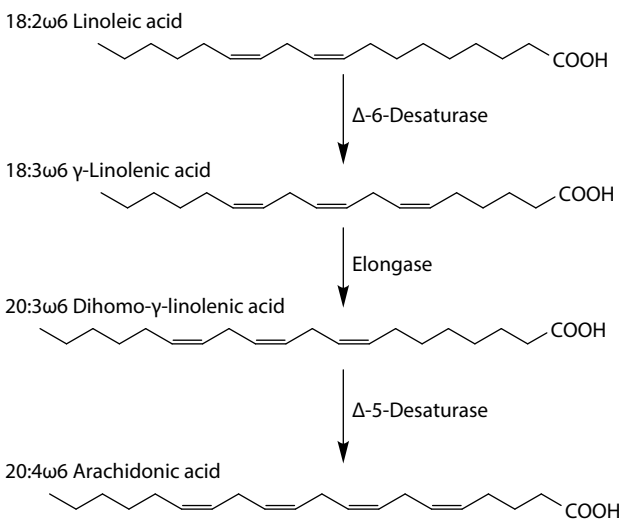


FIGURE 1.3 Pathway of 18:2ω6 metabolism to 20:4ω6.

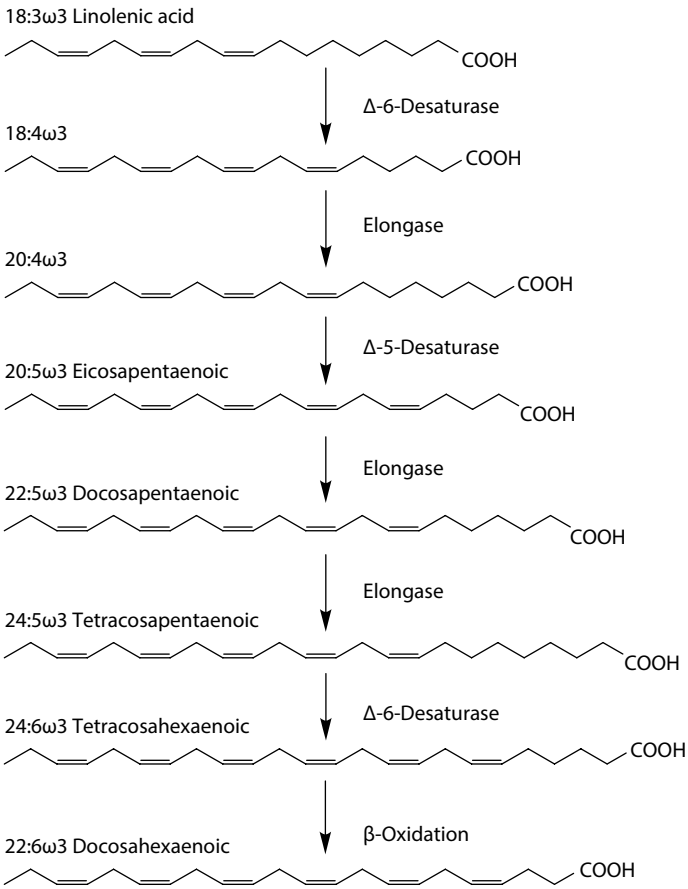


FIGURE 1.4 Pathway of 18:3ω3 metabolism to 22:6ω3.

One of the main functions of the EFAs is their conversion to metabolically active prostaglandins and leukotrienes [14,15]. Examples of some of the possible conversions from 20:4ω6 are shown in Figures 1.5 and 1.6 [15]. The prostaglandins are called eicosanoids as a class and originate from the action of cyclooxygenase on 20:4ω6 to produce PGG_2. The standard nomenclature of prostaglandins allows usage of the names presented in Figure 1.5. For a name such as PGG_2, the PG represents prostaglandin, the next letter (G) refers to its structure (Figure 1.7), and the subscript number refers to the number of double bonds in the molecule.

The parent structure for most of the prostaglandins is prostanoic acid (Figure 1.7) [14]. Thus, the prostaglandins can be named based on this parent structure. In addition, they can be named using standard nomenclature rules. For example, prostaglandin E_2 (PGE_2) is named (5*Z*,11α,13*E*,15*S*)-11,15-dihydroxy-9-oxoprosta-5,13-dienoic acid using the prostanoic acid template. It can also be named using standard nomenclature as 7-[3-hydroxy-2-(3-hydroxy-1-octenyl)-5-oxocyclopentyl]-*cis*-5-heptenoic acid.

The leukotrienes are produced from 20:4ω6 via 5-, 12-, or 15-lipoxygenases to a wide range of metabolically active molecules. The nomenclature is shown in Figure 1.6.

It is important to realize that there are 1-, 2-, and 3-series prostaglandins originating from 20:3ω6, 20:4ω6, and 20:5ω3, respectively. The structures of the 1-, 2-, and 3-prostaglandins differ by the removal or addition of the appropriate double bonds. Leukotrienes of the 3-, 4-, and 5-series are formed via lipoxygenase activity on 20:3ω6, 20:4ω6, and 20:5ω3. A great deal of interest has

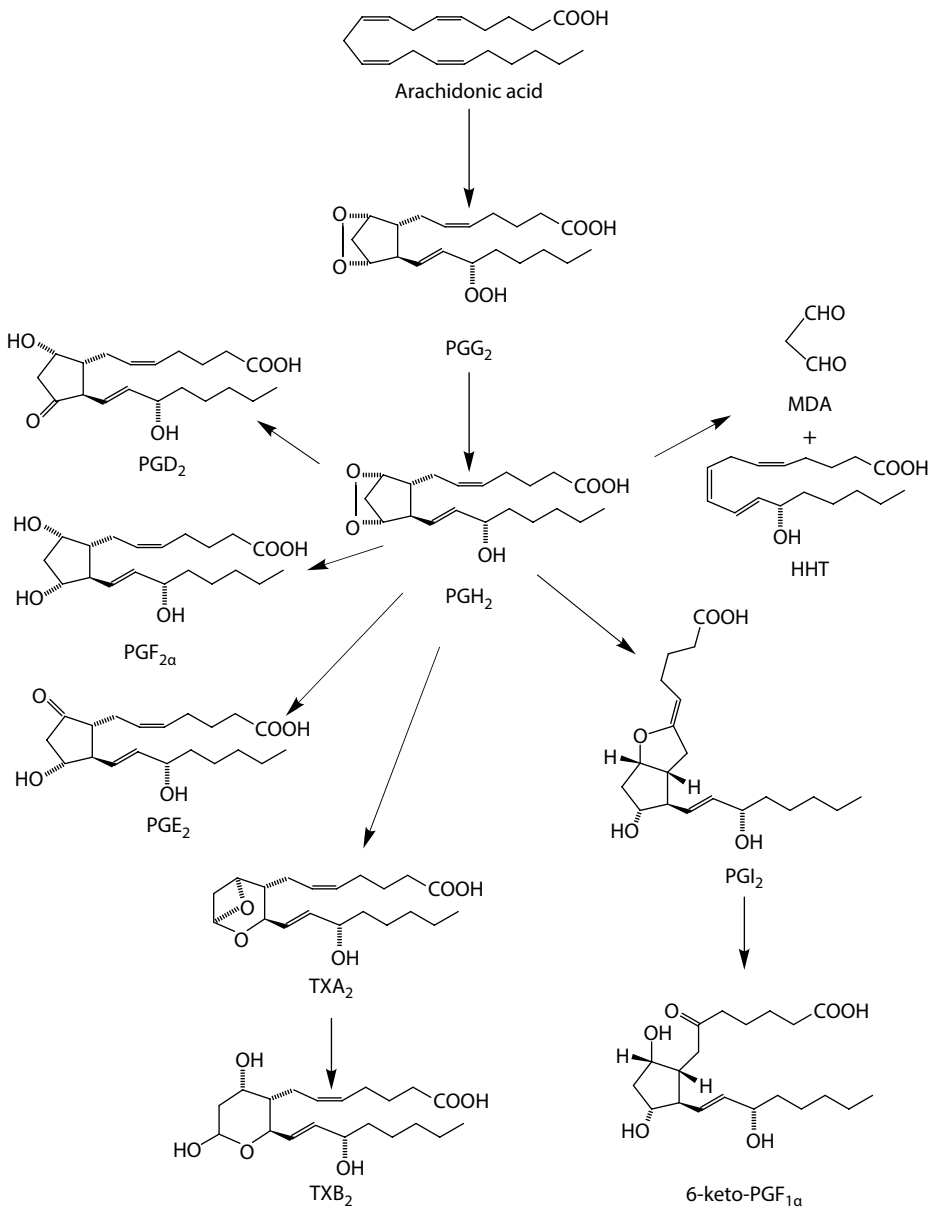


FIGURE 1.5 Prostaglandin metabolites of 20:4ω6.

been focused on changing proportions of the prostaglandins and leukotrienes of the various series by diet to modulate various diseases.

3. Acetylenic Fatty Acids

A number of different fatty acids have been identified having triple bonds [16]. The nomenclature is similar to double bonds, except that the -ane ending of the parent alkane is replaced with -ynoic acid, -diynoic acid, etc.

Shorthand nomenclature uses a lowercase "a" to represent the acetylenic bond; 9*c*,12a-18:2 is an octadecynoic acid with a double bond in position 9 and the triple bond in position 12. Figure 1.8 shows the common names and standard nomenclature for some acetylenic fatty acids. Since the

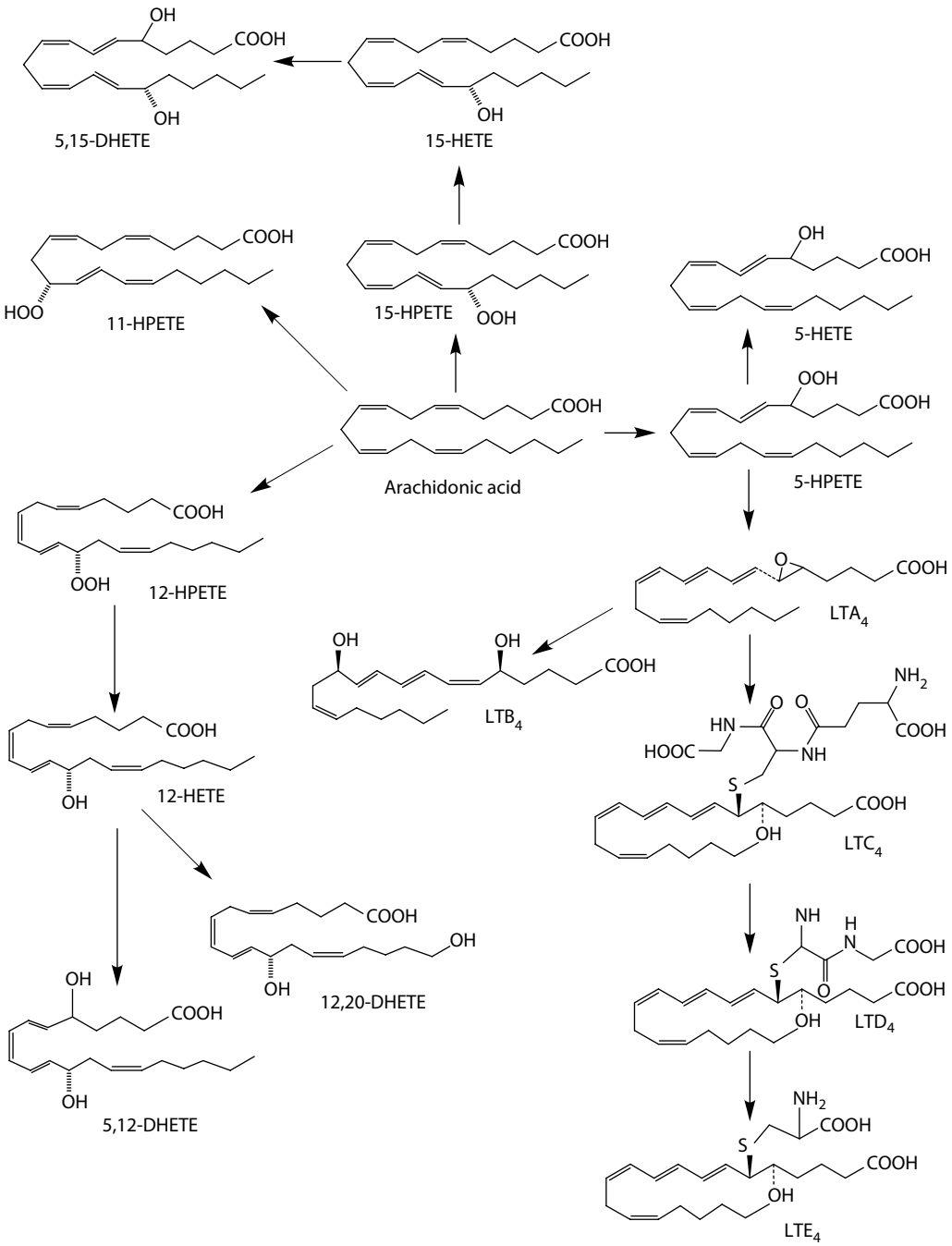


FIGURE 1.6 Leukotriene metabolites of 20:4ω6.

ligands attached to triple-bonded carbons are 180° from one another (the structure through the bond is linear), the second representation in Figure 1.8 is more accurate.

The acetylenic fatty acids found in nature are usually 18-carbon molecules with unsaturation starting at Δ9 consisting of conjugated double–triple bonds [9,16]. Overall, acetylenic fatty acids are rare in nature.

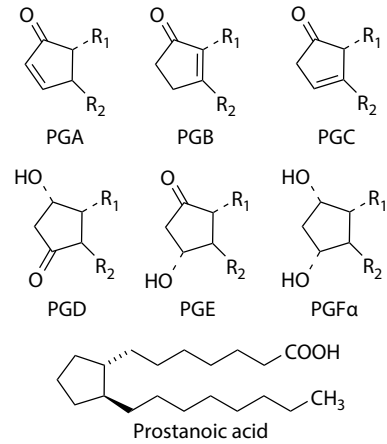


FIGURE 1.7 Prostanoic acid and prostaglandin ring nomenclature.

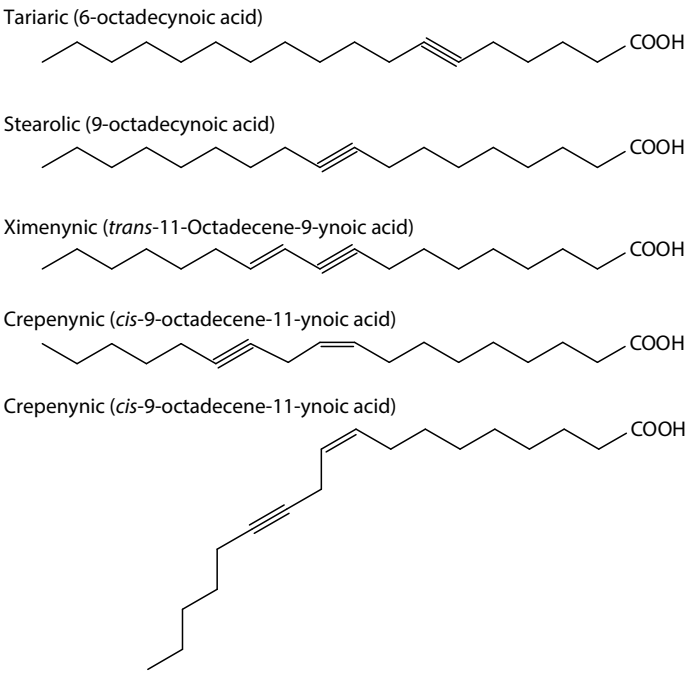


FIGURE 1.8 Some acetylenic acid structures and nomenclature.

4. *Trans* Fatty Acids

Trans fatty acids include any unsaturated fatty acid that contains double-bond geometry in the E (*trans*) configuration. Nomenclature differs from normal *cis* fatty acids only in the configuration of the double bonds.

The three main origins of *trans* fatty acids in our diet are from bacteria, deodorized oils, and partially hydrogenated oils. The preponderance of *trans* fatty acids in our diets is derived from the hydrogenation process.

Hydrogenation is used to stabilize and improve the oxidative stability of oils and to create plastic fats from oils [17]. The isomers that are formed during hydrogenation depend on the nature and

amount of catalyst, the extent of hydrogenation, and other factors. The identification of the exact composition of a partially hydrogenated oil is extremely complicated and time-consuming. The partial hydrogenation process produces a mixture of positional and geometrical isomers. Identification of the fatty acid isomers in a hydrogenated menhaden oil has been described [18]. The 20:1 isomers originally present in the unhydrogenated oil were predominantly *cis*-Δ11 (73% of total 20:1) and *cis*-Δ13 (15% of total 20:1). After hydrogenation from an initial iodine value of 159–96.5, the 20:1 isomers were distributed broadly across the molecules from Δ3 to Δ17 (Figure 1.9). The major *trans* isomers were Δ11 and Δ13, whereas the main *cis* isomers were Δ6, Δ9, and Δ11. Similar broad ranges of isomers are produced in hydrogenated vegetable oils [17].

Geometrical isomers of essential fatty acids linoleic and linolenic were first reported in deodorized rapeseed oils [19]. The geometrical isomers that result from deodorization are found in vegetable oils and products made from vegetable oils (infant formulas) and include 9*c*,12*t*-18:2; 9*t*,12*c*-18:2; and 9*t*,12*t*-18:2, as well as 9*c*,12*c*,15*t*-18:3; 9*t*,12*c*,15*c*-18:3; 9*c*,12*t*,15*c*-18:3; and 9*t*,12*c*,15*t*-18:3 [19–22]. These *trans*-EFA isomers have been shown to have altered biological effects and are incorporated into nervous tissue membranes [23,24], although the importance of these findings has not been elucidated. Geometrical isomers of long-chain v3 fatty acids have been identified in deodorized fish oils.

A mounting body of scientific evidence has linked industrially produced *trans* fats to elevated levels of cholesterol and a major contributor of heart disease [25,26]. The scientific evidence against *trans* fats has led the U.S. FDA to make a decision in June 2015 to remove generally recognized as safe (GRAS) status from industrial *trans* fats. Food companies must remove *trans* fats from their food products by June 2018, although food with less than 0.5 g of *trans* fat per serving can be labeled 0 g of *trans* fat [26]. The ban of *trans* fats extends only to industrially produced *trans* fats as naturally occurring *trans* fats such as ruminant *trans* fatty acids are exempt from the ban.

Trans fatty acids are formed naturally by some bacteria, primarily under anaerobic conditions [27]. It is believed that the formation of *trans* fatty acids in bacterial cell membranes is an adaptation

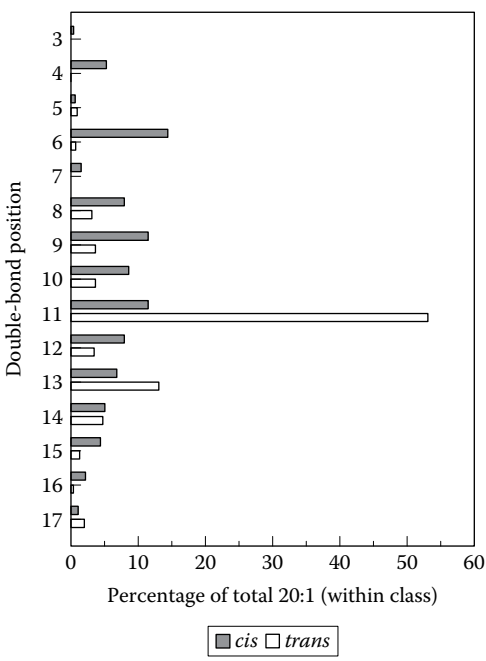


FIGURE 1.9 Eicosanoid isomers in partially hydrogenated menhaden oil. (From Sebedio, J.L. and Ackman, R.G., *J. Am. Oil Chem. Soc.*, 60, 1986, 1983.)

response to decrease membrane fluidity, perhaps as a reaction to elevated temperature or stress from solvents or other lipophilic compounds that affect membrane fluidity.

Not all bacteria produce appreciable levels of *trans* fatty acids. The *trans*-producing bacteria are predominantly gram negative and produce *trans* fatty acids under anaerobic conditions. The predominant formation of *trans* is via double-bond migration and isomerization, although some bacteria appear to be capable of isomerization without bond migration. The action of bacteria in the anaerobic rumen results in biohydrogenation of fatty acids and results in *trans* fatty acid formation in dairy fats (2%–6% of total fatty acids). The double-bond positions of the *trans* acids in dairy fats are predominantly in the Δ11 position, with smaller amounts in Δ9, Δ10, Δ13, and Δ14 positions [28].

5. Branched Fatty Acids

A large number of branched fatty acids have been identified [16]. The fatty acids can be named according to rules for branching in hydrocarbons (Table 1.2). Besides standard nomenclature, several common terms have been retained, including iso-, with a methyl branch on the penultimate (ω2) carbon, and anteiso, with a methyl branch on the antepenultimate (ω3) carbon. The iso and anteiso fatty acids are thought to originate from a modification of the normal *de novo* biosynthesis, with acetate replaced by 2-methyl propanoate and 2-methylbutanoate, respectively [16]. Other branched fatty acids are derived from isoprenoid biosynthesis including pristanic acid (2,6,10,14-tetramethylpentadecanoic acid) and phytanic acid (3,7,11,15-tetramethylhexadecanoic acid).

6. Cyclic Fatty Acids

Some fatty acids that exist in nature contain cyclic carbon rings [29]. Ring structures contain either three (cyclopropyl and cyclopropenyl), five (cyclopentenyl), or six (cyclohexenyl) carbon atoms and may be saturated or unsaturated. Also, cyclic fatty acid structures resulting from heating the vegetable oils have been identified [29–31].

In nomenclature of cyclic fatty acids, the parent fatty acid is the chain from the carboxyl group to the ring structure. The ring structure and additional ligands are considered a substituent of the parent fatty acid. An example is given in Figure 1.10. The parent in this example is nonanoic acid (not pentadecanoic acid, which would result if the chain were extended through the ring structure). The substituted group is a cyclopentyl group with a 2-butyl ligand (2-butylcyclopentyl). Thus, the correct standard nomenclature is 9-(2-butylcyclopentyl)nonanoic acid. The 2 is sometimes

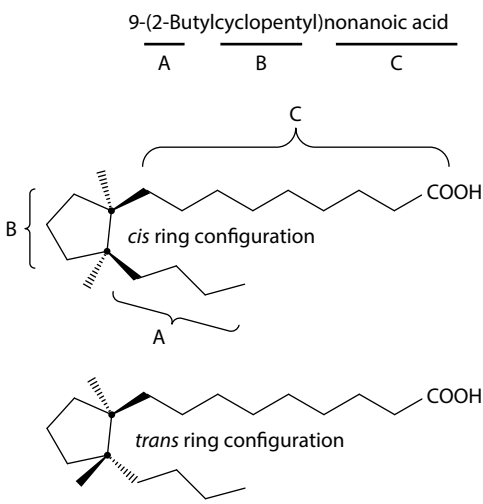
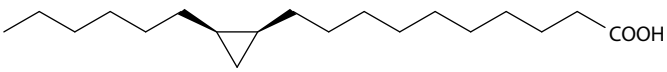


FIGURE 1.10 Nomenclature of cyclic fatty acids.

expressed as 2′ to indicate that the numbering is for the ring and not for the parent chain. The C-1 and C-2 carbons of the cyclopentyl ring are chiral, and two possible configurations are possible. Both the carboxyl and the longest hydrocarbon substituents can be on the same side of the ring, or they can be on opposite sides. These are referred to as *cis* and *trans*, respectively.

The cyclopropene and cyclopropane fatty acids can be named by means of the standard nomenclature noted in the previous example. They are also commonly named using the parent structure that carries through the ring structure. In the example in Figure 1.11, the fatty acid (commonly named lactobacillic acid or phycomonic acid) is named 10-(2-hexylcyclopropyl) decanonic acid in standard nomenclature. An older naming system would refer to this fatty acid as *cis*-11,12-methyleneoctadecanoic acid, where *cis* designates the configuration of the ring structure. If the fatty acid is unsaturated, the term "methylene" is retained but the double-bond position is noted in the parent fatty acid structure (*cis*-11,12-methylene-*cis*-octadec-9-enoic acid) (Figure 1.12).

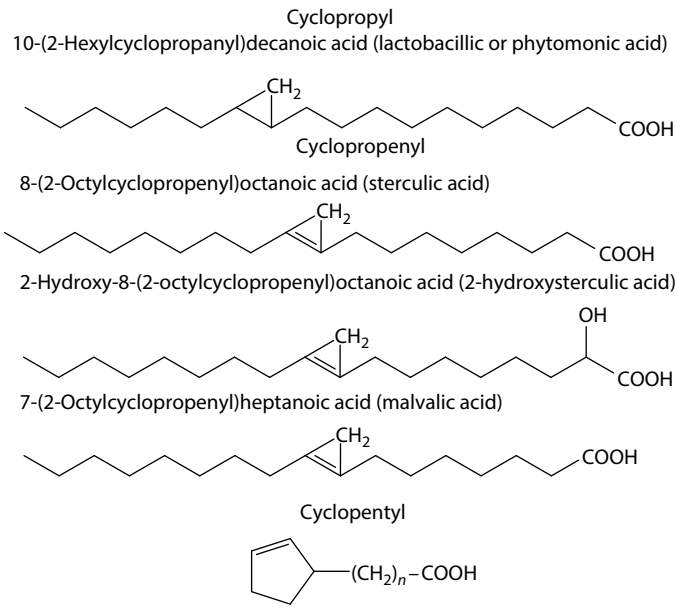


cis-11, 12-Methyleneoctadecanoic acid

cis-10-(2-Hexylcyclopropyl)decanoic acid

Lactobacillic acid / phytomonic acid

FIGURE 1.11 Nomenclature for a cyclopropenoid fatty acid.



n =

2 3-(2-Cyclopentenyl)propanoic acid (aleprolic acid)
4 5-(2-Cyclopentenyl)pentanoic acid (aleprestic acid)
6 7-(2-Cyclopentenyl)heptanoic acid (aleprylic acid)
8 9-(2-Cyclopentenyl)nonanoic acid (alepric acid)
10 11-(2-Cyclopentenyl)undecanoic acid (hyndocarpic acid)
12 13-(2-Cyclopentenyl)tridecanoic acid (chaulmoogric acid)

FIGURE 1.12 Cyclic fatty acid structures and nomenclature.

7. Hydroxy and Epoxy Fatty Acids

Saturated and unsaturated fatty acids containing hydroxy and epoxy functional groups have been identified [1,16]. Hydroxy fatty acids are named by means of the parent fatty acid and the hydroxy group numbered with its Δ location. For example, the fatty acid with the trivial name ricinoleic (Figure 1.13) is named *R*-12-hydroxy-*cis*-9-octadecenoic acid. Ricinoleic acid is found in the seeds of *Ricinus* species and accounts for about 90% of the fatty acids in castor bean oil.

Because the hydroxy group is chiral, stereoisomers are possible. The *R*/*S* system is used to identify the exact structure of the fatty acid. Table 1.6 reviews the rules for *R*/*S* nomenclature. The *R*/*S* system can be used instead of the α/β and *cis*/*trans* nomenclature systems. A fatty acid with a hydroxy substituent in the Δ2 position is commonly called an α-hydroxy acid; fatty acids with hydroxy substituents in the Δ3 and Δ4 positions are called β-hydroxy acids and γ-hydroxy acids, respectively. Some common hydroxy acids are shown in Figure 1.13. Cutins, which are found in the outer layer of fruit skins, are composed of hydroxy acid polymers, which may also contain epoxy groups [16].

Epoxy acids, found in some seed oils, are formed on prolonged storage of seeds [16]. They are named similarly to cyclopropane fatty acids, with the parent acid considered to have a substituted oxirane substituent. An example of epoxy fatty acids and their nomenclature is shown in Figure 1.14. The fatty acid with the common name vernolic acid is named (using standard nomenclature) 11-(3-pentyloxyranyl)-9-undecenoic acid. In older nomenclature, where the carbon chain is carried through the oxirane ring, vernolic acid would be called 12,13-epoxyoleic acid or 12-13-epoxy-9-octadecenoic acid. The configuration of the oxirane ring substituents can be named in the *cis*/*trans*, *E*/*Z*, or *R*/*S* configuration systems.

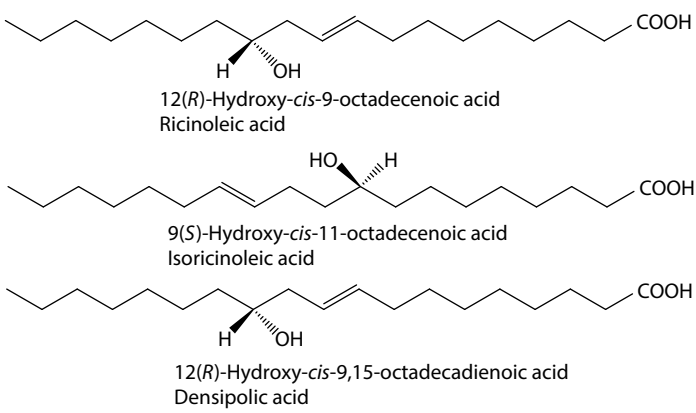
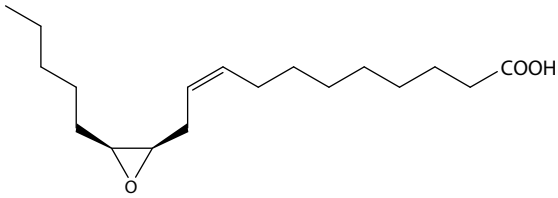


FIGURE 1.13 Hydroxy fatty acid structures and nomenclature.

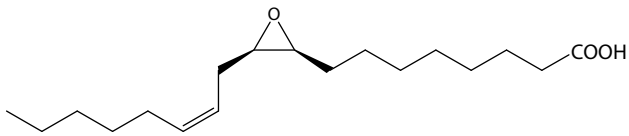
TABLE 1.6
Summary of Rules for *R*/*S* Nomenclature

1. The sequence priority rules (Table 1.3) are used to prioritize the ligands attached to the chiral center ($a > b > c > d$).
2. The molecule is viewed with the *d* substituent facing away from the viewer.
3. The remaining three ligands (*a*, *b*, *c*) will be oriented with the order *a*–*b*–*c* in a clockwise or counterclockwise direction.
4. Clockwise describes the *R* (*rectus*, right) conformation, and counterclockwise describes the *S* (*sinister*, left) conformation.

Source: Streitwieser Jr., A. and Heathcock, C.H., *Introduction to Organic Chemistry*, Macmillan, New York, 1976, p. 111.



11-(3-Pentyloxiranyl)-9-undecanoic acid
cis-12-13-Epoxy-*cis*-9-octadecenoic acid
Vernolic acid
Both the (+) 12*S*, 13*R* and (–) 12*R*, 13*S* forms are found in nature



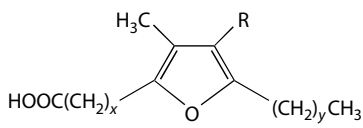
8-(3-*cis*-2′-Nonenyloxiranyl)-octanoic acid
cis-9,10-Epoxy-*cis*-12-octadecenoic acid
Coronaric acid

FIGURE 1.14 Epoxy fatty acid structures and nomenclature.

8. Furanoid Fatty Acids

Some fatty acids contain an unsaturated oxolane heterocyclic group. There are more commonly called furanoid fatty acids because a furan structure (diunsaturated oxolane) is present in the molecule. Furanoid fatty acids have been identified in *Exocarpus* seed oils. They have also been identified in plants, algae, and bacteria and are a major component in triacylglycerols (TAGs) from latex rubber [1,16]. They are important in marine oils and may total several percentage points or more of the total fatty acids in liver and testes [1,32].

Furanoid fatty acids have a general structure as shown in Figure 1.15. A common nomenclature describing the furanoid fatty acids (as F1, F2, etc.) is used [32]. The naming of the fatty acids in this nomenclature is arbitrary and originated from elution order in gas chromatography. A shorthand



Name	*x*	*y*	*R*
F1	8	2	CH_3
F2	8	4	H
F3	8	4	CH_3
F4	10	2	CH_3
F5	10	4	H
F6	10	4	CH_3
F7	12	4	H
F8	12	4	CH_3

FIGURE 1.15 Furanoid fatty acid structure and shorthand nomenclature.

notation that is more descriptive gives the methyl substitution followed by F and then the carbon lengths of the carboxyl and terminal chains in parentheses: MeF(9,5). Standard nomenclature follows the same principles outlined in Section III.A.6. The parent fatty acid chain extends only to the furan structure, which is named as a ligand attached to the parent molecule. For example, the fatty acid named F5 in Figure 1.15 is named 11-(3,4-dimethyl-5-pentyl-2-furyl)undecanoic acid. Shorthand notation for this fatty acid would be F_5 or MeF(11,5). Numbering for the furan ring starts at the oxygen and proceeds clockwise.

B. Acylglycerols

Acylglycerols are the predominant constituent in oils and fats of commercial importance. Glycerol can be esterified with one, two, or three fatty acids, and the individual fatty acids can be located on the three different carbons of glycerol. The terms *monoacylglycerol*, *diacylglycerol*, and *triacylglycerol* (TAG) are preferred for these compounds over the older and confusing names mono-, di-, and triglycerides [6,7].

Fatty acids can be esterified on the primary or secondary hydroxyl groups of glycerol. Although glycerol itself has no chiral center, it becomes chiral if different fatty acids are esterified to the primary hydroxyls or if one of the primary hydroxyls is esterified. Thus, terminology must differentiate between the two possible configurations (Figure 1.16). The most common convention to differentiate these stereoisomers is the *sn* convention of Hirshmann (see Reference 33). In the numbering that describes the hydroxyl groups on the glycerol molecule in Fisher projection, *sn*1, *sn*2, and *sn*3 designations are used for the top (C1), middle (C2), and bottom (C3) OH groups (Figure 1.17). The *sn* term indicates stereospecific numbering [1].

In common nomenclature, esters are named α on primary and β on secondary OH groups. If the two primary-bonded fatty acids are present, the primary carbons are called α and α′. If one or two acyl groups are present, the term "partial glyceride" is sometimes used. Nomenclature of the common partial glycerides is shown in Figure 1.18.

Standard nomenclature allows several different names for each TAG [6]. A TAG with three stearic acid esters can be named as glycerol tristearate, tristearoyl glycerol, or tri-*O*-stearoyl glycerol. The *O* locant can be omitted if the fatty acid is esterified to the hydroxyl group. More commonly,

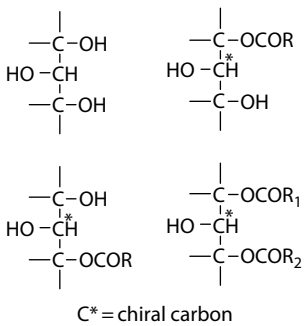


FIGURE 1.16 Chiral carbons in acylglycerols.

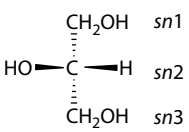


FIGURE 1.17 Stereospecific numbering (*sn*) of triacylglycerols.

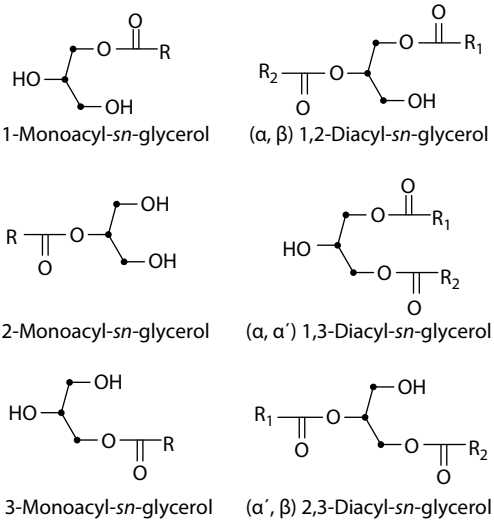


FIGURE 1.18 Mono- and diacylglycerol structures.

TAG nomenclature uses the designation -in to indicate the molecule in a TAG (e.g., tristearin). If different fatty acids are esterified to the TAG—for example, the TAG with *sn*-1 palmitic acid, *sn*-2 oleic acid, and *sn*-3 stearic acid—the name replaces the -ic in the fatty acid name with -oyl, and fatty acids are named in *sn*1, *sn*2, and *sn*3 order (1-palmitoyl-2-oleoyl-3-stearoyl-*sn*-glycerol). This TAG can also be named as *sn*-1-palmito-2-oleo-3-stearin or *sn*-glycerol-1-palmitate-2-oleate-3-stearate. If two of the fatty acids are identical, the name incorporates the designation di- (e.g., 1,2-dipalmitoyl-3-oleoyl-*sn*-glycerol, 1-stearoyl-2,3-dilinolenoyl-*sn*-glycerol).

To facilitate TAG descriptions, fatty acids are abbreviated using one or two letters (Table 1.7). The TAGs can be named after the EFAs using shorthand nomenclature. For example, *sn*-POSt

TABLE 1.7
Short Abbreviations for Some Common Fatty Acids

AC	Acetic	Ln	Linolenic
Ad	Arachidic	M	Myristic
An	Arachidonic	N	Nervonic
B	Butyric	O	Oleic
Be	Behenic	Oc	Octanoic
D	Decanoic	P	Palmitic
E	Erucic	Po	Palmitoleic
El	Elaidic	R	Ricinoleic
G	Eicosenoic	S	Saturated (any)
H	Hexanoic	St	Stearic
L	Linoleic	U	Unsaturated (any)
La	Lauric	V	Vaccenic
Lg	Lingnoceric	X	Unknown

Source: Litchfield, C., *Analysis of Triglycerides*, Academic Press, New York, 1972, 355pp.

is shorthand description for the molecule 1-palmitoyl-2-oleoyl-3-stearoyl-*sn*-glycerol. If the *sn*- is omitted, the stereospecific positions of the fatty acids are unknown. POSt could be a mixture of *sn*-POSt, *sn*-StOP, *sn*-PStO, *sn*-OStP, *sn*-OPSt, or *sn*-StPO in any proportion. An equal mixture of both stereoisomers (the racemate) is designated as *rac*. Thus, *rac*-OPP represents equal amounts of sn-OPP and *sn*-PPO. If only the *sn*-2 substituent is known with certainty in a TAG, the designation β- is used. For example, β-POSt is a mixture (unknown amounts) of *sn*-POSt and *sn*-StOP.

TAGs are also sometimes described by means of the ω nomenclature. For example, *sn*-18:0–18:2ω6–16:0 represents 1-stearoyl-2-linoleoyl-3-palmitoyl-*sn*-glycerol.

C. Sterols and Sterol Esters

The steroid class of organic compounds includes sterols of importance in lipid chemistry. Although the term "sterol" is widely used, it has never been formally defined. The following working definition was proposed some years ago: "Any hydroxylated steroid that retains some or all of the carbon atoms of squalene in its side chain and partitions nearly completely into the ether layer when it is shaken with equal volumes of ether and water" [34]. Thus, for this definition, sterols are a subset of steroids and exclude the steroid hormones and bile acids. The importance of bile acids and their intimate origin from cholesterol make this definition difficult. In addition, nonhydroxylated structures such as cholestane, which retain the steroid structure, are sometimes considered sterols.

The sterols may be derived from plant (phytosterols) or animal (zoosterols) sources. They are widely distributed and are important in cell membranes. The predominant zoosterol is cholesterol. Although a few phytosterols predominate, the sterol composition of plants can be very complex. For example, as many as 65 different sterols have been identified in corn (*Zea mays*) [35].

In the standard ring and carbon numbering (Figure 1.19) [35], the actual 3D configuration of the tetra ring structure is almost flat, so the ring substituents are either in the same plane as the rings or in front or behind the rings. If the structure in Figure 1.19 lacks one or more of the carbon atoms, the numbering of the remainder will not be changed.

The methyl group at position 10 is axial and lies in front of the general plane of the molecule. This is the β configuration and is designated by connection using a solid or thickened line. Atoms or groups behind the plane of the molecule are joined to the ring structure by a dotted or broken line and are given the α configuration. If the stereochemical configuration is not known, a wavy line is used and the configuration is referred to as ε. Unfortunately, actual 3D position of the substituents may be in plane, in front of, or behind the plane of the molecule. The difficulties with this nomenclature have been discussed elsewhere [34,35].

The nomenclature of the steroids is based on parent ring structures. Some of the basic steroid structures are presented in Figure 1.20 [6]. Because cholesterol is a derivative of the cholestane structure (with the H at C-5 eliminated because of the double bond), the correct standard nomenclature

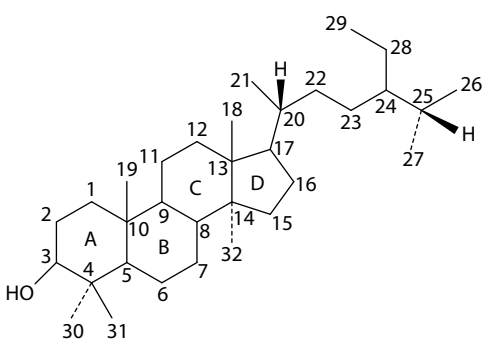


FIGURE 1.19 Carbon numbering in cholesterol structure.

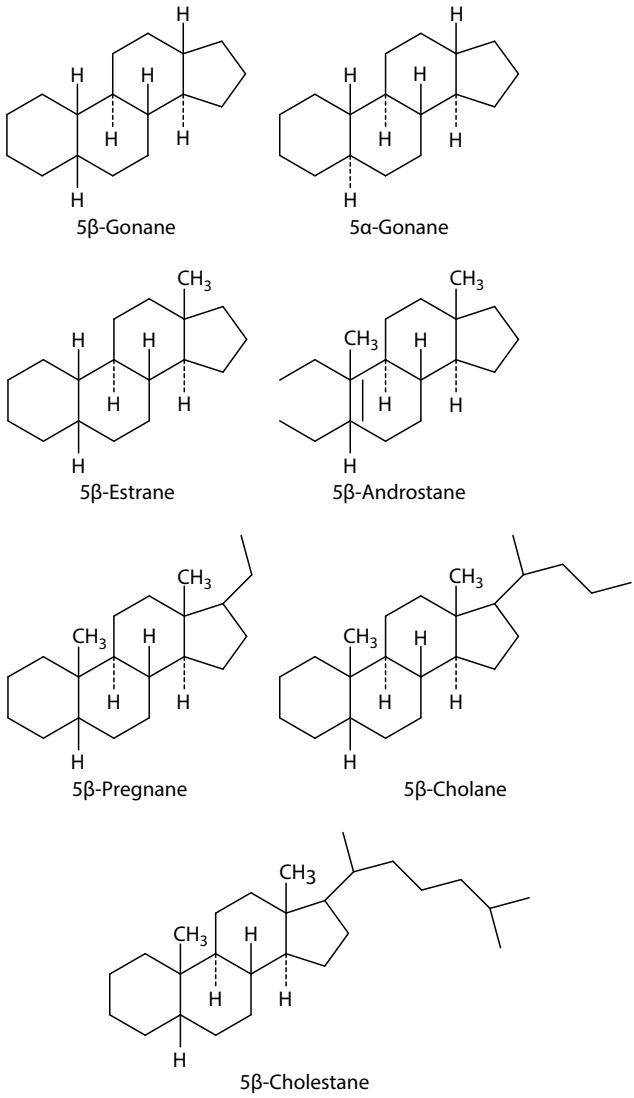


FIGURE 1.20 Steroid nomenclature.

for cholesterol is 3β-cholest-5-en-3-ol. The complexity of standardized nomenclature has led to the retention of trivial names for some of the common structures (e.g., cholesterol). However, when the structure is changed—for example, with the addition of a ketone group to cholesterol at the 7 position—the proper name is 3β-hydroxycholest-5-en-7-one, although this molecule is also called 7-ketocholesterol in common usage.

A number of other sterols of importance in foods are shown in Figure 1.21. The trivial names are retained for these compounds, but based on the nomenclature system discussed for sterols, stigmasterol can be named 3β-hydroxy-24-ethylcholesta-5,22-diene. Studies have suggested that plant sterols and stanols (saturated derivatives of sterols) have cholesterol-lowering properties in humans [36].

Cholesterol has been reported to oxidize *in vivo* and during food processing [37–40]. These cholesterol oxides have come under scrutiny because they have been implicated in the development of atherosclerosis. Some of the more commonly reported oxidation products are shown in Figures 1.22 and 1.23.

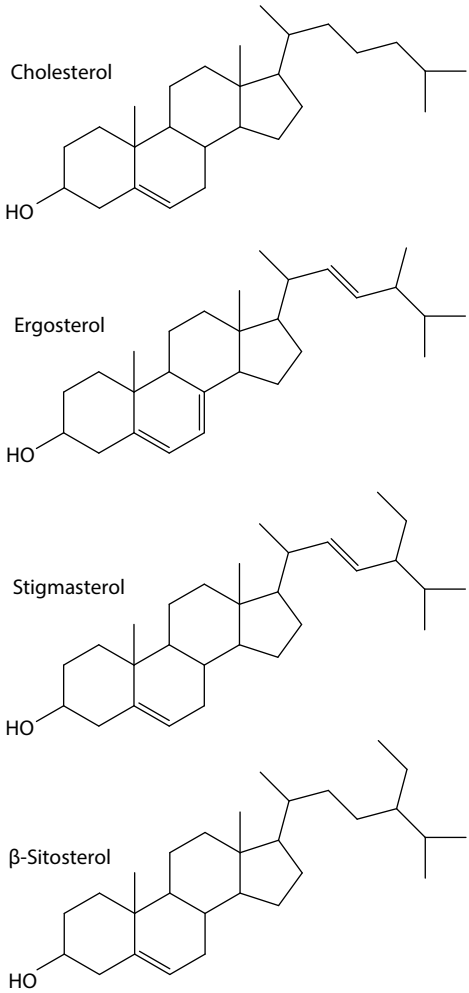


FIGURE 1.21 Common steroid structures.

Nomenclature in common usage in this field often refers to the oxides as derivatives of the cholesterol parent molecule: 7-β-hydroxycholesterol, 7-ketocholesterol, 5,6β-epoxycholesterol, etc. The standard nomenclature follows described rules and is shown in Figures 1.22 and 1.23.

Sterol esters exist commonly and are named using standard rules for esters. For example, the ester of cholesterol with palmitic acid would be named cholesterol palmitate. The standard nomenclature would also allow this molecule to be named 3-*O*-palmitoyl-3β-cholest-5-en-3-ol or 3-palmitoyl-3β-cholest-5-en-3-ol.

D. Waxes

Waxes (commonly called wax esters) are esters of fatty acids and long-chain alcohols. Simple waxes are esters of medium-chain fatty acids (16:0, 18:0, 18:1ω9) and long-chain aliphatic alcohols. The alcohols range in size from C8 to C18. Simple waxes are found on the surfaces of animals, plants, and insects and play a role in prevention of water loss. Complex waxes are formed from diols or from alcohol acids. Di- and triesters as well as acid and alcohol esters have been described.

Simple waxes can be named by removing the -ol from the alcohol and replacing it with -yl, and replacing the -ic from the acid with -oate. For example, the wax ester from hexadecanol and

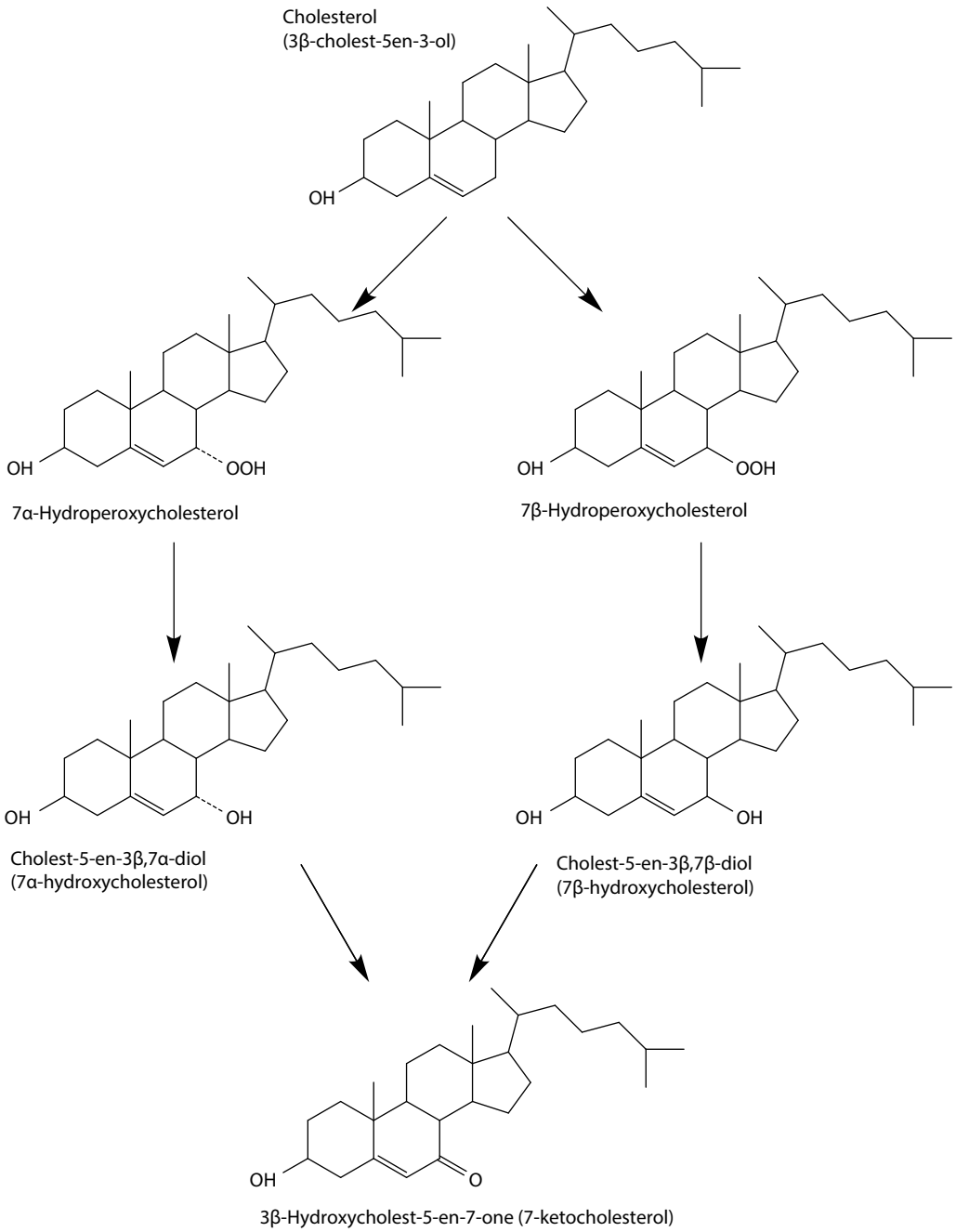


FIGURE 1.22 Cholesterol oxidation products and nomenclature I. (From Smith, L.L., *Chem. Phys. Lipids*, 44, 87, 1987.)

oleic acid would be named hexadecyl oleate or hexadecyl-*cis*-9-octadecenoate. Some of the long-chain alcohols have common names derived from the fatty acid parent (e.g., lauryl alcohol, stearyl alcohol). The C16 alcohol (1-hexadecanol) is commonly called cetyl alcohol. Thus, cetyl oleate is another acceptable name for this compound.

Waxes are found in animal, insect, and plant secretions as protective coatings. Waxes of importance in foods as additives include beeswax, carnauba wax, and candelilla wax.

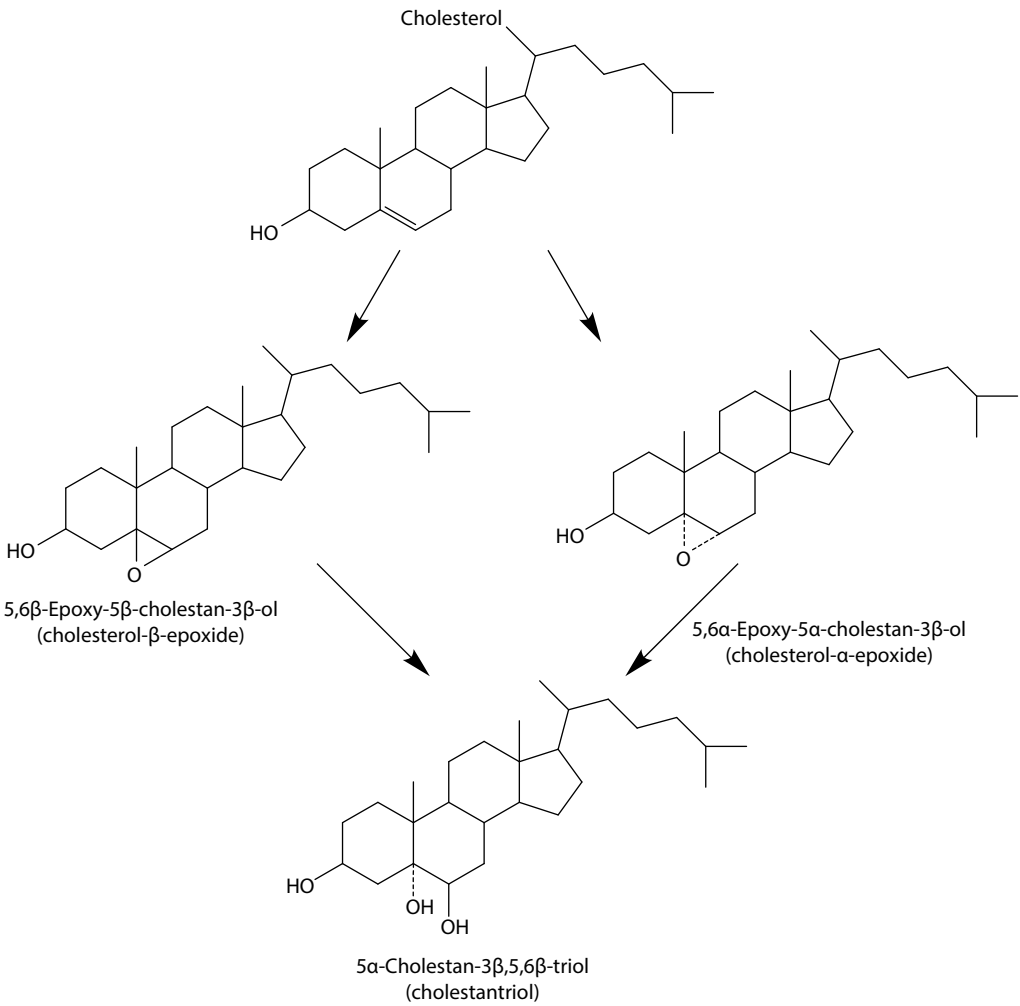


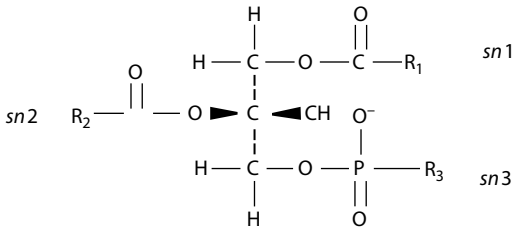
FIGURE 1.23 Cholesterol oxidation products and nomenclature II. (From Smith, L.L., *Chem. Phys. Lipids*, 44, 87, 1987.)

E. Phosphoglycerides (Phospholipids)

Phosphoglycerides (PLs) are composed of glycerol, fatty acids, phosphate, and (usually) an organic base or polyhydroxy compound. The phosphate is almost always linked to the *sn*-3 position of glycerol molecule.

The parent structure of the PLs is phosphatidic acid (*sn*-1,2-diacylglycerol-3-phosphate). The terminology for PLs is analogous to that of acylglycerols except that there is no acyl group at *sn*-3. The prefix lyso-, when used for PLs, indicates that the *sn*-2 position has been hydrolyzed, and a fatty acid is esterified to the *sn*-1 position only.

Some common PL structures and nomenclature are presented in Figure 1.24. Phospholipid classes are denoted using shorthand designation (PC = phosphatidylcholine, etc.). The standard nomenclature is based on the PL type. For example, a PC with an oleic acid on *sn*-1 and linolenic acid on *sn*-2 would be named 1-oleoyl-2-linolenoyl-*sn*-glycerol-3-phosphocholine. The name phosphorylcholine is sometimes used, but it is not recommended [8]. The terms *lecithin* and *cephalin* are sometimes used for PC and PE, respectively, but are also not recommended [8].



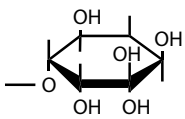
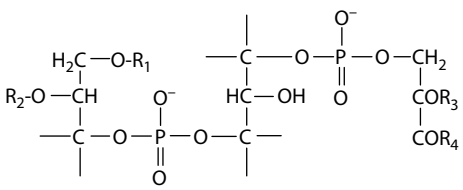
R_3	= Phospholipid class/nomenclature
–OH	Phosphatidic acid (PA)
$–O–CH_2–CH(NH_2)–COOH$	(3-*sn*-phosphatidyl)-L-serine (PS) 1,2-diacyl-*sn*-glycero(3)phospho-L-serine
$–O–CH_2–CH_2–NH_2$	(3-*sn*-phosphatidyl)ethanolamine (PE) 1,2-diacyl-*sn*-glycero(3)phosphoethanolamine
$–O–CH_2–CH_2–N^+(CH_3)_3$	(3-*sn*-phosphatidyl)choline (PC) 1,2-diacyl-*sn*-glycero(3)phosphocholine
	(3-*sn*-phosphatidyl)-L-*myo*-inositol (PI) 1,2-diacyl-*sn*-glycero(3)phospho-L-*myo*-inositol

FIGURE 1.24 Nomenclature for glycerophospholipids.



Cardiolipin
1′, 3′-di-*O*-(3-*sn*-phosphatidyl)-*sn*-glycerol
R1–R4 are fatty acids

FIGURE 1.25 Cardiolipin structure and nomenclature.

Cardiolipin is a PL that is present in heart muscle mitochondria and bacterial membranes. Its structure and nomenclature are shown in Figure 1.25. Some cardiolipins contain the maximum possible number of 18:2ω6 molecules (4 mol/mol).

F. Ether(Phospho)Glycerides (Plasmalogens)

Plasmalogens are formed when a vinyl (1-alkenyl) ether bond is found in a phospholipid or acylglycerol. The 1-alkenyl-2,3-diacylglycerols are termed *neutral plasmalogens*. A 2-acyl-1-(1-alkenyl)-*sn*-glycerophosphocholine is named a plasmalogen or plasmenylcholine. The related 1-alkyl compound is named plasmanylcholine.

G. Glyceroglycolipids (Glycosylglycolipids)

The glyceroglycolipids or glycolipids are formed when a 1,2-diacyl-*sn*-3-glycerol is linked via the *sn*-3 position to a carbohydrate molecule. The carbohydrate is usually a mono- or a disaccharide,

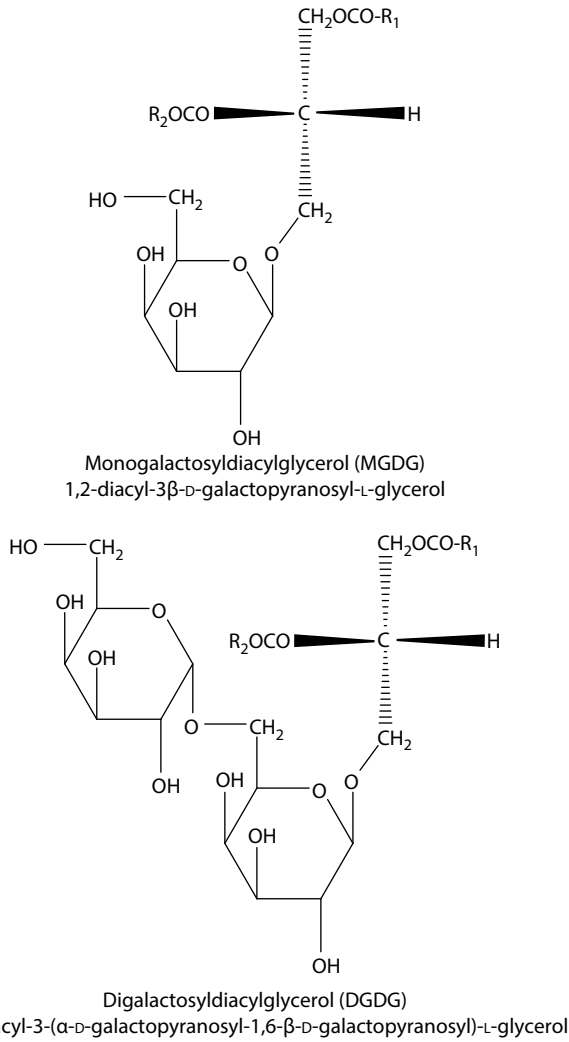


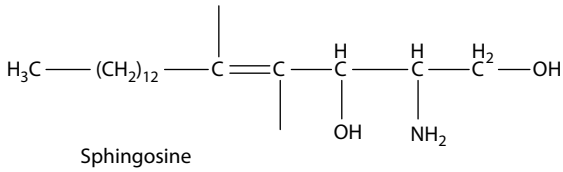
FIGURE 1.26 Glyceroglycolipid structures and nomenclature.

less commonly a tri- or tetrasaccharide. Galactose is the most common carbohydrate molecule in plant glyceroglycolipids.

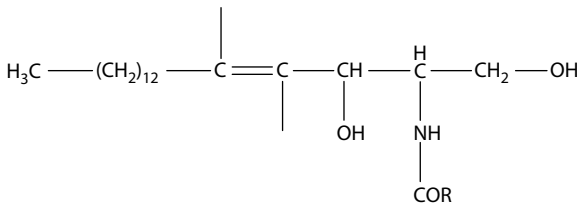
Structures and nomenclature for some glyceroglycolipids are shown in Figure 1.26. The names monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) are used in common nomenclature. The standard nomenclature identifies the ring structure and bonding of the carbohydrate groups (Figure 1.26).

H. Sphingolipids

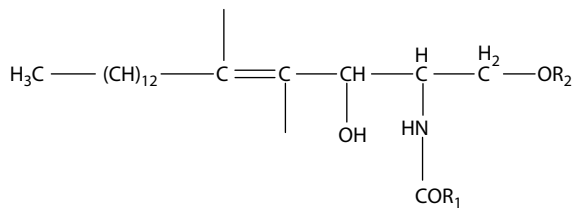
The glycosphingolipids are a class of lipids containing a long-chain base, fatty acids, and various other compounds, such as phosphate and monosaccharides. The base is commonly sphingosine, although more than 50 bases have been identified. The ceramides are composed of sphingosine and a fatty acid (Figure 1.27). Sphingomyelin is one example of a sphingophospholipid. It is a ceramide with a phosphocholine group connected to the primary hydroxyl of sphingosine. The ceramides can also be attached to carbohydrate molecules (sphingoglycolipids or cerebrosides) via the



Sphingosine
D-*erythro*-1,3-dihydroxy-2-amino-*trans*-4-octadecene

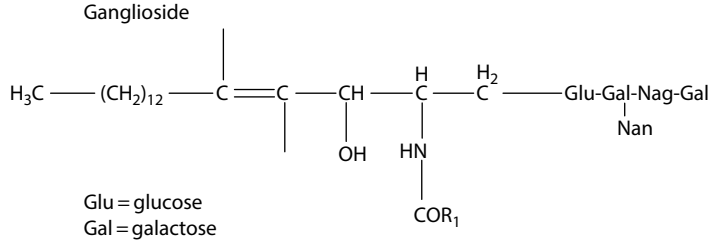


Ceramide
D-*erythro*-1,3-dihydroxy-2(*N*-acyl)-amino-*trans*-4-octadecene
(*N*-acyl-sphingosine)



Cerebroside
1-*O*-β-D-galactopyranosyl-*N*-acyl-sphingosine
R1 = fatty acid
R2 = galactopyranose

Ganglioside



Glu = glucose
Gal = galactose
Nag = *N*-acetylgalactosamine
Nan = *N*-acetylneuraminic acid

FIGURE 1.27 Sphingolipid structures and nomenclature.

primary hydroxyl group of sphingosine. Gangliosides are complex cerebrosides with the ceramide residue connected to a carbohydrate-containing glucose-galactosamine-*N*-acetylneuraminic acid. These lipids are important in cell membranes and the brain, and they act as antigenic sites on cell surfaces. Nomenclature and structures of some cerebrosides are shown in Figure 1.27.

I. Fat-Soluble Vitamins

1. Vitamin A

Vitamin A exists in the diet in many forms (Figure 1.28). The most bioactive form is the all-*trans* retinol, and *cis* forms are created via light-induced isomerization (Table 1.8). The 13-*cis* isomer is the most biopotent of the mono- or di-*cis* isomers. The α- and β-carotenes have biopotencies of

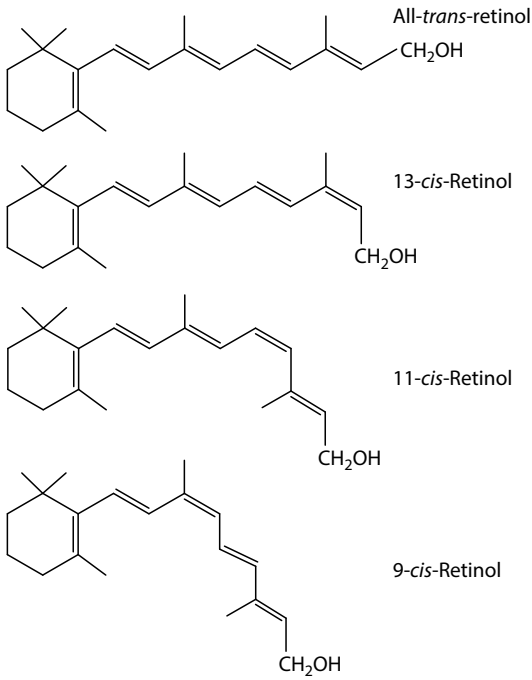


FIGURE 1.28 Structures of some vitamin A compounds.

TABLE 1.8
Approximate Biological Activity Relationships of Vitamin A Compounds

Compound	Activity of All-*trans* Retinol (%)
All-*trans* retinol	100
9-*cis* Retinol	21
11-*cis* Retinol	24
13-*cis* Retinol	75
9,13-Di-*cis* retinol	24
11,13-Di-*cis* retinol	15
α-Carotene	8.4
β-Carotene	16.7

about 8.7% and 16.7% of the all-*trans* retinol activity, respectively. The daily value (DV) for vitamin A is 1000 retinol equivalents (RE), which represents 1000 mg of all-*trans* retinol or 6000 μg of β-carotene. Vitamin A can be toxic when taken in levels exceeding the %DV. Some reports suggest that levels of 15,000 RE/day can be toxic [39].

Toxic symptoms of hypervitaminosis A include drowsiness, headache, vomiting, and muscle pain. Vitamin A can be teratogenic at high doses [41]. Vitamin A deficiency results in night blindness and ultimately total blindness, abnormal bone growth, increased cerebrospinal pressure, reproductive defects, abnormal cornification, loss of mucus secretion cells in the intestine, and decreased growth. The importance of beef liver, an excellent source of vitamin A, in cure of night blindness was known to the ancient Egyptians about 1500 BC [42].

2. Vitamin D

Although as many as five vitamin D compounds have been described (Figure 1.29), only two of these are biologically active: ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3). Vitamin D_3 can be synthesized in humans from 7-dehydrocholesterol, which occurs naturally in the skin, via light irradiation (Figure 1.30).

The actual hormonal forms of the D vitamins are the hydroxylated derivatives. Vitamin D is converted to 25-OH-D in the kidney and further hydroxylated to 1,25-diOH-D in the liver. The dihydroxy form is the most biologically active form in humans.

3. Vitamin E

Vitamin E compounds include the tocopherols and tocotrienols. Tocotrienols have a conjugated triene double-bond system in the phytyl side chain, whereas tocopherols do not. The basic nomenclature is shown in Figure 1.31. The bioactivity of the various vitamin E compounds is shown in Table 1.9. Methyl substitution affects the bioactivity of vitamin E, as well as its *in vitro* antioxidant activity.

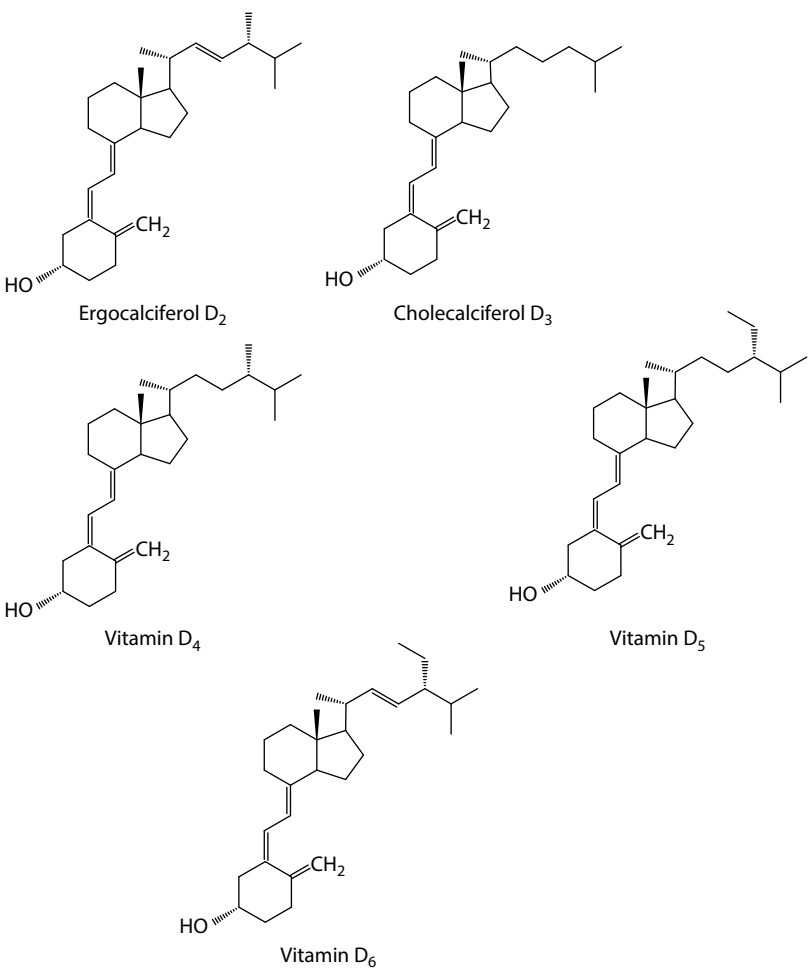


FIGURE 1.29 Structures of some vitamin D compounds.

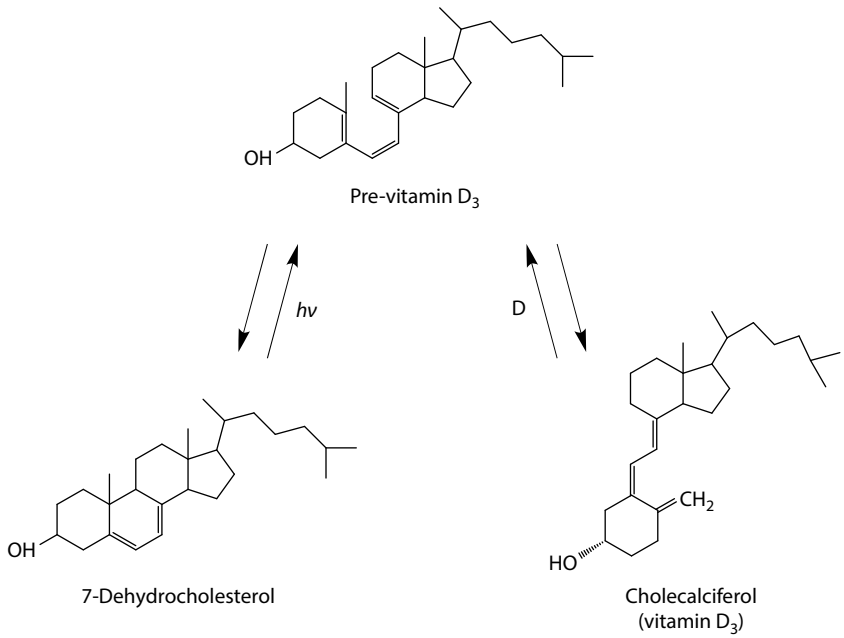
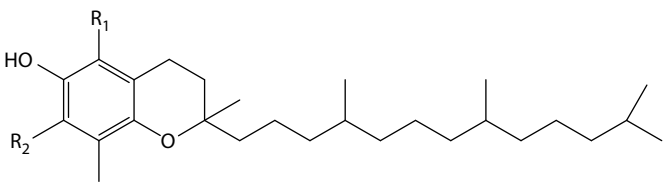
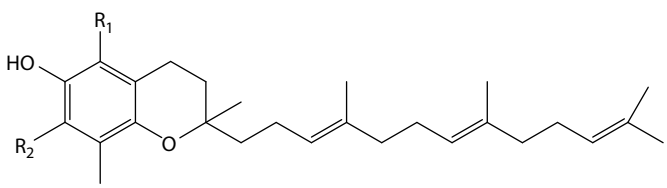


FIGURE 1.30 Formation of vitamin D *in vivo*.



Tocopherol	R_1	R_2
α	CH_3	CH_3
β	CH_3	H
γ	H	CH_3
δ	H	H



Tocotrienol	R_1	R_2
α	CH_3	CH_3
β	CH_3	H
γ	H	CH_3
δ	H	H

FIGURE 1.31 Structures of some vitamin E compounds.

TABLE 1.9
Approximate Biological Activity Relationships of Vitamin E Compounds

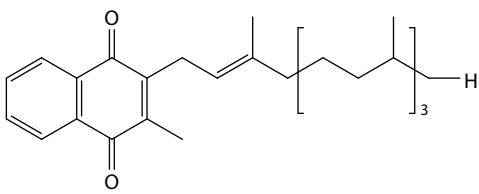
Compound	Activity of D-α-Tocopherol (%)
D-α-Tocopherol	100
L-α-Tocopherol	26
DL-α-Tocopherol	74
DL-α-Tocopheryl acetate	68
D-β-Tocopherol	8
D-γ-Tocopherol	3
D-δ-Tocopherol	—
D-α-Tocotrienol	22
D-β-Tocotrienol	3
D-γ-Tocotrienol	—
D-δ-Tocotrienol	—

4. Vitamin K

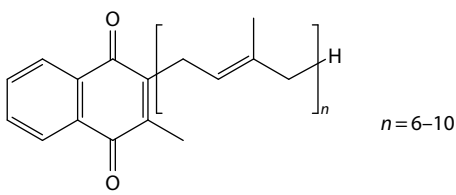
Several forms of vitamin K have been described (Figure 1.32). Vitamin K (phylloquinone) is found in green leaves, and vitamin K_2 (menaquinone) is synthesized by intestinal bacteria. Vitamin K is involved in blood clotting as an essential cofactor in the synthesis of γ-carboxyglutamate necessary for active prothrombin. Vitamin K deficiency is rare due to intestinal microflora synthesis. Warfarin and dicoumerol prevent vitamin K regeneration and may result in fatal hemorrhaging.

J. Hydrocarbons

Hydrocarbons include normal, branched, saturated, and unsaturated compounds of varying chain lengths. The nomenclature for hydrocarbons has already been discussed. The hydrocarbons of most interest to lipid chemists are the isoprenoids and their oxygenated derivatives.



Vitamin K_1
Phylloquinone
2-Methyl-3-phytyl-1,4-naphthoquinone



$n = 6–10$

Vitamin K_2
Menaquinone-*n*
2-Methyl-3-multiprenyl-1,4-naphthoquinone

FIGURE 1.32 Structures of some vitamin K compounds.

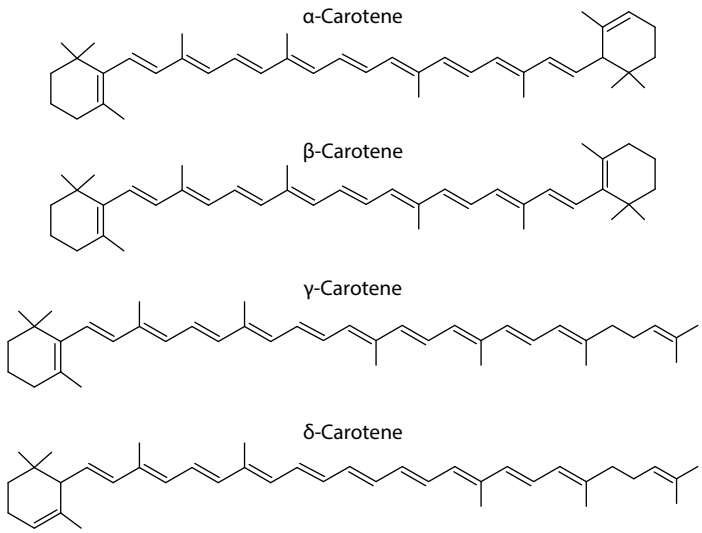


FIGURE 1.33 Structures and nomenclature of carotenoids.

The basic isoprene unit (2-methyl-1,3-butadiene) is the building block for a large number of interesting compounds, including carotenoids (Figure 1.33), oxygenated carotenoids or xanthophylls (Figure 1.34), sterols, and unsaturated and saturated isoprenoids (isopranes). Some 15-carbon and 20-carbon isoprenoids are covalently attached to some proteins and may be involved in control of cell growth [43]. Members of this class of protein-isoprenoid molecules are called prenylated proteins.

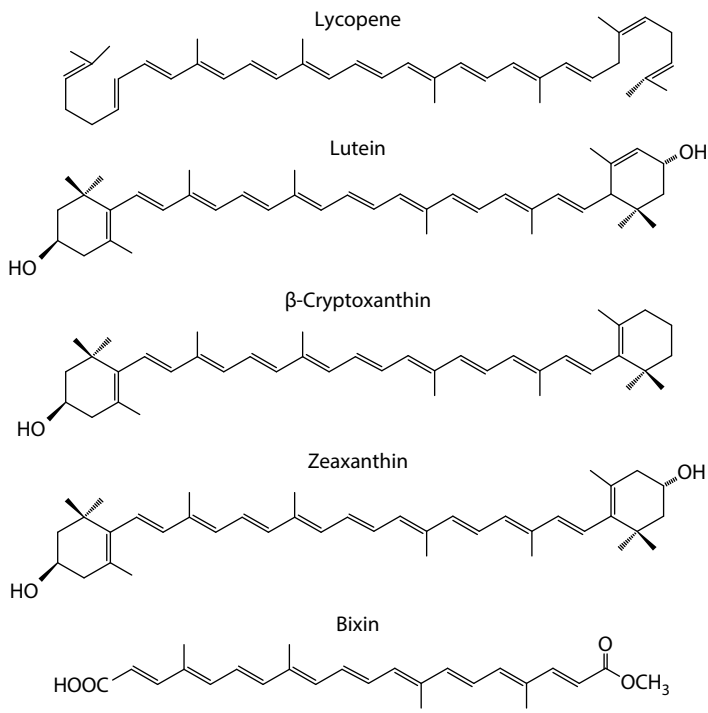


FIGURE 1.34 Structures and nomenclature of some oxygenated carotenoids.

IV. SUMMARY

It would be impossible to describe the structures and nomenclature of all known lipids even in one entire book. The information presented in this chapter is a brief overview of the complex and interesting compounds we call lipids.

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2 Chemistry and Properties of Lipids and Phospholipids

Bianca Pérez, Jingbo Li, and Zheng Guo

CONTENTS

I. INTRODUCTION

Lipids are referred to as a large and diverse group of naturally occurring compounds. The term "lipid" is restricted to long-chain fatty acids, their derivatives, and compounds that are related structurally and functionally to them [1,2]. They are highly soluble in organic solvents (e.g., hexane, diethyl ether, and chloroform) and slightly soluble/nonsoluble in water [3]. Lipids are found ubiquitously in plants, animals, and microorganisms, as well as the human body. They are the major constituents of the cell membrane, which is responsible for holding the cell together [4]. Lipids can be classified as polar and neutral lipids [5]. The International Lipid Classification and Nomenclature Committee developed a more detailed classification scheme (Lipid Classification System LIPID Metabolites and Pathways Strategy; http://www.lipidmaps.org) based on the concept that lipids may result from complete or partial carbanion-based condensations of ketoacyl thioesters or carbocation-based condensations of isoprene units [6]. Accordingly, lipids can be classified into eight groups, namely, fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids, and polyketides (Figure 2.1) [7,8]. This classification scheme is applicable to archaea and synthetic lipids, covers eukaryotic and prokaryotic sources, and allows the subdivision of the main categories into subclasses to include the new lipids structures.

Fatty acyls are biosynthesized by the chain elongation of an acetyl-CoA primer with malonyl-CoA or methyl-CoA groups [5]. The two main classes of lipids are (1) glycerolipids, which encompass mainly acylglycerols, and (2) glycerophospholipids, which are characterized by the presence of phosphate. In addition, steroid and prenoid lipids constitute lipids derived from the polymerization of dimethylallyl pyrophosphate/isopentenyl pyrophosphate. Other well-known categories of lipids are sphingolipids, saccharolipids, and polyketides. Sphingolipids contain a long aliphatic amine with two or more hydroxyl groups as their core structure and saccharolipids are lipids that have a fatty acyl linked to a sugar molecule forming structures that are compatible with membrane bilayers. Finally, the last category of lipids is polyketides. These are lipids derived from plants and microbial sources.

The physicochemical properties of lipids depend on their structural features [9]. For instance, the length of the fatty acyl chain, number of unsaturation in the molecules, and size of the polar head determine the physical states, existing as solid, liquid, or liquid crystal. In addition, the structural features provide insight into the oxidative stability of lipids. In relation to the application of lipids in food, the lipid property will to a large extent influence food quality and stability, and thus, in some cases structural modification of lipids is needed to meet the demand of food processing [10].

Modification of lipids has produced a wide variety of fats, lowered production cost, and enabled food products to meet nutritional demands [9,11]. For example, when modifying oils for food applications, producers attempted to meet both nutritional requirements (e.g., increasing concentration of unsaturated fatty acids) and technological requirements to facilitate production and to improve oxidative stability. Although unsaturated fatty acids are preferable over saturated fatty acids from a nutritional point of view [12], they are prone to oxidation. Therefore, to improve their oxidative stability, the produced oils are modified to contain less polyunsaturated fatty acids and more monounsaturated fatty acids such as oleic acid. Thus, modification of fats for the production of higher melting point fats is a strategy to increase food quality as the replacement or redistribution of the fatty acyls in glycerol backbone may alter the melting point, crystallization behavior, and oxidative stability of fats in food processing. Although hydrogenation of vegetable oil has been a widely used approach to generate plastic fat from liquid oil, now this strategy has been increasingly replaced by other procedures such as fatty acyl exchanges through enzymatic modifications of oils and fats because they prevent the production of *trans*-fatty acids that have been associated with coronary diseases [13].

Other examples of physical strategies to modify fats are fractionation of fats at different temperatures and extraction with CO_2 under supercritical conditions [14] or using organic solvents. Furthermore, if the fatty acid composition of different oils is known, they can be combined through physical blending to generate oil with the ratio of fatty acid desired [15]. Other strategies involve the use of genetic

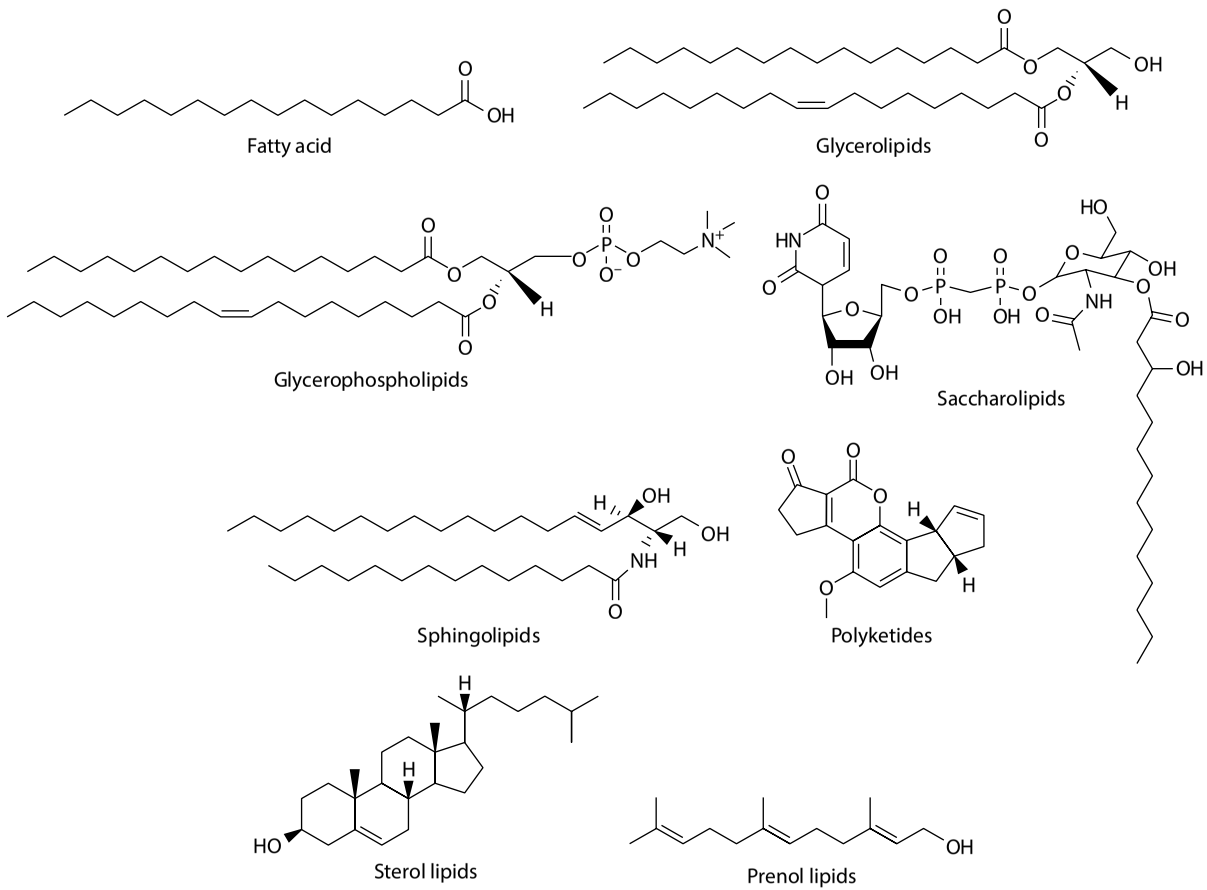


FIGURE 2.1 Representative structures of different classes of lipids. (From Fahy, E. et al., *J. Lipid Res.*, 46, 839, 2005.)

engineering to cultivate crops that are highly rich in specific and desired oil [16]. However, ecological safety regarding the cultivation of these types of crops has not been investigated.

On the other hand, examples of chemical synthesis and modification of lipids constituting hydrogenation or chemical esterification or transesterification of oils are still the technology in use today [17,18]. However, chemical approaches are increasingly being replaced by environmentally friendly strategies such as enzymatic synthesis (e.g., enzymatic esterification and transesterification) [19,20]. The latter decreases the amount of hazardous wastes that can be generated in high-scale production of lipids. Nonetheless, enzyme-based production is still expensive and less competitive in price and this is taken into account when designing production line to obtain lipids of relevance for food production.

Analysis and characterization strategies are also important to guarantee the quality of lipids as food ingredients [21–24]. High-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), and gas chromatography (GC) are the most common techniques used for the qualitative and quantitative analyses of lipids [25,26]. However, other techniques that are used for structural elucidation of lipids have also proved to be of value for the quantitative analyses of lipids such as Fourier transform infrared spectroscopy [27]. Generally, the use of multiple techniques is necessary to ensure the quality of lipids used for food production. For instance, in terms of physical characterization it is required to combine different techniques such as x-ray diffraction [28], differential scanning calorimetry [29], and nuclear magnetic resonance [30] to understand in detail the physicochemical properties of the lipids in question.

This chapter mainly focuses on the two most abundant classes of lipids in nature: acylglycerol and phospholipids. It describes the physicochemical properties of these lipids and envisages their molecular basis, structural features, and the types of chemical reactions that these classes of lipids undergo. In addition, this chapter reviews the most common strategies used for the synthesis, isolation, analysis, and characterization of lipids. Specifically, the enzymatic and chemical synthesis and modifications of acylglycerols and phospholipids are presented. Accordingly, this chapter provides useful insight for the generation of novel lipids of relevance to food applications.

II. ACYLGLYCEROLS

Acylglycerols are predominant components in oils and fats for food and oleochemical industries [31]. They represent all lipids containing a glycerol backbone covalently linked to fatty acyls. These lipids can be monoacylglycerols, diacylglycerols, and triacylglycerols (Figure 2.2), where the latter represents the main occurring form of acylglycerols for both plants and animals. In nature, acylglycerols also exist in partial glyceride form in relatively small quantity, including monoacylglycerols and diacylglycerols. The presence of hydroxyl group in partial acylglycerols gives them different polarity and properties from triacylglycerols. Natural triglycerides (triacylglycerols) are a mixture of triacylglycerols containing different fatty acids. Depending on the lipids, sources, the types, numbers, and relative contents of

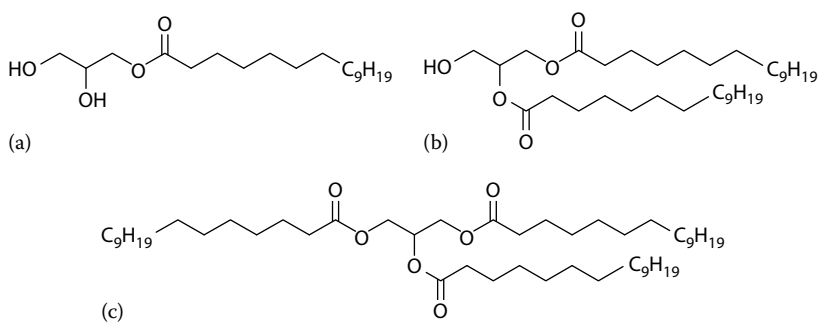


FIGURE 2.2 Representative structures of (a) mono-, (b) di-, and (c) triacylglycerols (MAG, DAG, and TAG, respectively).

fatty acids in triacylglycerol can vary greatly; for example, in milk fat the number of different fatty acids adds up to 400 [32]. Therefore, the total number of triacylglycerol molecules can be very high (400^3). However, for most plant oils they constitute no more than 100 different fatty acids; even so, the number of total triacylglycerol types can also be very high [23]. This complexity in composition of triacylglycerols makes it very difficult to accurately predict their property, while their properties are largely governed by dominating fatty acids. By changing the fatty acyl, the physicochemical properties of triacylglycerols can be altered for specific purpose; for example, by introducing saturated or monounsaturated fatty acids into highly polyunsaturated oils the oxidation stability of the products can be improved.

III. PHYSICAL PROPERTIES OF ACYLGLYCEROLS

The physical properties of acylglycerols depend on the molecular structure of fatty acyls linked to the glycerol backbone. The melting point and intermolecular interaction of lipid is determined by the saturated degree and carbon chain length of fatty acyls. For example, the melting point of saturated fatty acid is governed by the weak dispersion forces or noncovalent interactions known as van der Waals forces as molecules need to overcome these forces in order to be melted. In branched saturated fatty acid chain, these forces are weaker, lowering the energy required for these compounds to be melted [9]. In addition, increasing the number of double bonds and length of the lipid chain decreases the melting point and lowers the organization of lipid chains. For instance, linoleic acid, which contains two double bonds, displayed a lower melting point (–5°C) than oleic acid (m.p. = 13°C) and stearic acid (m.p. = 69°C), which contain 1 and no unsaturation, respectively (Figure 2.3).

In food formulation, the crystallization behavior of triacylglycerols is highly important as crystallization of lipids in food products might influence food structure, taste, and quality. Controlling the crystallization process of triacylglycerols can yield a product with desired physical characteristics [33]. For instance, the tempering process of chocolate determines whether the product will have or not have the desired appearance, snap, and melting properties since the crystallization process can lead to more or less stable crystal forms. During the crystallization process, the main steps that determine the crystal structure of lipids are nucleation and crystal growth. However, it is important to know that the liquid phase prior to crystal forms can retain some degree of ordering. Thus, heating up mixtures of lipids well above the melting point is of importance to erase crystal memory and to obtain the desired crystal structure by controlling the process of nucleation and crystal growth.

A. Nucleation

Nucleation takes place in the formation of crystalline phase from liquid phase. The nucleation rate can determine the number and size of the crystals formed and the resulting polyphorm. Nucleation can

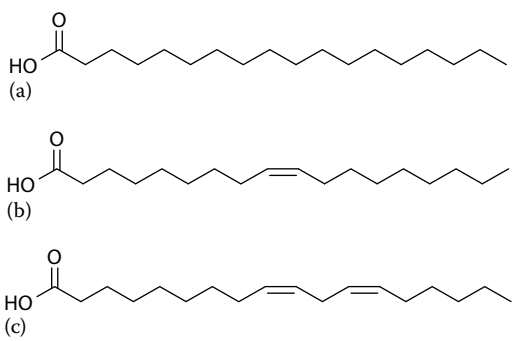


FIGURE 2.3 The effect of the degree of unsaturation on melting point of fatty acids. (a) Stearic acid, m.p. = 69°C; (b) oleic acid, m.p. = 13°C; and (c) linoleic acid, m.p. = –5°C.

occur homogeneously or heterogeneously, or when crystalline elements break from existing crystal and subsequently act as nuclei to promote crystallization [34,35]. Homogeneous nucleation, which is unlikely to occur, takes place without the assistance of a foreign interface. Instead, heterogeneous nucleation is catalyzed by foreign nucleating site such as dust particles or other interfaces that can reduce the free energy required for nucleation to occur. Rapid cooling of lipids in liquid state leads to high nucleation rate and does not allow enough time for the molecules to be organized into lamellae to form well-arranged crystal structures. As a consequence, a rapid cooling process will generate a less stable molecular organization. Other parameters that can alter nucleation are nucleation time, nucleation temperature, and crystal lattice of the nuclei formed.

B. Crystal Growth

Crystal growth occurs when molecules from the liquid phase start to migrate to the crystal surface [33]. For mixed triacylglycerols, the probability of two molecules crystallizing together will depend on the structural similarities such as chain length, degree of saturation, configuration of the double bond, and the position of the fatty acids in the glycerol backbone. For instance, mixed triacylglycerols may crystallize more slowly than pure triacylglycerol forms. Furthermore, cooling and agitation rate, temperature, and composition of lipid phase can also affect the crystal growth. In addition, the presence of some minor components might retard crystal growth.

C. Polymorphism

Polymorphism is the ability of a compound to form more than one crystalline structure [33]. The lipid polymorphism is determined by the coherence force, which results from the exclusion volume, head group interaction, polarity, and van der Waals forces between hydrocarbon chains. The main polymorphic forms of triacylglycerols are alpha (α) form, beta prime (β′) form, and beta (β) form (Figure 2.4). These packing structures differ in their stability, as the most organized form is the most stable crystal. The most densely packed forms constitute the β polymorphs. Accordingly, β form is more stable and closely packed than β′ and α form (Table 2.1). Thus, the β forms have higher melting point and higher heat of fusion than the α forms. Transformation from a least stable polymorph to a more stable form can be achieved by slowly increasing the temperature above the melting point of the least stable form and allowing the liquid to recrystallize at this temperature.

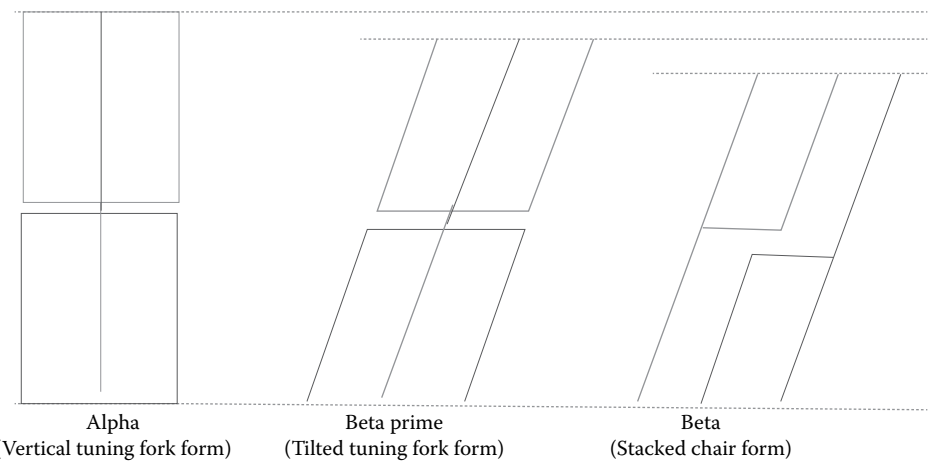


FIGURE 2.4 Triacylglycerols polymorphs.

TABLE 2.1
Physical Features of Triacylglycerols Polymorphs

Alpha	Beta Prime	Beta
Obtained after rapid cooling of liquid fat	Obtained after slow cooling of liquid fat	Obtained after very slow cooling of liquid fat
—	Polymorphic transformation of the α form	Polymorphic transformation of the β′ form
Thermodynamically most unstable	Thermodynamically unstable	Most stable form
Lowest melting point	Intermediate melting point	Highest melting point
Most loosely packed	More closely packed	Most closely packed

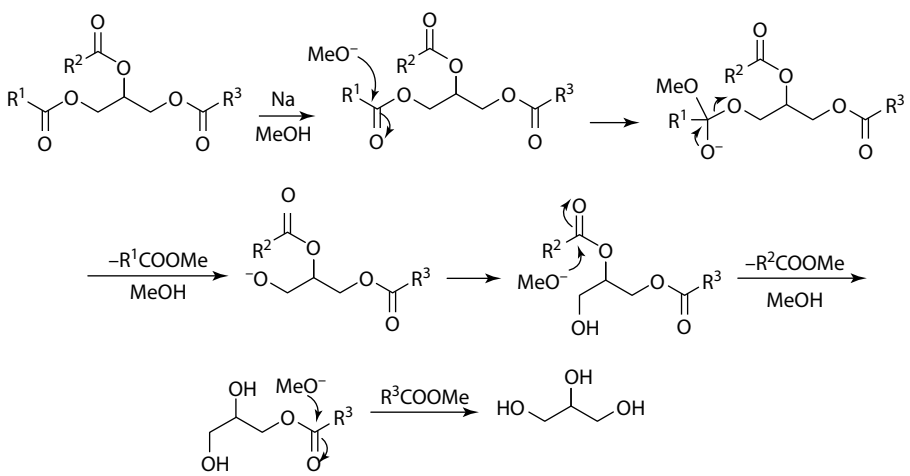
However, this transformation may also take place without melting and in some mixtures of triacylglycerols no β polymorphs may be observed or more than one β′ polymorph could be identified. For example, cocoa butter may display two β′ forms and two β forms [36] that have slightly different melting points but present an x-ray spectra that make them fall into one of the categories [33].

IV. CHEMICAL AND ENZYMATIC SYNTHESIS OF ACYLGLYCEROLS

Acylglycerols can undergo a series of chemical reactions including hydrolysis, esterification, interesterification, hydrogenation, and so forth. The structural modifications of oils and fatty acids can alter the packing behavior of the molecules generating lipids of higher or lower melting point based on the structural modification of the lipid in question [37]. For instance, fully hydrogenated unsaturated oils will yield a product with a higher melting point and more thermodynamically stable.

A. Alcoholysis

Alcoholysis, also known as transesterification, is the reaction between an alcohol and an ester [37]. This normally requires the presence of a catalyst to initiate the reaction. The mechanism of this reaction is displayed in Scheme 2.1. The alcoholysis of triacylglycerols by methanol can generate fatty acid methyl esters and glycerol. Methyl esters are favorable derivatives in oleochemistry (as biodiesels) and gas chromatography analysis because they are less reactive and more volatile than



SCHEME 2.1 Mechanism of triacylglycerols methanolysis.

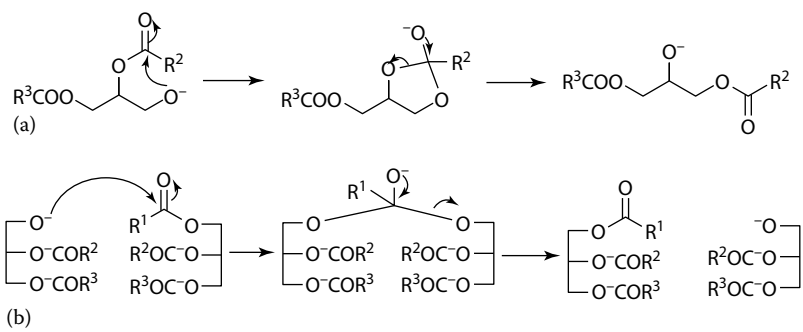
fatty acids or triacylglycerols. Furthermore, the development of the process for the production of methyl esters is of increasing importance due to demand for biodiesels [38]. Alcoholysis of high-purity triacylglycerols under dry condition can be completed in a few minutes with a strong base (such as sodium methoxide) as catalyst. The presence of free fatty acids may retard or completely terminate the reaction if Brønsted or Lewis acid is added as catalyst. Alcoholysis can also be carried out using glycerol as the alcohol donor with lipases or base as catalyst and this is called glycerolysis. Glycerolysis of oils is an important reaction to produce partial glycerides [39]. Monoacylglycerols and diacylglycerols are important food emulsifiers that are produced through glycerolysis reaction of oils and fats, followed by fractionation process.

Recently, Guo's research group reported the enzymatic glycerolysis of sardine oil to produce monoacylglycerols rich in ω-3 polyunsaturated fatty acids (n-3 PUFAs) [40]. In this work, they combined glycerolysis with molecular distillation to yield the desired monoacylglycerols. The reaction conditions used were a mole ratio of 3:1 glycerol:sardine oil, a 5% load of lipase based on the mass of total reactants, 50°C, and 2 h reaction time. In addition, when performing the reaction in larger scale in a 1 L batch reactor under 200 rpm stirring, approximately the same yields were reached. Accordingly, the work generated a scalable experimental procedure to obtain omega-3-enriched monoacylglycerols.

B. Interesterification

Interesterification is the exchanging reaction of two acyl groups between two esters [20,41]. This reversible reaction also requires a catalyst to speed up the reaction to equilibration. Interesterification is generally used to alter the melting profile of fats, crystalline characteristics, solid fat content, and plasticity. For triacylglycerols, interesterification can take place in an intramolecular or intermolecular mode and both modes at the same time (Scheme 2.2). The interesterification in the presence of an alcoholate can also generate monoalcohol esters, as in the methanolysis reaction described earlier (Scheme 2.1). Thus, formed glycerol ion can react with the acyl group in the adjacent carbon in the glycerol molecule to yield an intermediary cyclic species with a negative charge that will promote acyl migration as shown in Scheme 2.2b. However, this can also take place in an intermolecular mode. Lipase-catalyzed interesterification represents a more environmentally friendly way that also offers the advantage of region selectivity; for example, *sn*-1,3-specific lipases such as Lipozyme TL IM (*Thermomyces lanuginosus* lipase) will not, in principle, affect the acyl bond in the *sn*-2 position of glycerol backbone.

Ifeduba et al. [42] recently reported the enzymatic interesterification to generate low saturated, zero-*trans*, interesterified fats with 20% or 30% saturated fatty acids. Accordingly, tristearin or tripalmitin was initially blended with sunflower oil rich in oleic acid at different ratios (0.1:1, 0.3:1,



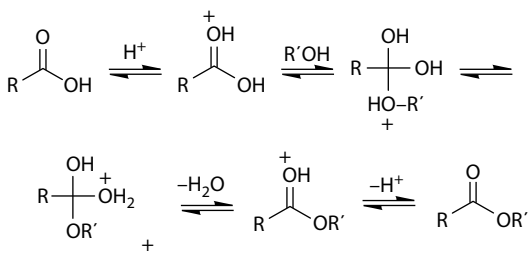
SCHEME 2.2 (a) Intramolecular reaction between a glycerate ion and acylate carbonyl R2; (b) intermolecular reaction between a glycerate ion and the acylate carbonyl R1 of another triacylglycerol molecule.

and 0.5:1 [w/w]). By plotting the total composition of the blended mixture against the molar ratio used, they were able to determine which blended mixture will yield the desired fat. Posteriorly, they carried out the enzymatic interesterification using Lipozyme TL IM. Based on their results, the interesterified fat contained 5%–10% more saturated fatty acids in the *sn*-2 position than the blended mixture and had broader melting profiles.

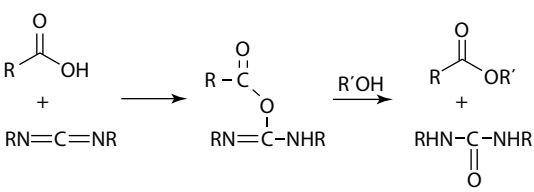
C. Esterification

Esterification is the reaction of an alcohol with a fatty acid; for example, the reaction of one molecule of glycerol with one to three molecules of fatty acids yields acylglycerols [43,44]. This reaction can take place in the presence of a catalyst or by activating fatty acid. For example, esterification can be catalyzed by 1%–5% of a Brønsted acid or by a Lewis acid (e.g., boron trifluoride etherate). The mechanism involves the nucleophilic reaction of an alcohol with a protonated carbonyl, followed by a rapid exchange of protons between oxygen atoms, and the elimination of a water molecule (Scheme 2.3). The direct esterification of alcohol with fatty acid is a reversible reaction and the yield can be improved by increasing the amount of the reactants or by removing the product (e.g., removal of water). This reaction can be performed in an industrial scale where water may be removed by distillation.

Another strategy for esterification constitutes the activation of the carbonyl group of a fatty acid prior to nucleophilic attack by a hydroxyl group [37]. Activating the carbonyl group of a fatty acid can transform the hydroxyl group into a better leaving group. One of the most used synthetic strategies to activate a carbonyl group is the transformation of a fatty acid into an acyl chloride. Other strategies involve performing the reaction in the presence of pyridine, which can trap the generated acid and also act as a cocatalyst by forming an acylium ion, which makes carbonyl more prone to react with alcohols. However, 4-dimethyl aminopyridine is more effective. Carbodiimide is also frequently used as a coupling agent to activate carboxylic acid and generate desired ester bond. Basically, this agent reacts on the diimide to generate an isourea *O*-acyl that can react with alcohols yielding the ester product and dialkyl urea (Scheme 2.4).



SCHEME 2.3 Direct esterification.



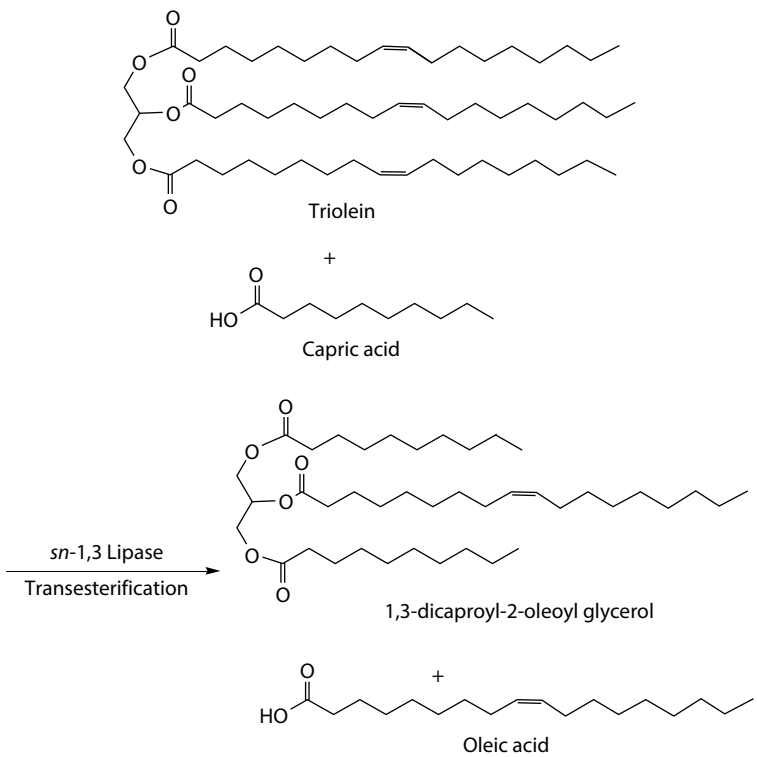
SCHEME 2.4 Carbonyl activation by carbodiimide.

D. Transesterification

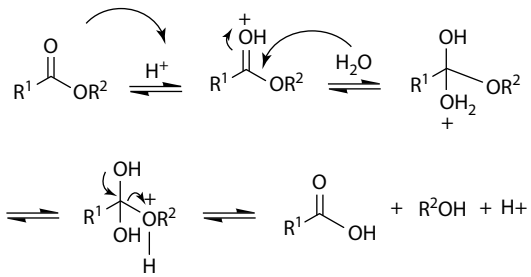
Transesterification refers to the reaction between triacylglycerol and acyl donors (fatty acids or fatty acid short-chain alcohol ester, e.g., fatty acid ethyl esters). The reaction between triacylglycerols and fatty acids is also called acidolysis [45]. As reviewed elsewhere [46], transesterification is an important strategy to produce structured lipids. With *sn*-1,3-specific lipases as biocatalysts, ABA-type structured lipids can be produced. A typical example is illustrated in Scheme 2.5 where the fatty acid exchange takes place at *sn*-1,3 positions of glycerol backbone under catalysis by *sn*-1,3-specific lipase. By applying excessive dosage of external fatty acids, most of the original fatty acyl groups in *sn*-1,3 positions of triacylglycerols will be replaced by external acid. A new type of structured lipids can be produced by the removal of replaced fatty acids and unreacted fatty acids with distillation. This reaction has found a variety of applications in producing milk fat equivalents and low-calorie fats [47].

E. Hydrolysis

Hydrolysis is one of the most used reaction procedures in lipids chemistry. Hydrolysis of triacylglycerols involves the cleavage of an ester bond by a water molecule leading to a fatty acid and a compound containing a hydroxyl group, for example, diacylglycerols, monoacylglycerols, and glycerol [48]. This reaction can take place in acidic, basic, or neutral medium. This reaction is reversible under acidic or neutral conditions but irreversible under basic conditions. The mechanism of the reaction in acidic medium is displayed in Scheme 2.6. The solubility of the reagents can determine the temperature and pressure required for the reaction to proceed. For example, tristearin that is insoluble in water needs high temperature and pressure to be hydrolyzed. Hydrolysis can also take



SCHEME 2.5 Transesterification of triolein and capric acid.



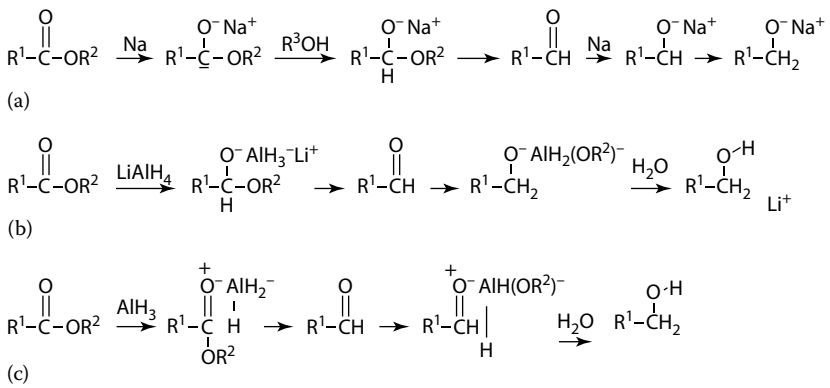
SCHEME 2.6 Mechanism of the hydrolysis of fatty esters.

place through an enzymatic pathway. For instance, lipolytic enzyme and lipase of bacterial and fungal origin can hydrolyze different lipids and fatty acid esters.

A recent example of using enzymatic approach for the hydrolysis of soybean oil was reported by Li et al. [25]. A lipase from *Rhizopus oryzae* (rProROL) produced by recombinant *Pichia pastoris* was used for the hydrolysis of soybean to generate diacylglycerols. They found that pH, temperature, water content, and enzyme concentration could affect reaction progress. The maximal content of diacylglycerols (33.11%) was achieved at a pH of 6.5, 20% water content, enzyme concentration of 30 U/g, 40°C, and 6 h. During the hydrolysis process, the enzyme displayed 1,3-regioselectivity and high hydrolytic activity demonstrating the enzyme potential for industrial processing of oils and fats.

F. Hydrogenolysis

Hydrogenolysis is the reduction of carboxylic acids, esters, ketone, aldehydes, acyl chloride, or unsaturated fatty acid chains into alcohols, aldehydes, or hydrocarbons, depending on the selectivity of the reagents and the solvent used [37]. There are mainly three kinds of reduction agents: alkali metals, hydrides, and hydrogen in the presence of a metal catalyst. For instance, reduction of ester, ketone, and aldehydes can take place in the presence of sodium and an alcohol, where an electron transfer occurs from the metal to the carbonyl as intermediary step for the generation of the product (Scheme 2.7a). Other popular reagents frequently used to reduce esters include anionic (e.g., $LiAlH_4$) or neutral (e.g., AlH_3) hydride. For example, while anionic hydride transfer a hydride through a nucleophilic substitution on an activated carbonyl reducing an aldehyde into an alcoholate, neutral hydride transfers a hydride inside a reducing agent-substrate (Scheme 2.7b and c).



SCHEME 2.7 Multiple mechanisms for the reduction of esters functionalities using Na (a), $LiALH_4$ (b), or AlH_3 (c) as reduction agent.

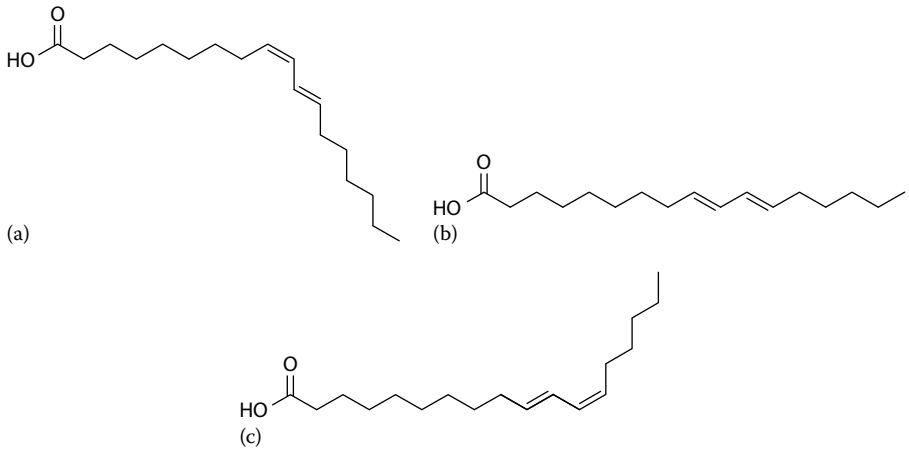
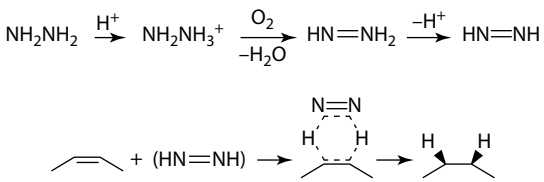


FIGURE 2.5 Structure of three conjugated linoleic acid (CLA) isomers (a) c9,t11; (b) t9,t11; and (c) t10,c12.



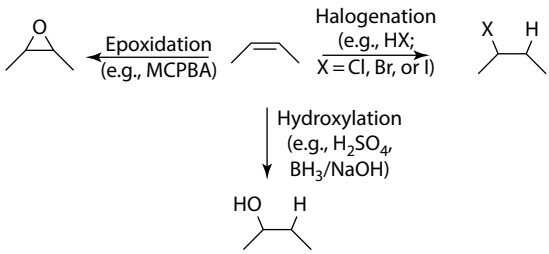
SCHEME 2.8 Noncatalytic hydrogenation using hydrazine.

Hydrogenation of double bonds can be full or partial depending on the catalyst used. This can be carried out in heterogeneous or homogeneous reaction conditions. The former is the most used since it facilitates the removal of the catalyst. Hydrogenation can proceed as partial hydrogenation and full hydrogenation. While full hydrogenation yields a fully saturated product, partial hydrogenation may generate isomerization (e.g., *cis* → *trans*) or migration of double bonds. Isomerization or conjugation reaction for conjugated unsaturated fatty acids/oils is an important reaction to produce biologically active conjugated linoleic acids (CLAs); for example, the beneficial health effects associated with the c9,t11; t9,t11; and t10,c12 CLA isomers (Figure 2.5) [49]. The latter can depend on the catalyst and the operating conditions used. For instance, nickel leads to the formation of more *trans* isomers than copper and the use of low temperature can limit the number of *trans* configuration generated.

Nonetheless, hydrogenation can also take place in a noncatalytic way but with a chemical hydrogen donor conferring a higher stereoselectivity [37]. Examples of reagents that can act as hydrogen donor constitute hydrazine (NH_2–NH_2), azodicarboxylic acid (HOOC–N=N–COOH), tosylhydrazine (CH_3–C_6H_4–SO_2NHNH_2), and hydroxylamine (NH_2–OH). In this type of reaction, the *cis* configuration is not shifted and only the ethylenic and acetylenic bonds are reduced by the hydrogen transfer to sp^2 carbons (Scheme 2.8).

G. Addition Reactions to Double Bond

The most common reaction of double bond is the addition reaction. Different reagents, both inorganic and organic, have proved to add to alkene (Scheme 2.9). This reaction is generally exothermic as the ethylenic pi bond is relatively weak compared to the sigma bond. The most used addition reactions are epoxidation where oxygen is transferred to olefin. Epoxidation of oils and unsaturated fatty acids is used for the generation of oxiranes valuable for industrial application [50]. Other addition reactions include halogenation where halogens are added to the ethylenic bond and hydroxylation



SCHEME 2.9 Examples of addition reactions to double bond.

to generate alcohol. However, halogenation is mostly used for analysis purpose (e.g., to determine iodine values of unsaturated oils and fats) or to form intermediate products in synthesis.

V. PHOSPHOLIPIDS

Phospholipids are the major constituent lipids in biomembranes [51]. Membranes are the barriers that constitute the outer boundary of cell (plasma membrane) and the inner compartment (organelles). They are selectively permeable and control the flow of substances in and out of the cell [52]. Membranes are mainly composed of proteins, sugar, and lipids. The phospholipids are generally organized as a bilayer structure where the protein molecules are totally or partially embedded into the bilayers (Figure 2.6). Noncovalent interactions keep together the lipids and proteins while sugars

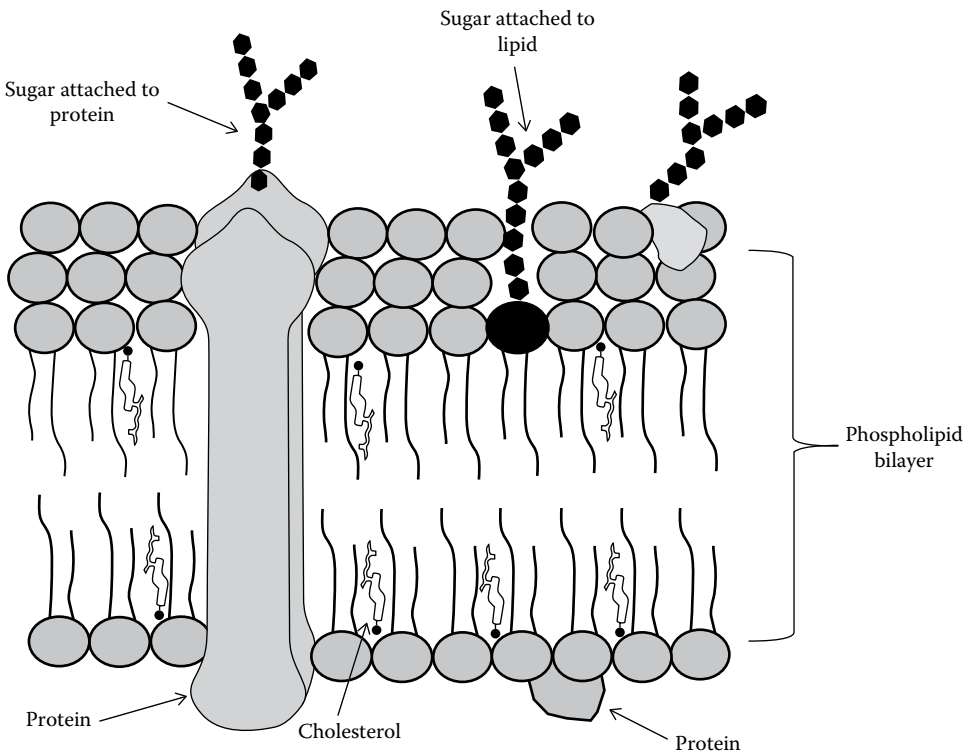


FIGURE 2.6 Graphical representation of a biological membrane. (From Brown, B.S., Biological membranes, The Biochemical Society, http://www.biochemistry.org/portals/0/education/docs/basc08_full.pdf, 1996, accessed October 10, 2016.)

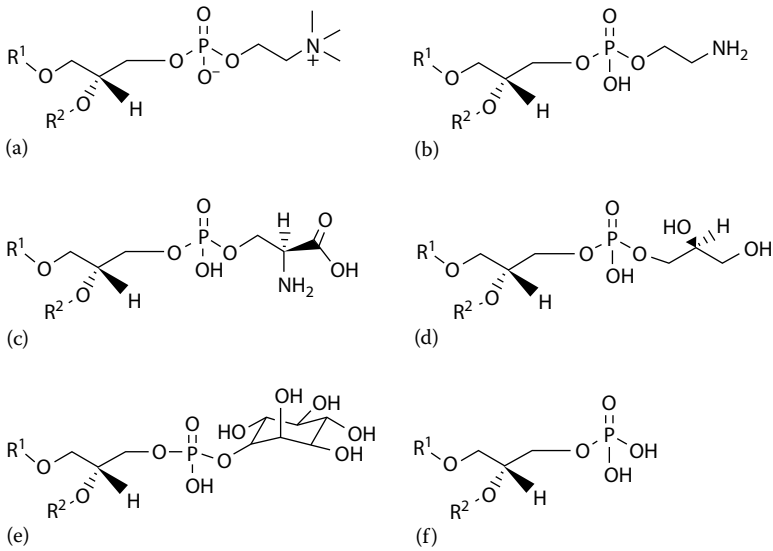


FIGURE 2.7 Structure of glycerophospholipids. (a) PC, (b) PE, (c) PS, (d) PG, (e) PI, and (f) PA. R^1 and R^2 are fatty acids.

are covalently linked to some of the lipids and proteins within the membranes. Different lipids have specific functions in the membrane as their structure will determine the molecular organization influencing membrane fluidity [4].

The most common phospholipids, known as glycerophospholipids, are based on a glycerol backbone. Glycerophospholipids can be subdivided into distinct classes, based on the nature of the polar head group at the *sn*-3 position of the glycerol backbone in eukaryotes and eubacteria or the *sn*-1 position in the case of archaebacteria. Accordingly, the different classes of glycerophospholipids are phosphatidylcholine (PC; Figure 2.7a), phosphatidylethanolamine (PE; Figure 2.7b), phosphatidylserine (PS; Figure 2.7c), phosphatidylglycerol (PG; Figure 2.7d), phosphatidylinositol (PI; Figure 2.7e), and phosphatidic acid (PA; Figure 2.7f). If the *sn*-1 position (R^1 in Figure 2.7) is hydrolyzed, the corresponding lysophospholipid is formed. A second glycerol unit constitutes part of the head group in the glycerophosphoglycerols (PG; Figure 2.7d) and glycerophosphoglycerophosphates (Figure 2.8), whereas for the glycerophosphoglycerophosphoglycerols (cardiolipins; Figure 2.8), a third glycerol unit is typically acylated at the *sn*-1′ and *sn*-2′ positions to create a pseudo-symmetrical molecule.

VI. PHYSICAL PROPERTIES OF PHOSPHOLIPIDS

The packing behavior of lipids is determined by the intermolecular repulsive and attractive forces of the polar head group of the lipid [53]. In phospholipids, the hydrogen bonding between hydrogen bond donor functional group such as −P−OH, −OH, and $-NH_3^+$ and hydrogen bond acceptor including −P−O^- and $-COO^-$ can account for the molecular phase transition to go into a hexagonal phase. If submerged in water, these zwitterionic amphiphilic lipids can assemble to form a bilayer where the polar head group points toward the water and the hydrophobic tails locate in between the hydrophilic heads. This bilayer will form sealed compartments, known as liposomes, to eliminate the lipid tails contact with water (Figure 2.9). Accordingly, liposomes can be absorbed by many cells by interaction with the cell plasma membrane and used to deliver drugs to specific organs in the body.

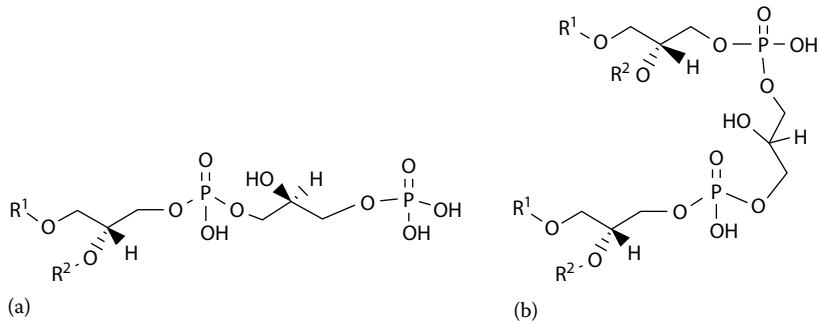


FIGURE 2.8 Structure of (a) glycerophosphoglycerophosphates and (b) glycerophosphoglycerophospho glycerols.

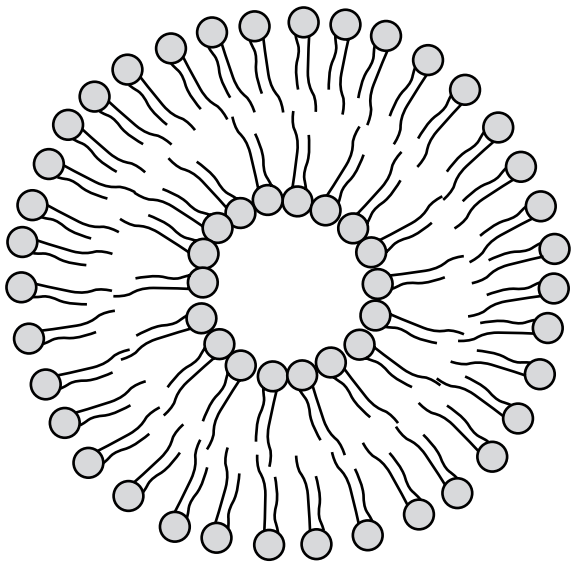


FIGURE 2.9 Representation of a liposome structure.

In membranes, the fluidity of a lipid bilayer depends on temperature, fatty acyl composition, and cholesterol content [54]. At low temperature, the lipid chains are more closely organized. However, as temperature increases the lipid bilayer becomes more fluid, forming a more disordered arrangement. The temperature range at which membranes are more rigid is determined by the length of the fatty acyl chain and the degree of unsaturation. For instance, the temperature at which the membrane becomes fluid is lower if fatty acid chains are shorter or have more double bonds. In addition, the membrane fluidity is also dependent on the cholesterol content. For example, eukaryotic membranes contain one cholesterol molecule per two phospholipid molecules [54]. In membranes, cholesterol locates in between the phospholipid chain with the hydroxyl group pointing toward the polar head of phospholipids and the steroid ring in between the lipid chains.

Phospholipids are zwitterionic surfactants that have GRAS (Generally Recognized as Safe) status, which enables their use in food [55]. For instance, phospholipids can also be used in food nanotechnology. Nanotechnologies can encapsulate, protect, and deliver lipophilic ingredients such as omega-3

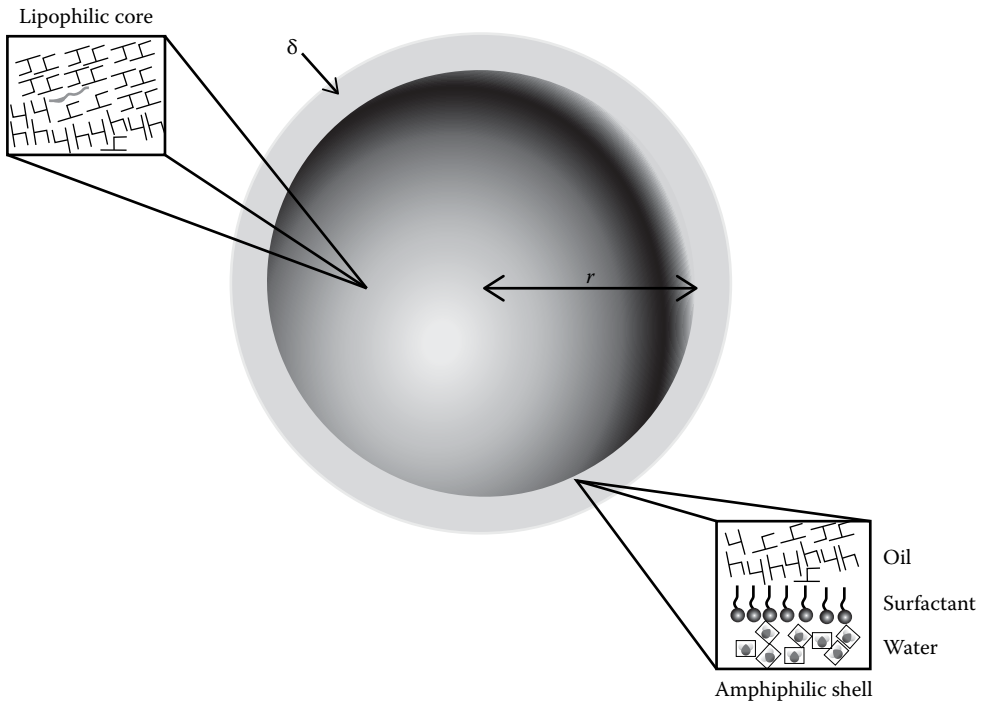


FIGURE 2.10 Schematic representation of nanoemulsions' core shell. (From McClements, D.J. and Rao, J., *Crit. Rev. Food Sci. Nutr.*, 51, 285, 2011.)

fatty acids. In addition, the small particle size of these nanoparticles confers higher stability, optical clarity, and bioavailability. For instance, since nanoparticles such as nanoemulsions poorly scatter light, they can be incorporated into food products such as fortified drinks that are required to be optically clear or only slightly turbid [56]. Nanoemulsions contain (1) an oil phase composed of a nonpolar component such as acylglycerol and free fatty acids, (2) an aqueous continuous phase that mainly consists of water and other water-soluble components, and (3) an interface between oil/water phases, consisting of emulsifiers, stabilizers, or surface-active compounds to improve the long-term stability of the nanoemulsions (Figure 2.10). Emulsifiers adsorb to droplets' surface, prompting droplets' disruption and preventing droplets' aggregation. The stability of nanoemulsions under environmental stresses, including heating, cooling, pH changes, and storage is largely influenced by the property of emulsifiers. In food industry, phospholipids are commonly used as zwitterionic surfactants. However, natural phospholipids usually require the use of a cosurfactant, the compounds containing a hydrocarbon chain and a polar head, to ensure a better stability of nanoemulsions.

Nanoliposome is a type of liposome with the smaller diameter in nano range and is assumed to offer better encapsulation or controlled release of food material so as to ensure better bioavailability and stability and longer shelf-life of oxidation-sensitive food ingredients [55]. Nanoliposomes are formed by the auto-assembly of amphiphilic molecules such as phospholipids when energy is supplied (e.g., heating, sonication). These amphiphilic molecules assemble to shield the hydrophobic chains from the aqueous environment, while the polar heads maintain contact with the water layer and when sufficient energy is provided, a closed organized vesicle (nanoliposomes) could be formed (Figure 2.11). The structural feature of phospholipids responsible for bilayer-forming molecules is their amphiphilicity, whereas the hydrophobic domain between the bilayer of vesicles enables them to entrap and deliver lipophilic, hydrophobic, or amphiphilic compounds. Nanoliposomes have

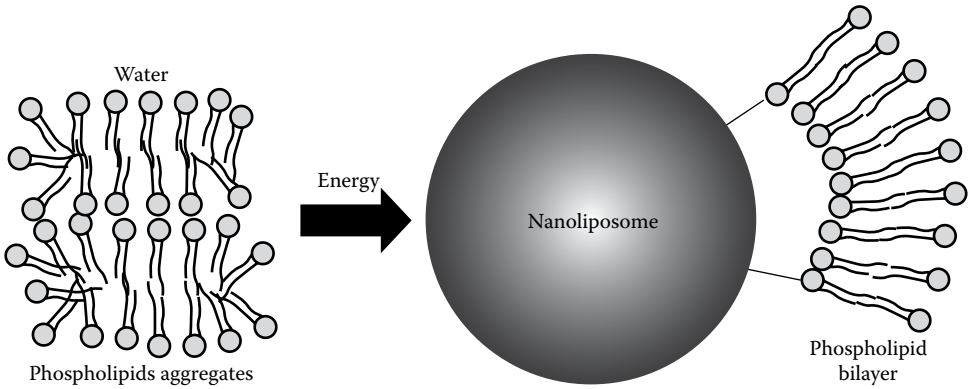


FIGURE 2.11 Simplified representation of the mechanism of nanoliposome formation. (From Mozafari, M.R. et al., *J. Liposome Res*., 18, 309, 2008.)

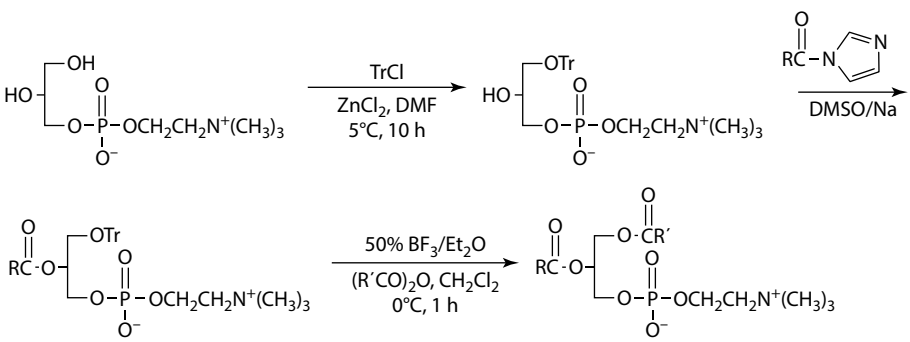
demonstrated its application potential, particularly when used to encapsulate antimicrobials in food products to prevent microbial contamination [55].

VII. CHEMICAL AND ENZYMATIC SYNTHESIS OF PHOSPHOLIPIDS

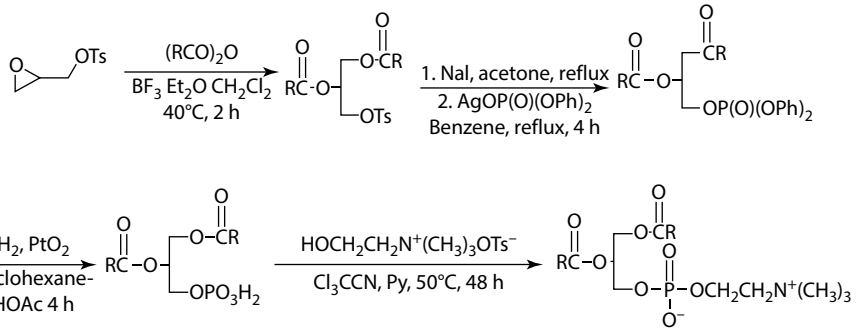
A. Chemical Synthesis of Phospholipids

Introducing specific functional groups in phospholipids has become of interest for the generation of structurally relevant phospholipid derivatives for membrane-associated processes [57]. However, relying on enzymatic process to cleave the acyl bond and later to introduce new functional group through chemical reactions limits the yield obtained for the desired products. Therefore, pure chemical strategies have come into play to address this problem. In this type of synthetic pathway, glycidyl, protected glycerol, and *sn*-glycero-phosphatidylcholine (GPC) are common starting materials used for the synthesis of phospholipids. They can generate mixed chain diacyl phospholipids. For instance, GPC is used to prepare 1-*O*-trityl-*sn*-glycero-3-phosphocholine using trityl chloride and zinc chloride, and 1-*O*-trityl-GPC is posteriorly acylated at *sn*-2 position using oleoyl imidazolide [58]. The resulted product is deprotected using boron trifluoride (BF_3) and acylated with palmitic anhydride (Scheme 2.10).

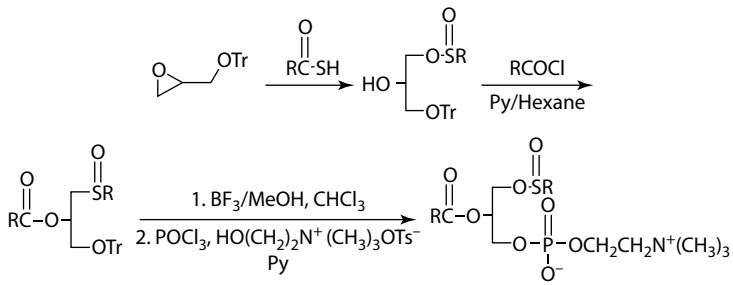
Optically pure diacyl-PCs can be generated using (R)- or (S)-glycidyl tosylate as starting material (Scheme 2.11) [59]. Basically, the arene sulfonate works as a protecting group in the *sn*-3



SCHEME 2.10 Preparation of phospholipids via 1-lyso-2-acyl-*sn*-glycero-3-phosphocholine.



SCHEME 2.11 Preparation of optically pure diacyl-PC from (R)- or (S)-glycidyl tosylate.



SCHEME 2.12 Preparation of 1-thioester-2-ester-PC.

position, thereby preventing acyl migration from *sn*-2 position while a Lewis acid–catalyzed reaction takes place to open and acylate the epoxide functionality using fatty acid anhydride and BF_3. Subsequently, the tosylate group can be converted to phosphate ether using sodium iodide and later silver diphenyl phosphate and the resulting diphenyl phosphate is converted into phosphatidic acid and posteriorly into phosphatidylcholine using choline tosylate in the presence of trichloroacetonitrile. However, chiral glycidyl is expensive, making the synthesis of optically pure diacyl-PCs through this methodology impractical.

Other analogues of phospholipids can also be prepared from protected glycidol [60]. For instance, the 1-thioester-2-ester and 1,2-dithioester-PC can be generated using trityl glycidyl ether as starting material (Scheme 2.12). The ring opening takes place in the presence of thioacid to yield the thioester at *sn*-1 position and later acylation occurs at the *sn*-2 position with acylchloride. After detritylation, the resulted product can be converted into phosphocholine derivatives using phosphorus oxychloride and choline tosylate. Although commercially available, trityloxyglycerols can be prepared from allyl alcohol or from glycidol.

B. Enzymatic Synthesis of Phospholipids

Phospholipids can be structurally modified with the appropriate enzymes (Figure 2.12) [61]. Although phospholipases are found ubiquitously in nature, only a few enzymes have been produced in large scale [62]. Examples of natural enzymes applied for the modification of phospholipids include phospholipases A_1 (EC 3.1.1.32; PLA_1) and A_2 (EC 3.1.1.4, PLA_2) and lipases. These enzymes can be used for acyl modification purposes. *Sn*-1,3 lipases can specifically modify position 1 but not *sn*-2. Only PLA_2 has demonstrated to be valuable for the latter purpose. For instance, PLA_1 hydrolysis yields a 2-acyl lysophospholipid and PLA_2 hydrolysis produces 1-acyl lysophospholipid. Phospholipase D (PLD) can modify polar head and phospholipase C (PLC) can hydrolyze the bond

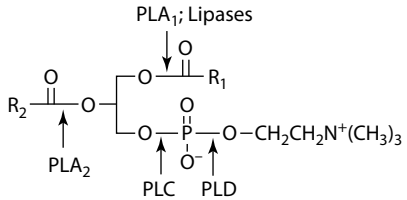


FIGURE 2.12 Enzymatic modification of phospholipids. (From Xu, X. et al., Enzymatic modification of phospholipids and related polar lipids, in: Gunstone, F.D., ed., *Phospholipid Technolology and Applications*, The Oily Press, 2008, pp. 41–82.)

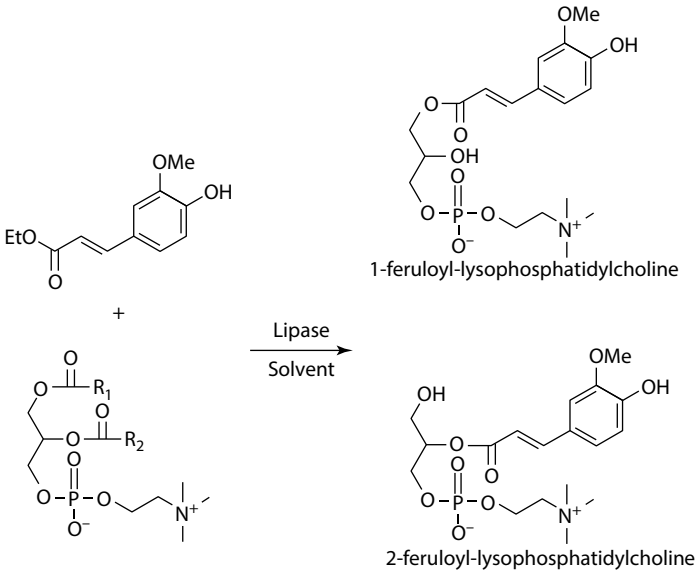
between glycerol OH and the phosphate group. Accordingly, structured phospholipids with specific fatty acid composition and polar head group and with desired chemical and physical properties can be generated by enzyme-catalyzed reactions such as hydrolysis, transesterification, and transphosphatidylation [63].

1. Hydrolysis

PLA_1 has been used for the enzymatic modifications of phospholipids for industrial processes [61]. For instance, PAL_1 is used in the degumming process of edible oils. Removal of one of the acyl chains of phosphatidylcholine generates a more hydrophilic compound known as lyso-PC. This compound possesses higher emulsifying properties since the molecule becomes more hydrophilic and consequently is easy to form oil-in-water emulsions. The enzymatic hydrolysis of naturally occurring lecithin is one of the processes to generate high amounts of lysophospholipids. Both lipases and phospholipases are useful for this process. Different conditions and enzymes have been used to obtain lysophospholipids from low to high yields. Recently, Lim et al. [64] reported an experimental procedure to produce lyso-PC containing high content of unsaturated fatty acids (78% linoleic acid) in relation to the total content of fatty acids. Accordingly, they optimized different reaction parameters such as temperature, water content, and enzyme load to increase yield and minimize acyl migration. Optimal reaction conditions were 10% of water content and 1% of enzyme load based on PC content, 60°C, and 3 h reaction time, under which 84% yield of lyso-PC was obtained [64].

2. Transesterification

Transesterification or replacement of fatty acyl chain by exogenous fatty acids can be performed using PAL_1, PAL_2, and lipases. Several parameters including enzyme dosage, water content, solvents, reaction time, temperature, acyl donor, and PL polar head group can influence the reaction performance [61]. For instance, increasing the fatty acid concentration to perform lipase-catalyzed esterification or transesterification inhibits hydrolysis and increases the yield of the reaction [65]. Although reaction time needed is independent of fatty acid concentration in esterification reaction, the reaction time has to be increased with fatty acid concentration in transesterification. The substrate molar ratio used can influence the cost of removal of the fatty acids from the product. Therefore, a compromise is required to select the substrate mole ratio of the reaction. Water content also plays an important role on enzyme activity. For instance, PAL_2 requires a higher water content than lipases to function and as such yields might be lower since major hydrolysis occurs in parallel [65]. Other strategies are to use water-mimicking molecules such as ethylene glycol or propylene glycol that promote esterification by activating the enzyme but do not contribute to hydrolysis. In addition, if the use of solvent can be avoided, the reaction should be carried out in a solvent-free system, especially, in the case of organic solvents that can generate hazardous wastes. Still organic solvents have demonstrated to increase mass transfer. Accordingly, organic solvents lower the viscosity of the system and allow better accessibility of enzyme to substrate. Nonetheless, the polarity of solvent has strong water solvation effect and thus deactivate enzyme. Therefore, generally used organic solvents have a $\log P > 2$.



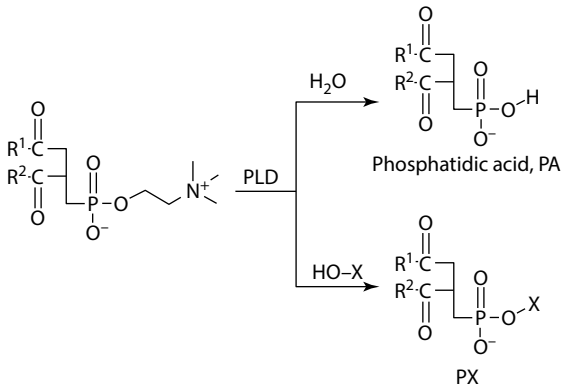
SCHEME 2.13 Lipase-catalyzed synthesis of feruloylated lysophospholipids.

A recent example of the introduction of functional compound into phospholipids to generate a new phospholipid derivative was reported by Yang et al. [66]. The authors reported the enzymatic synthesis of feruloylated lysophospholipids using phosphatidylcholine and ethyl ferulate (EF) as ferulic acid has demonstrated antioxidant properties (Scheme 2.13). Different lipases and solvents have been examined, and Novozym 435 and toluene were found to be good starting point for further optimization. Subsequently, binary solvents were used in combination with toluene and 90:10 (v/v) of toluene: chloroform afforded the optimal enzyme activity. In addition, the optimal conditions for the generation of feruloylated lysophospholipids were 60 mg/mL of enzyme load, 1.5 h of PC's hydrolytic time, and a molar ratio of 5:1 PC:EF.

Recently, Chojnacka et al. [67] reported the lipase-catalyzed transesterification of egg-yolk phosphatidylcholine with pomegranate seed oil (PSO) containing over 77% of conjugated linolenic acid (punicic acid; 18:3, 9c,11t,13c). Three different immobilized lipases were used as biocatalyst and the enzyme load, reaction time, and mole ratio of substrates were optimized. Accordingly, the best results were obtained when using CALB, 20% of enzyme load, 72 h of reaction time, and a mole ratio of PC:PSO, 1:3. In addition, the resulted product contained up to 50% of polyunsaturated fatty acids.

3. Transphosphatidylation

Different from enzymatic modification of fatty acyls in phospholipids, modification of the polar head group of glycerophospholipids can only be carried out using enzymes in the same category as PLD (Scheme 2.14). This modification is generally carried out to increase the content of one particular phospholipid species (e.g., PS, phosphatidylserine) using phospholipids present in lecithin as starting materials [68]. Another type of modification is also performed using alcohol acceptors (HO−X; Figure 2.13) to generate phosphatidyl derivatives of drugs such as genipin, ascorbic acid, and arbutin with different physicochemical properties. In addition, it can also be used to prepare novel derivatives such as alkylphosphate ester of potential pharmaceutical value [61].



SCHEME 2.14 Enzymatic transphosphatidylation of phosphatidylcholine using water or an alcohol acceptor.

VIII. SEPARATION, ANALYSIS, AND CHARACTERIZATION OF LIPIDS AND PHOSPHOLIPIDS

Multiple techniques are used for separation, analysis, and characterization of acylglycerols and phospholipids. The main reasons for lipid analysis and characterization are to ensure food quality, identify and prevent lipid-oxidation products, and optimize processing [69]. Well-known and highly used techniques for lipid analysis are TLC, HPLC, and GC. However, to investigate the structural features and physical properties of lipids, nuclear magnetic resonance spectroscopy (NMR), differential scanning calorimetry (DSC), Fourier transform infrared (FTIR), and x-ray diffraction studies are also required. The combination of multiple techniques enables the isolation and determination of physicochemical features of a lipid. Consequently, the use of multiple techniques helps to disclose the specific functionalities pertaining to acylglycerols or phospholipids.

A. Separation of Acylglycerols and Phospholipids

Several separation techniques are available today to enrich, isolate, or purify lipids and phospholipids. These techniques can be used to modify the properties of lipids. For instance, palm oil, milk fat, and palm-kernel oil can be fractionated by crystallization to generate different functional lipids [70]. The most used techniques for lipid separation in industrial-relevant scale are crystallization, fractionation, and short path distillation (SPD). In the case of SPD, also known as molecular distillation, it is a thermal separation technique that operates at pressure down to 0.001 mbar that allows the separation of heat-sensitive and high-boiling-point products minimizing thermal stress and product decomposition. Figure 2.14 displayed an example of the use of this technique for the separation of different acylglycerol products from the glycerolysis of sardine oil [40]. In laboratory scale, the techniques mainly used are preparative thin-layer chromatography (PTLC) and column chromatography. In both techniques, lipids and phospholipids are isolated based on their polarity. However, PTLC allows the isolation of lipids in milligrams scale. In contrast, column chromatography can be used both from the mg to the industrial scale. However, column chromatography is avoided in food industry due to the use of large quantity of organic solvent that may be needed during the purification process.

B. Analysis of Acylglycerols and Phospholipids

The main techniques used for qualitative and quantitative analyses of lipids in a mixture of lipids are TLC, HPLC, and GC. These techniques separate lipids based on their affinity to a stationary and a mobile phase. However, these techniques rely on the detection technique used. For instance,

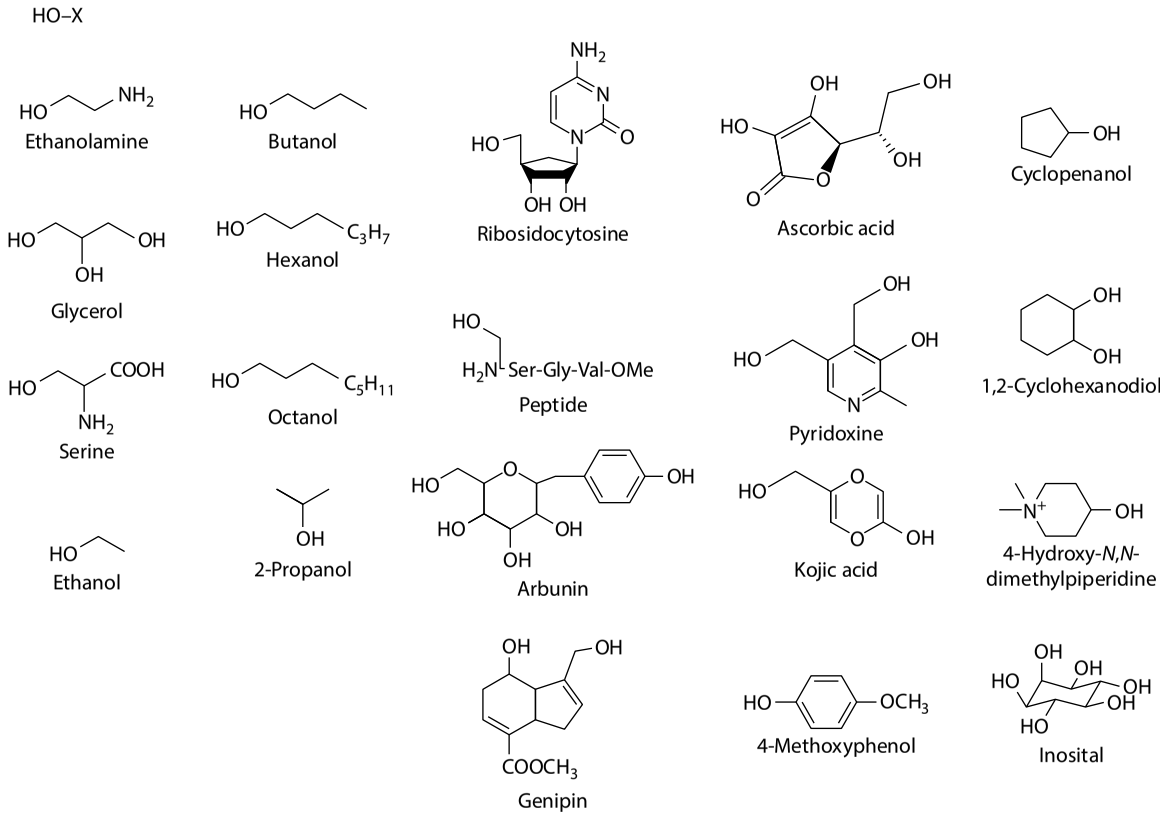


FIGURE 2.13 Alcohol acceptors used in enzymatic transphosphatidylation. (From Xu, X. et al., Enzymatic modification of phospholipids and related polar lipids, in: Gunstone, F.D. ed., *Phospholipid Technolology and Applications*, The Oily Press, 2008, pp. 41–82.)

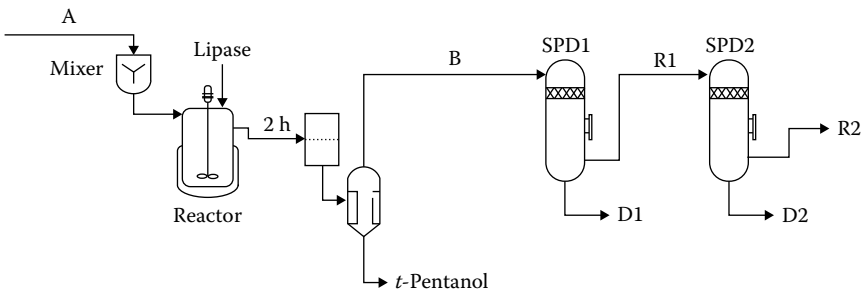


FIGURE 2.14 Representation of the molecular distillation of the product of glycerolysis of sardine oil at selected temperatures, where D and R stand for distillate and residue, respectively. A, Sardine oil + glycerol + *t*-pentanol; B, TAG, DAG, MAG, FFA, glycerol; R1, TAG, DAG, MAG; D1, glycerol, FFA; R2, TAG, DAG; D2, MAG. (From Solaesa, Á.G. et al., *Food Chem*., 190, 960, 2016.)

GC-FID (FID: flame ionization detector) helps measure the concentration of lipids in a sample of interest, while GC-MS (MS: mass spectroscopy) both measures concentrations and provides information regarding the structure of the lipids in the same sample.

1. Thin-Layer Chromatography

TLC is a technique in which compounds are separated based on their affinity to an absorbent material (stationary phase), typically silica gel. Kahovcova and Odavić developed a rapid, simple, and reproducible method for the quantitative determination of phospholipids by TLC [71]. After separation, each phospholipid class was detected by spraying 50% (w/w) sulfuric acid, direct mineralization at high temperature and subsequent determination of the liberated inorganic phosphate with the Hand and Luckhaus reagent. Chloroform–methanol–water (65:25:4, v/v/v) was used as developing solvent. However, in this report only PC, lyso-PC, and PE were involved, meaning that the separation was easier than more complex samples.

For separation of more complex samples, 2D TLC is required. One example was reported by Parsons and Patton [72]. They selected chloroform–methanol–water–28% aqueous ammonia (130:70:8:0.5) in the first dimension development, followed by chloroform–acetone–methanol–acetic acid–water (100:40:20:20:10) in the second. The resultant chromatogram is shown in Figure 2.15a. Another example of the use of 2D-TLC for the determination of phospholipids is the work reported by Rouser et al. [73]. They used chloroform–methanol–28% aqueous ammonia (65:25:5) for first dimension development. Later, the chromatograms were dried for about 10 min in a TLC chamber flushed with nitrogen and then developed in the second dimension with chloroform–acetone–methanol–acetic acid–water (3:4:1:0.5). The resulting chromatograms are shown in Figure 2.15b and c.

TLC is a separation method, meaning it cannot quantify the content directly. Thus, several different quantification methods are developed such as FID (i.e., TLC-FID and high-performance TLC-FID) and colorimetric method to quantify, for instance, phosphorus content of individual spot scraped from the developed plate. The main drawbacks of TLC-based methods are that they are time-consuming and involve multisteps of operation. However, it is an accurate method and is recommended as the AOCS standard method.

2. High-Performance Liquid Chromatography

Similar to TLC, HPLC method also includes two main parts, namely, separation and detection. Both are very critical steps in the analysis of lipids and phospholipids by HPLC. If separation is not good enough, it is not possible to quantify different classes of phospholipids. On the one hand, separation can be carried out using either normal-phase or reversed-phase columns. The latter is capable

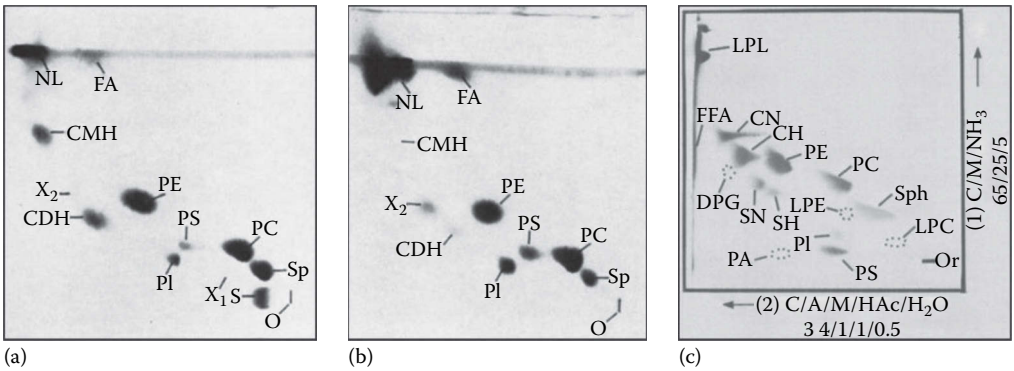


FIGURE 2.15 Two-dimensional thin-layer chromatography (TLC) of bovine milk polar lipids (a) and mammary tissue total lipids (b). The chromatograms were developed from right to left with chloroform–methanol–water–28% aqueous ammonia 130:70:8:0.5 and then in the vertical direction with chloroform–acetone–methanol–acetic acid–water 100:40:20:20:10. O, origin; S, carbohydrate (lactose) and protein; Sp, sphingomyelin; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; CDH, cerebroside dihexoside; CMH, cerebroside monohexoside; FFA, free fatty acids; NL, neutral lipid; and unknown substances listed as X1 and X2. X2 has been tentatively identified as cardiolipin. (c) Two-dimensional TLC of normal human (23-year-old male) whole brain lipids. LPL, less polar lipid (cholesterol, triacylglycerol, etc.); CN and CH, cerebroside with normal and hydroxy fatty acids; SN and SH, sulfatide with normal and hydroxy fatty acids; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; LPE, lysophosphatidylethanolamine; PA, phosphatidic acid; Sph, sphingomyelin; DPG, diphosphatidylglycerol.

of separating different molecular species within the same lipid class. On the other hand, as lipids and phospholipids are eluted out of the column, they can be detected through several types of detectors. There are different HPLC detection techniques. However, they have to be selected based on the mixture of lipids or phospholipids that are analyzed by HPLC. Ultraviolet (UV) detectors working with fixed or variable wavelengths are commonly used in HPLC. However, lipids possess weak UV absorption or none at all. Therefore, some mass detectors, such as evaporative light-scattering detector (ELSD), are generally used instead. In the latter detection mechanism, the eluents from the column pass through a nebulizer, and the formed droplets are evaporated from the nonvolatile analytes, which are detected as particles by a light-scattering device. Any solvents can be used for this detector. Recently, a relatively new detector, charge aerosol detector (CAD) has been proven to show greater sensitivity and better precision than ELSD. Additionally, CAD is quite user-friendly since it does not require any optimization of operating parameters [74].

In general, "normal-phase" chromatography is used when different PL classes have to be separated. A highly common method for the analysis of phospholipid consists of the use of silica normal-phase Zorbax Rx-SIL (Agilent-Technologies) for separation [74,75]. In this method, a linear binary gradient: t_0 min: 0%B, t_{14} 100%B, and finally isocratic conditions (100%B) for 9 min was employed. Total chromatographic run time was 40 min per sample, which consisted of a 23 min analysis, 12 min to restore initial conditions, and 5 min for re-equilibration. Eluent A: $CHCl_3$:MeOH:30% ammonium hydroxide (80:19:0.5) and eluent B: $CHCl_3$:MeOH:30% ammonium hydroxide:H_2O (60:34:0.5:5.5) are used at a flow rate of 1 mL/min. In addition, a Sedex ELSD detector is used for signal detection. For instance, Avalli and Contarini [75] found these conditions to be optimal for the separation of PE, PI, PS, PC, and SM. However, Stith et al. [76] found that these HPLC conditions yield a varying baseline and did not separate certain phospholipids well such as PI or PS from PC. Therefore, they developed another gradient elution strategy to solve the problem. Three eluents were introduced and finally the separation was optimized. The result, indicating well separation of each class of phospholipids, is shown in Figure 2.16.

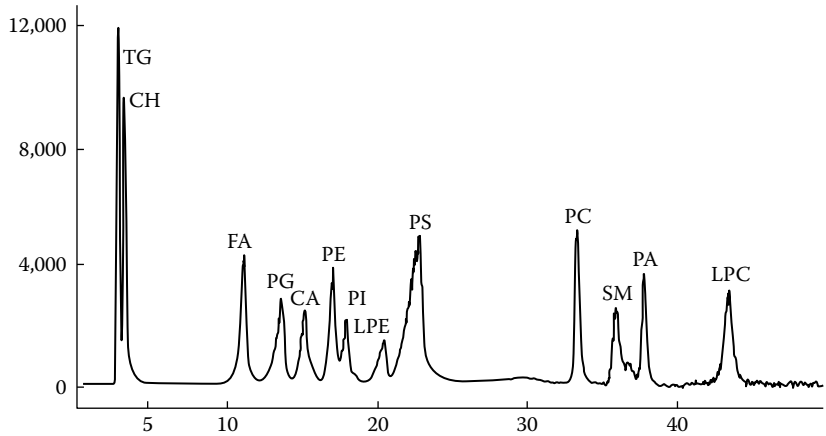


FIGURE 2.16 Separation of lipid standards by the heated diol column and three-pump high-performance liquid chromatography (HPLC) system in the Avanti Polar Lipids analytical laboratories. The abscissa represents time (min), whereas the ordinate presents the evaporative light-scattering detector (ELSD) response (μV). TG, CH, FA, PG, CA, PE, soy PI, LPE, PS, PC, SM, PA, and LPC (20 μg of each standard in a total volume of 20 μL) were injected. Note that, as is commonly found, neutral lipids produce a larger ELSD response than phospholipids or SM.

For the analysis of acylglycerol, reversed-phase HPLC is generally used instead. In this technique, the stationary phase is an *n*-alkyl hydrocarbon chain or aromatic ring immobilized on the chromatography support. Reverse-phase HPLC is a useful technique for the analysis of hydrophobic molecules including lipids, proteins, peptides, and nucleic acids with excellent recovery and resolution [77]. The resolution of the chromatograms may be determined by (1) the hydrophobic interaction of the solute molecules in the mobile phase with the stationary phase and (2) the desorption of the solute from the stationary phase back into the mobile phase. This type of chromatography uses generally gradient elution instead of isocratic elution. For lipid analysis, the lipophilic stationary phase in the column retards and separates lipophilic molecules depending on the combination and structure of the fatty acyl groups. The methylene groups in the chain contribute to an increasing retention time and the double bonds have the opposite effect, decreasing retention time. Accordingly, this technique can also be used for the differentiation of PLs with varying fatty acid composition.

3. Gas Chromatography

GC partitions a lipid mixture between a nonvolatile stationary liquid phase and a mobile gas phase, which is generally nitrogen or helium for packed columns and helium or hydrogen for capillary columns. Through GC technique, it is possible to analyze the fatty acid composition of any given lipids or phospholipids. Analysis of the fatty acid composition of a sample by GC generally requires the transformation of fatty acids into fatty acid methyl esters to yield more volatile compounds. The different fatty acids are first separated on GC columns and later detected by FID. GC-FID determines the fatty acids composition based on the comparison of the retention times displayed in the chromatogram with those obtained by reference samples. Instead, for structure elucidation GC coupled to a mass spectrometer (GC-MS) is the technique required since it provides information regarding molecular structure. However, to determine the distribution of the fatty acid in the glycerol backbone, the use of multiple techniques will be needed. For instance, mixture of *sn*-1 and *sn*-2 monoacylglycerols has to be separated by TLC and later analyzed by GC-FID. Figure 2.17 is an example of a GC chromatogram of seed oil of *Cassia artemisioides* [78].

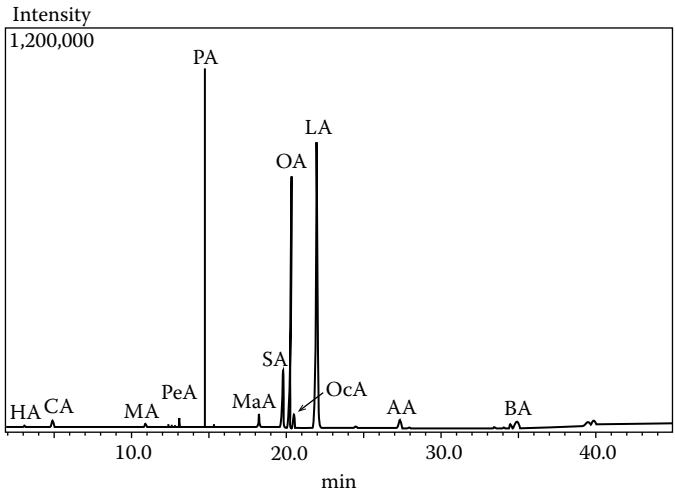


FIGURE 2.17 Gas chromatogram of seed oil of *Cassia artemisioides*. HA, hexanoic acid; CA, caprylic acid; MA, myristic acid; PeA, pentadecanoic acid; PA, palmitic acid; MaA, margaric acid; SA, stearic acid; OA, oleic acid; OcA, octadecenoic acid; LA, linoleic acid (ω-6); AA, arachidic acid; BA, behenic acid. (From Qureshi, M.N. et al., *J. Food Sci. Technol*., 52, 7530, 2015.)

C. Characterization of Lipids and Phospholipids

1. NMR

NMR is definitely a quantitative spectroscopic tool because the intensity of a resonance line is directly proportional to the number of resonant nuclei (spins). This fact enables a precise determination of the quantity of substances in solids as well as in liquids. For example, ^{1}H-NMR is a very useful technique for the structural characterization and quantification of lipids (http://lipidlibrary.aocs.org/nmr/1NMRintr/index.htm). This technique can work for the characterization of mixture of oils without prior isolation of individual components [79]. The chemical shifts obtained are based on the structure of the functional group containing the proton(s) and the intensity of the signals indicates the molar percentage of each component in the studied system. Recently, this technique demonstrated to be novel approach for the determination of glycidyl fatty acid esters, which are contaminants of refined oils and fats that can generate a carcinogenic molecule called glycidol during the digestion process [80]. The quantification formula developed relied on the signal expected for the two epoxy methylene (CH_2) protons at chemical shifts 2.56 and 2.76 ppm. This technique provides a new and very useful approach for the detection of GDE in edible oils (Figure 2.18).

Although ^{1}H-NMR is the most exploited technique due to ^{1}H nucleus abundance in an organic molecule, ^{31}P-NMR and ^{13}C-NMR are of increased interest because they can help solve specific problems in food science. For instance, ^{31}P-NMR can determine the phospholipid content in milk because phospholipids present a polar head group containing a phosphorus atom. Since phosphorus atom can be detected and quantified by ^{31}P-NMR, it becomes possible to quantify the amount and kind of phospholipids in a mixture. Figure 2.19 displays a ^{31}P-NMR spectrum of commercial lecithin in $CHCl_3/CH_3OH/H_2O$–EDTA (10/4/1). Accordingly, the signal corresponding to the phosphorus atom in different phospholipid classes can be distinguished as each displays different chemical shifts. The latter permits the quantification of phospholipids of a sample of interest.

One-dimensional ^{31}P-NMR is no doubt a powerful tool for quantitative and qualitative analyses of some simple phospholipids samples. In this method, standards need to be prepared and run to help assign the peaks to individual phospholipid species. It is of high cost firstly and also some metabolites do not even have a commercial standard. Thus, an automatic method for the

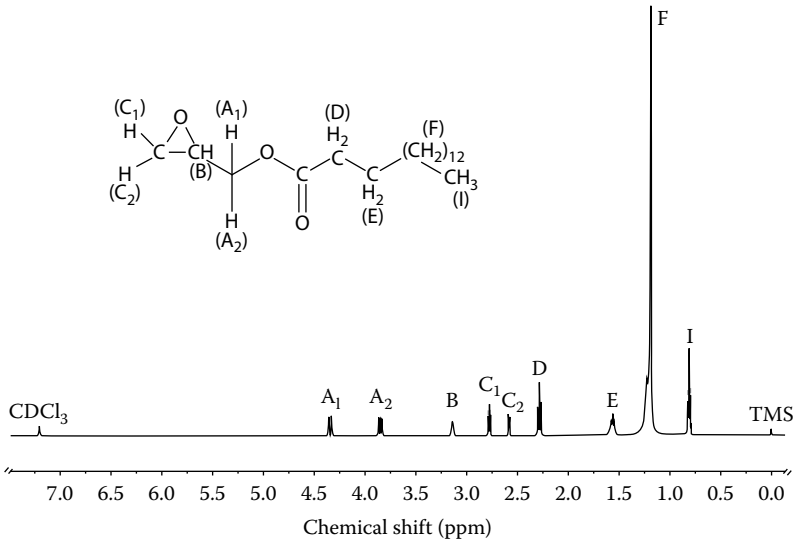


FIGURE 2.18 ^{1}H-NMR of a glycidyl palmitate. (From Song, Z. et al., *Eur. J. Lipid Sci. Technol*., 117, 1, 2015.)

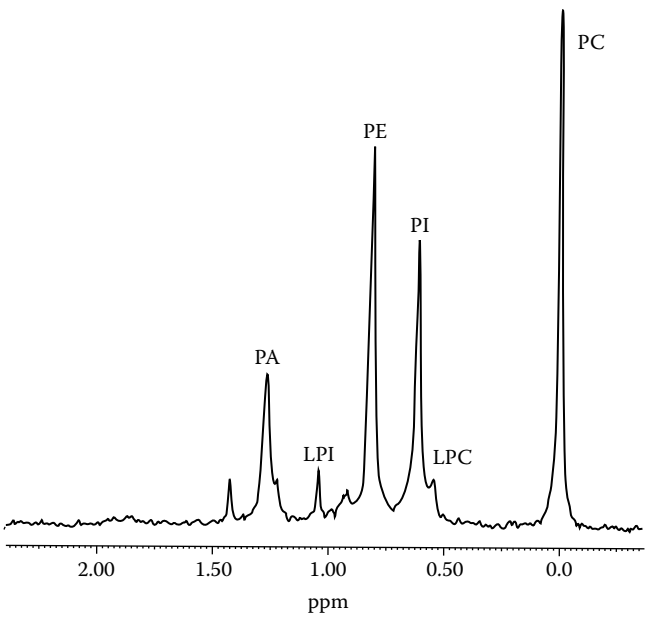


FIGURE 2.19 ^{31}P-NMR (121.5 MHz) of commercial lecithin in $CHCl_3/CH_3OH/H_2O$–EDTA (10/4/1).

identification and quantification of phospholipids in complex lipid mixtures from 2D ^{1}H-^{31}P NMR was developed [81]. In this method, ^{31}P intensities are used to quantify the phospholipid species of interest, while ^{1}H chemical shifts are used to assign the peaks. With the aid of this technique, no standards are needed and a global map of phospholipids can be obtained in a short time and with sufficient accuracy. Figure 2.20 shows the 2D NMR spectra of approximately 10 different ^{31}P signals in the range 0–2 ppm. The corresponding traces in the ^{1}H dimension typically show up to 10

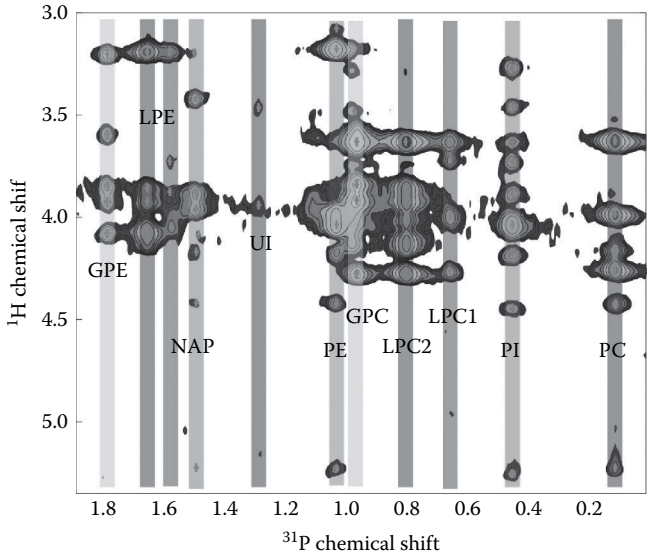


FIGURE 2.20 Contour plot of a 2D ^{1}H-^{31}P TOCSY spectrum of a lipid mixture with assignment of the different signals. The bands represent the lipids identified during the peak picking, and the individual peaks are highlighted by magenta dots. The width of the bands illustrates that the peak positions may fluctuate in the ^{31}P dimension because of their imperfect line shapes or overlap with neighboring peaks. Both the chemical shifts and the intensity distribution in the ^{1}H dimension are important observables for a reliable peak picking algorithm. The band labeled UI (unidentified) represents signals that do not match any entries in the database. LPC1, 1-lysophosphatidylcholine; PI, phosphatidylinositol; LPC2, 2-lysophosphatidylcholine; GPC, glycerophosphorylcholine; PE, phosphatidylethanolamine; NAP, N-acyl phosphatidylethanolamine; LPE, 1-lysophosphatidylethanolamine; GPE, glycerophosphorylethanolamine. (From Balsgart, N.M. et al., *Anal. Chem*., 88, 2170, 2016.)

resonances from the different hydrogen atoms in the vicinity of the phosphorus atoms in the lipids. With combination of all information, the individual phospholipid can be identified and quantified. This modern technique will attract more and more attention in phospholipid analysis.

2. Fourier Transform Infrared Spectroscopy

FTIR is a technique that can be used to elucidate the chemical structure of a lipid. Table 2.2 displays typical signals expected based on chemical structure. In addition, FTIR can be used for quantification. In fact, FTIR demonstrated to be useful to determine the total phospholipid content in vegetable oil. Nzai and Proctor [82] found a linear relationship between the concentration of phospholipids and the area of the band from 1200 to 970 cm^{-1}. Accordingly, they used phospholipid absorption bands between 1200 and 970 cm^{-1} (P−O−C + PO_2 chemical bonds) for quantification.

FTIR is a relatively rapid and reliable method that can potentially provide substantial savings in the fats and oils industry in terms of time and labor. Another advantage of this method is that only a very small amount of solvent or no solvent is needed to run the analysis. However, the main drawback of this method is the vibration due to interference from other nonphospholipid components. Additionally, only total phospholipid content can be obtained by using this method. Recently, Meng et al. [83] quantified the total phospholipid content in edible oils by in-vial solvent extraction coupled with FTIR analysis. They found that the second-derivative differential spectra were better than the differential spectra. Various bands were used to investigate the linear regression, and finally, the asymmetric phosphate diester PO_2^- stretching band at 1243 cm^{-1} in second-derivative spectra was regarded to be sufficient for the accurate measurement of total phospholipids with minimal co-extracted triacylglycerol interferences being encountered.

TABLE 2.2
Mid-Infrared Phospholipid Band Assignments

Wavenumber (cm^{-1})	Assignments
970	Asymmetric C–N stretching of $(CH_3)N^+$
1025	Symmetric ester C–O–P stretch
1068	Asymmetric ester C–O–P stretch in $C–O–PO_2^-$
1037	Symmetric ester C–O stretch in C=O–O–C
1173	Asymmetric C–O stretch in C=O–O–C
1090	Symmetric phosphate diester stretch PO_2^-
1243	Asymmetric phosphate diester PO_2^- stretch
1376	Symmetric CH_3 bending vibration
1451	δ CH_2 bending vibration
1740	C=O stretch

Source: Meng, X. et al., *J. Agric. Food Chem*., 62, 3101, 2014.

FTIR can also provide information regarding the molecular organization of lipid chains. This technique can be used to determine the interactions of the head groups and, consequently, yield information regarding the intra- and intermolecular interaction of the study system. FTIR spectra of a lipid sample can determine how well the lipid chains are packed in the system [84]. For instance, if the CH_2 scissoring is split in two bands, a doublet is expected between 1450 and 1500 cm^{-1} (Figure 2.21). The latter suggests the presence of a well-organized system forming orthorhombic packing. However, if no splitting is observed but instead the FTIR spectra display a singlet in the scissoring region, molecules are less organized forming a hexagonal packing. In addition, FTIR can also provide information regarding the stability of a system *versus* temperature. The latter can be measured by collecting the spectra of a specific sample at different temperatures, and when an

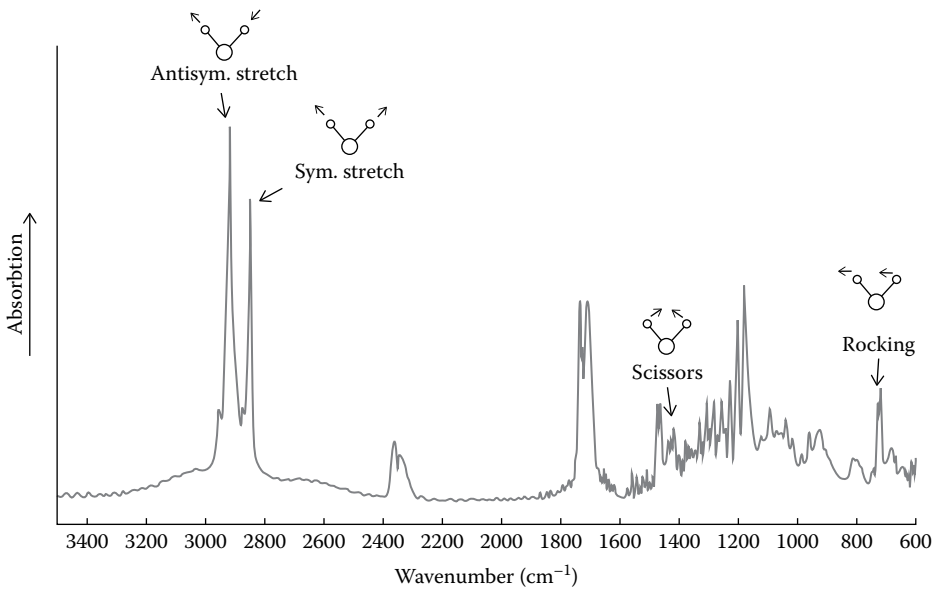


FIGURE 2.21 An example of a Fourier transform infrared (FTIR) spectrum.

increase of the frequency in the region between ~2850 and ~2920 cm^{-1} is registered, a transition from a more organized to a less organized system can be recorded.

3. X-Ray Diffraction

X-ray can be used to characterize the packing and phase behavior of lipids as it can provide information about the interaction of hydrocarbon chains [85]. One of the most used x-ray techniques is wide angle x-ray diffraction (WAXD) [86]. WAXD can detect electron density fluctuations and these fluctuations are related to the characteristic length of the crystalline structure by Bragg's law (Equation 2.1) where d represents the length, θ is the scattering angle, and λ is the wavelength of the beam. Accordingly, the shape and dimensions of the crystalline lattice are determined by the direction of the diffraction beam. This technique is sensitive to small structural changes, nondestructive, and can have high penetration in organic systems. X-ray techniques are of special importance for the characterization of lipids used to generate lipid nanoparticles for food application as the crystalline structure of these lipids will play a role in determining stability and digestibility of these nanosystems.

$$2d \sin \theta = \lambda \tag{2.1}$$

Other x-ray technique constitutes small-angle x-ray scattering (SAXS). The latter can provide information on the structure of complex food materials, for instance, hen-egg yolk and oil-in-water emulsions containing lipids and proteins. Recently, Reinke et al. [87] used microfocus SAXS to investigate the migration pathway of oil in different cocoa matrixes (Figure 2.22) as one of the

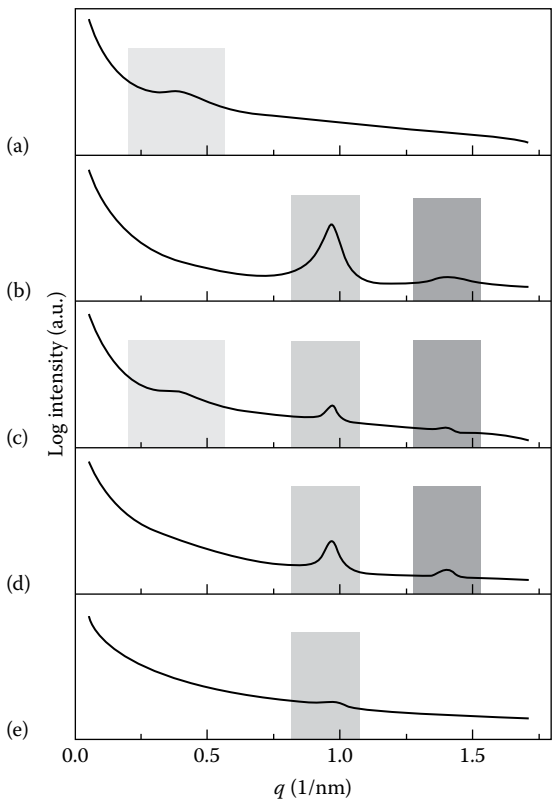


FIGURE 2.22 Microfocus small-angle x-ray scattering (SAXS) curves dry powders before migration of oil started. (a) Skim milk powder; (b) cocoa butter; (c) skim milk powder with cocoa butter; (d) sucrose with cocoa butter; (e) cocoa powder. (From Reinke, S.K. et al., *ACS Appl. Mater. Interfaces*, 7, 9929, 2015.)

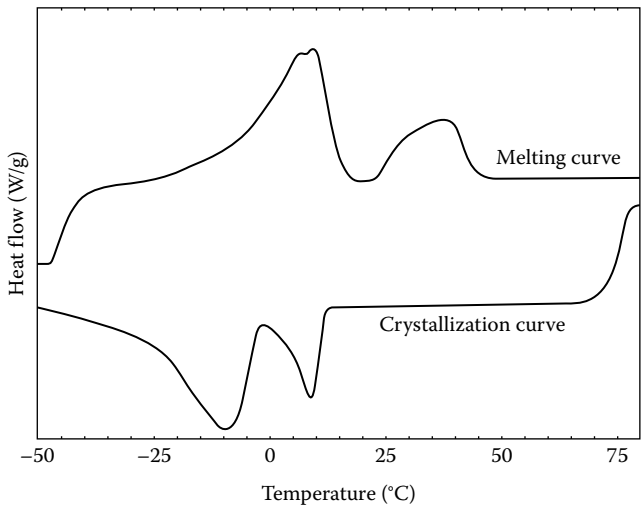


FIGURE 2.23 Differential scanning calorimetry (DSC) melting and crystallization curve of refined–bleached–deodorized palm oil. (From Tan, C.P. and Che Man, Y.B., *Food Chem*., 76, 89, 2002.)

major problems in chocolate production is the formation of white defects on the chocolate surface, also known as chocolate blooming [87]. Accordingly, the results suggested that the oil first migrates into the pores and crack and later chemical migration takes place through the fat phase prompting softening and partial dissolution of the crystalline cocoa butter. Therefore, this process could be prevented by decreasing porosity and defects in the chocolate matrix.

4. Differential Scanning Calorimetry

DSC provides information regarding lipids' molecular organization and polymorphism by cooling and heating of a lipid sample to break down the lipids' crystalline structure. It measures the thermal behavior of a sample, helping to establish the link between the temperature and the physical properties of a material. DSC can determine phase transitions, melting point, heat of fusion, oxidative stability, percent of crystallinity, and crystallization kinetics. Basically, energy is simultaneously applied to a sample cell (e.g., containing solid sample) and a reference cell (e.g., empty cell) and the temperature increased identically over time. The difference between the energy required for cells to be at the same temperature will be the amount of heat absorbed or released by the sample of interest. Since more energy is required to heat the sample cell to the same temperature as the reference cell, this can translate into heat excess [88]. Accordingly, the conformation of the lipid chains will determine the sharpness, position, and shape in DSC scan curve. Figure 2.23 displays the DSC melting and crystallization curves of refined–bleached–deodorized (RBD) palm oil [89].

IX. SUMMARY

Acylglycerols and phospholipids are molecules encountered in daily life; as such, understanding the chemical and physical features of these compounds is of high relevance to the scientific community. This chapter presented the chemical structure of different classes of lipids and phospholipids, and it discussed the most used chemical and enzymatic reactions to modify and obtain these compounds. It also addressed the role of phospholipids in membranes fluidity and explained the molecular structure of lipids and phospholipids and their self-assembled structures. It described the relevance of this self-assembly structures in food application such as in the case of nanotechnologies, which are generally used to encapsulate ingredients sensitive to oxidation, for example,

some essential polyunsaturated fatty acids. In addition, it presented different enzymatic and chemical approaches for the synthesis of lipids and phospholipids with desired physicochemical properties. Furthermore, the chapter covered the main techniques used for the separation, analysis, and characterization of lipids and phospholipids. Specifically, advantages and disadvantages of each analytical method are compared. For instance, although TLC is relatively inexpensive and straightforward to perform, it is not a good method for separation in large scale. In addition, lipids characterization involves the use of multiple techniques to elucidate the packing behavior, crystal structure, and stability of crystal. For example, though the use of NMR and FTIR provides information regarding the structural features of the lipids and can help to quantify lipid content, DSC and x-ray are the techniques most used to elucidate the packing behavior of these molecules. Overall, this chapter offered well-rounded background for the synthesis and characterization of relevant lipids for food application.

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3 Lipid-Based Emulsions and Emulsifiers

David Julian McClements

CONTENTS

I. INTRODUCTION

Many natural and processed foods exist either partly or wholly as emulsions or have been in an emulsified state at some time during their existence [1–5]. Milk is the most common example of a naturally occurring food emulsion [6]. Mayonnaise, salad dressing, cream, ice cream, butter, and margarine are all examples of manufactured food emulsions. Powdered coffee whiteners, sauces, and many desserts are examples of foods that were emulsions at one stage during their production but subsequently were converted into another form. The bulk physicochemical properties of food emulsions, such as appearance, texture, and stability, depend ultimately on the type of molecules the food contains and their interactions with one another. Food emulsions contain a variety of ingredients, including water, lipids, surfactants, proteins, carbohydrates, minerals, preservatives, colors, and flavors [3]. By a combination of covalent and physical interactions, these ingredients form the individual phases and structural components that give the final product its characteristic physicochemical properties [7]. It is the role of food scientists to untangle the complex relationship between the molecular, structural, and bulk properties of foods so that foods with improved properties can be created in a more systematic fashion.

II. EMULSIONS

An emulsion is a dispersion of droplets of one liquid in another liquid with which it is incompletely miscible [1,8]. In foods, the two immiscible liquids are oil and water. The diameter of the droplets in food emulsions is typically within the 100 nm to 100 μm range [2,3]. A system that consists of oil droplets dispersed in an aqueous phase is called an oil-in-water (O/W) emulsion. A system that consists of water droplets dispersed in an oil phase is called a water-in-oil (W/O) emulsion. The material that makes up the droplets in an emulsion is referred to as the *dispersed* or *internal phase*, whereas the material that makes up the surrounding liquid is called the *continuous* or *external phase*. Multiple emulsions can be prepared that consist of oil droplets contained in larger water droplets, which are themselves dispersed in an oil phase (O/W/O) or vice versa (W/O/W). Multiple emulsions can be used for protecting certain ingredients, for controlling the release of ingredients, or for creating low-fat products [9].

Emulsions are thermodynamically unstable systems because of the positive free energy required to increase the surface area between the oil and water phases [3]. The origin of this energy is the

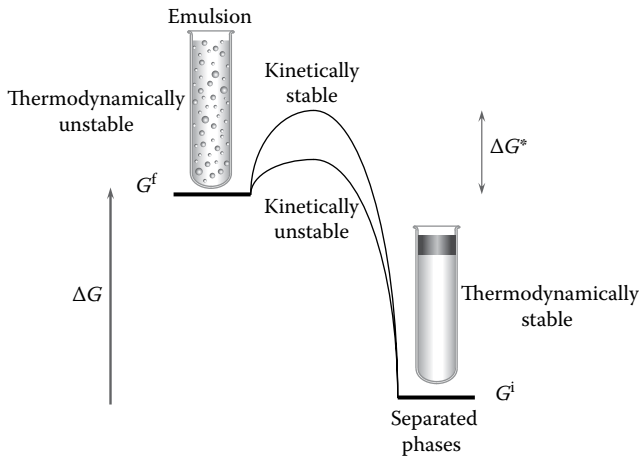

FIGURE 3.1 Emulsions are thermodynamically unstable systems that tend to revert back to the individual oil and water phases with time. To produce an emulsion, energy must be supplied.

unfavorable interaction between oil and water, which exists because water molecules are capable of forming strong hydrogen bonds with other water molecules but not with oil molecules [8,9]. Thus emulsions tend to reduce the surface area between the two immiscible liquids by separating into a system that consists of a layer of oil (lower density) on top of a layer of water (higher density). This is clearly seen if one tries to *homogenize* pure oil and pure water together: initially, an emulsion is formed, but after a few minutes phase separation occurs (Figure 3.1).

Emulsion instability can manifest itself through a variety of physicochemical mechanisms, including creaming, flocculation, coalescence, partial coalescence, Ostwald ripening, and phase inversion (Section VI). To form emulsions that are kinetically stable for a reasonable period (a few weeks, months, or even years), chemical substances known as *emulsifiers* must be added prior to homogenization. Emulsifiers are surface-active molecules that adsorb to the surface of freshly formed droplets during homogenization, forming a protective membrane that prevents the droplets from coming close enough together to aggregate [3]. Most food emulsifiers are *amphiphilic* molecules, that is, they have both polar and nonpolar regions on the same molecule. The most common types used in the food industry are lipid-based emulsifiers (small-molecule surfactants and phospholipids) and amphiphilic biopolymers (proteins and polysaccharides) [2,3]. In addition, some types of small solid particles are also surface active and can act as emulsifiers in foods, for example, granules from egg or mustard.

Most food emulsions are more complex than the simple three-component (oil, water, and emulsifier) system described earlier [3,5,9]. The aqueous phase may contain water-soluble ingredients of many different kinds, including sugars, salts, acids, bases, surfactants, proteins, polysaccharides, flavors, and preservatives [1]. The oil phase may contain a variety of lipid-soluble components, such as triacylglycerols, diacylglycerols, monoacylglycerols, fatty acids, vitamins, cholesterol, and flavors [1]. The interfacial region may be composed of surface-active components of a variety of types, including small-molecule surfactants, phospholipids, polysaccharides, and proteins. It should be noted that the composition of the interfacial region may evolve over time after an emulsion is produced, due to competitive adsorption with other surface-active substances or due to adsorption of oppositely charged substances, for example, polysaccharides [1a]. Some of the ingredients in food emulsions are not located exclusively in one phase but are distributed between the oil, water, and interfacial phases according to their partition coefficients. Despite having low concentrations, many of the minor components present in an emulsion can have a pronounced influence on its bulk physicochemical properties. For example, addition of small amounts (~few mM) of multivalent

mineral ions can destabilize an electrostatically stabilized emulsion [1]. Food emulsions may consist of oil droplets dispersed in an aqueous phase (e.g., mayonnaise, milk, cream, soups), or water droplets dispersed in an oil phase (e.g., margarine, butter, spreads). The droplets and/or the continuous phase may be fluid, gelled, crystalline, or glassy. The size of the droplets may vary from less than a micrometer to a few hundred micrometers, and the droplets themselves may be more or less polydisperse. In addition, many emulsions may contain air bubbles that have a pronounced influence on the sensory and physicochemical properties of the system, for example, ice cream, whipped cream, and desserts [1].

To complicate matters further, the properties of food emulsions are constantly changing with time because of the action of various chemical (e.g., lipid oxidation, biopolymer hydrolysis), physical (e.g., creaming, flocculation, coalescence), and biological (e.g., bacterial growth) processes. In addition, during their processing, storage, transport, and handling, food emulsions are subjected to variations in their temperature (e.g., via sterilization, cooking, chilling, freezing) and to various mechanical forces (e.g., stirring, mixing, whipping, flow through pipes, centrifugation high pressure) that alter their physicochemical properties. Despite the compositional, structural, and dynamic complexity of food emulsions, considerable progress has been made in understanding the major factors that determine their bulk physicochemical properties.

III. LIPID-BASED EMULSIFIERS

A. Molecular Characteristics

The most important types of lipid-based emulsifier used in the food industry are small-molecule surfactants (e.g., Tweens, Spans, monoglycerides, diglycerides, and modified fatty acids) and phospholipids (e.g., lecithin). The principal role of lipid-based emulsifiers in food emulsions is to enhance the formation and stability of the product; however, they may also alter the bulk physicochemical properties by interacting with proteins or polysaccharides or by modifying the structure of fat crystals [1,9]. All lipid-based emulsifiers are amphiphilic molecules that have a hydrophilic "head" group with a high affinity for water and lipophilic "tail" group with a high affinity for oil [1,8,10,11]. These emulsifiers can be represented by the formula RX, where X represents the hydrophilic head and R the lipophilic tail. Lipid-based emulsifiers differ with respect to type of head group and tail group. The head group may be anionic, cationic, zwitterionic, or nonionic. The lipid-based emulsifiers used in the food industry are mainly nonionic (e.g., monoacylglycerols, sucrose esters, Tweens, and Spans), anionic (e.g., fatty acids), or zwitterionic (e.g., lecithin). The tail group usually consists of one or more hydrocarbon chains, having between 10 and 20 carbon atoms per chain. The chains may be saturated or unsaturated, linear or branched, aliphatic and/or aromatic. Most lipid-based emulsifiers used in foods have either one or two linear aliphatic chains, which may be saturated or unsaturated. Each type of emulsifier has unique functional properties that depend on its chemical structure.

Lipid-based emulsifiers aggregate spontaneously in solution to form a variety of thermodynamically stable structures known as *association colloids* (e.g., micelles, bilayers, vesicles, reversed micelles) (Figure 3.2). These structural types are adopted because they minimize the unfavorable contact area between the nonpolar tails of the emulsifier molecules and water [10]. The type of association colloid formed depends principally on the polarity and molecular geometry of the emulsifier molecules (Section III.C.3). The forces holding association colloids together are relatively weak, and so they have highly dynamic and flexible structures [8]. Their size and shape are continually fluctuating, and individual emulsifier molecules rapidly exchange between the micelle and the surrounding liquid. The relative weakness of the forces holding association colloids together also means that their structures are particularly sensitive to changes in environmental conditions, such as temperature, pH, ionic strength, and ion type. Surfactant micelles are the most important type of association colloid formed in many food emulsions, and we focus principally on their properties.

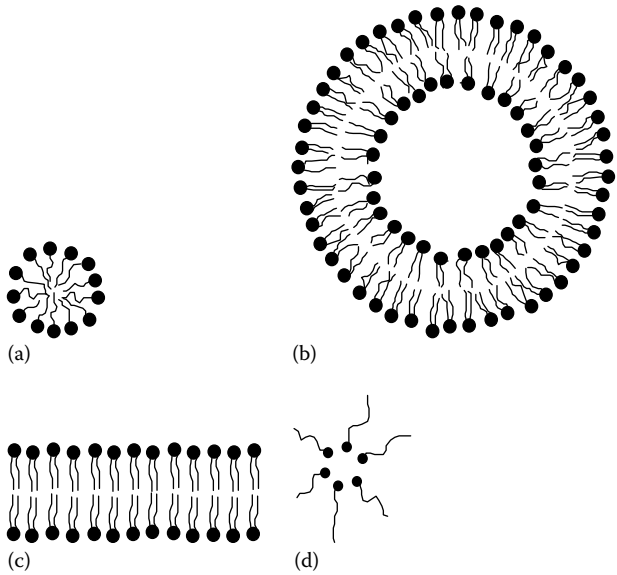

FIGURE 3.2 Association colloids formed by surfactant molecules: (a) micelles; (b) vesicles; (c) bilayers; and (d) reverse micelles.

B. Functional Properties

1. Critical Micelle Concentration

A surfactant forms micelles in an aqueous solution when its concentration exceeds some critical level, known as the *critical micelle concentration* (cmc). Below the cmc, surfactant molecules are dispersed predominantly as monomers, but once the cmc has been exceeded, any additional surfactant molecules form micelles, and the monomer concentration remains constant. Despite the highly dynamic nature of their structure, surfactant micelles do form particles that have a well-defined average size. Thus, when surfactant is added to a solution above the cmc, the number of micelles increases, rather than their size. When the cmc is exceeded, there is an abrupt change in the physicochemical properties of a surfactant solution (e.g., surface tension, electrical conductivity, turbidity, osmotic pressure) [12]. This is because the properties of surfactant molecules dispersed as monomers are different from those in micelles. For example, surfactant monomers are amphiphilic and have a high surface activity, whereas micelles have little surface activity because their surface is covered with hydrophilic head groups. Consequently, the surface tension of a solution decreases with increasing surfactant concentration below the cmc but remains fairly constant above it.

2. Cloud Point

When a surfactant solution is heated above a certain temperature, known as the *cloud point*, it becomes turbid. As the temperature is raised, the hydrophilic head groups become increasingly dehydrated, which causes the emulsifier molecules to aggregate. These aggregates are large enough to scatter light, and so the solution appears turbid. At temperatures above the cloud point, the aggregates grow so large that they sediment under the influence of gravity and form a separate phase. The cloud point increases as the hydrophobicity of a surfactant molecule increases; that is, the length of its hydrocarbon tail increases or the size of its hydrophilic head group decreases [13,14].

3. Solubilization

Nonpolar molecules, which are normally insoluble or only sparingly soluble in water, can be solubilized in an aqueous surfactant solution by incorporation into micelles or other types of

association colloid [9]. The resulting system is thermodynamically stable; however, equilibrium may take an appreciable time to achieve because of the activation energy associated with transferring a nonpolar molecule from a bulk phase to a micelle. Micelles containing solubilized materials are referred to as *swollen micelles* or *microemulsions*, whereas the material solubilized within the micelle is referred to as the *solubilizate*. The ability of micellar solutions to solubilize nonpolar molecules has a number of potentially important applications in the food industry, including selective extraction of nonpolar molecules from oils, controlled ingredient release, incorporation of nonpolar substances into aqueous solutions, transport of nonpolar molecules across aqueous membranes, and modification of chemical reactions [9]. Three important factors determine the functional properties of swollen micellar solutions: the location of the solubilizate within the micelles, the maximum amount of material that can be solubilized per unit mass of surfactant, and the rate at which solubilization proceeds [9].

4. Surface Activity and Droplet Stabilization

Lipid-based emulsifiers are used widely in the food industry to enhance the formation and stability of food emulsions. To do this, they must adsorb to the surface of emulsion droplets during homogenization and form a protective membrane that prevents the droplets from aggregating with each other [1]. Emulsifier molecules adsorb to oil–water interfaces because they can adopt an orientation in which the hydrophilic part of the molecule is located in the water, while the hydrophobic part is located in the oil. This minimizes the unfavorable free energy associated with the contact of hydrophilic and hydrophobic regions and therefore reduces the interfacial tension. This reduction in interfacial tension is important because it facilitates the further disruption of emulsion droplets; that is, less energy is needed to break up a droplet when the interfacial tension is lowered.

Once adsorbed to the surface of a droplet, the emulsifier must provide a repulsive force that is strong enough to prevent the droplet from aggregating with its neighbors. Ionic surfactants provide stability by causing all the emulsion droplets to have the same electric charge, hence to repel each other electrostatically. Nonionic surfactants provide stability by generating a number of short-range repulsive forces (e.g., steric overlap, hydration, thermal fluctuation interactions) that prevent the droplets from getting too close together [1,11]. Some emulsifiers form multilayers (rather than monolayers) at the surface of an emulsion droplet, which greatly enhances the stability of the droplets against aggregation.

In summary, emulsifiers must have three characteristics to be effective. First, they must rapidly adsorb to the surface of the freshly formed emulsion droplets during homogenization. Second, they must reduce the interfacial tension by a significant amount. Third, they must form a membrane that prevents the droplets from aggregating.

C. Ingredient Selection

A large number of different types of lipid-based emulsifier can be used as food ingredients, and a manufacturer must select the one that is most suitable for each particular product. Suitability, in turn, depends on factors such as an emulsifier's legal status as a food ingredient; its cost and availability, the consistency in its properties from batch to batch, its ease of handling and dispersion, its shelf life, its compatibility with other ingredients, and the processing, storage, and handling conditions it will experience, as well as the expected shelf life and physicochemical properties of the final product.

How does a food manufacturer decide which emulsifier is most suitable for a product? There have been various attempts to develop classification systems that can be used to select the most appropriate emulsifier for a particular application. Classification schemes have been developed that are based on an emulsifier's solubility in oil and/or water (Bancroft's rule), its ratio of hydrophilic to lipophilic groups (HLB number) [15,16], and its molecular geometry [17]. Ultimately, all of these properties depend on the chemical structure of the emulsifier, and so all the different classification schemes are closely related.

1. Bancroft's Rule

One of the first empirical rules developed to describe the type of emulsion that could be stabilized by a given emulsifier was proposed by Bancroft. Bancroft's rule states that the phase in which the emulsifier is most soluble will form the continuous phase of an emulsion. Hence, a water-soluble emulsifier will stabilize oil-in-water emulsions, whereas an oil-soluble emulsifier will stabilize water-in-oil emulsions.

2. Hydrophile–Lipophile Balance

The hydrophile–lipophile balance (HLB) concept underlies a semiempirical method for selecting an appropriate emulsifier or combination of emulsifiers to stabilize an emulsion. The HLB is described by a number, which gives an indication of the overall affinity of an emulsifier for the oil and/or aqueous phases [12]. Each emulsifier is assigned an HLB number according to its chemical structure. A molecule with a high HLB number has a high ratio of hydrophilic groups to lipophilic groups and vice versa. The HLB number of an emulsifier can be calculated from a knowledge of the number and type of hydrophilic and lipophilic groups it contains, or it can be estimated from experimental measurements of its cloud point. The HLB numbers of many emulsifiers have been tabulated in the literature [15,16]. A widely used semiempirical method of calculating the HLB number of a lipid-based emulsifier is as follows:

$$\text{HLB} = 7 + \Sigma(\text{Hydrophilic group numbers}) - \Sigma(\text{Lipophilic group numbers}) \tag{3.1}$$

As indicated in Table 3.1 [18], group numbers have been assigned to hydrophilic and lipophilic groups of many types. The sums of the group numbers of all the lipophilic groups and of all the hydrophilic groups are substituted into Equation 3.1, and the HLB number is calculated. The semiempirical equation mentioned earlier has been found to have a firm thermodynamic basis, with the sums corresponding to the free energy changes in the hydrophilic and lipophilic parts of the molecule when micelles are formed.

The HLB number of an emulsifier gives a useful indication of its solubility in the oil and/or water phases, and it can be used to predict the type of emulsion that will be formed. An emulsifier with a low HLB number (4–6) is predominantly hydrophobic, dissolves preferentially in oil, stabilizes water-in-oil emulsions, and forms reversed micelles in oil. An emulsifier with a high HLB number (8–18) is predominantly hydrophilic, dissolves preferentially in water, stabilizes oil-in-water emulsions, and forms micelles in water. An emulsifier with an intermediate HLB number (6–8) has no particular preference for either oil or water. Nonionic molecules with HLB numbers below 4 and above 18 are less surface active and are therefore less likely to preferentially accumulate at an oil–water interface.

Emulsion droplets are particularly prone to coalescence when they are stabilized by emulsifiers that have extreme or intermediate HLB numbers. At very high or very low HLB numbers, a nonionic

TABLE 3.1
Selected HLB Group Numbers

Hydrophilic Group	Group Number	Lipophilic Group	Group Number
$-SO_4NA^+$	38.7	—CH—	0.475
$-COO^-H^+$	21.2	$-CH_2-$	0.475
Tertiary amine	9.4	$-CH_3-$	0.475
Sorbitan ring	6.8		
—COOH	2.1		
—O—	1.3		

Source: Adapted from Davis, H.T., *Colloids Surf. A*, 91, 9, 1994.

emulsifier has such a low surface activity that it does not accumulate appreciably at the droplet surface and therefore does not provide protection against coalescence. At intermediate HLB numbers (6–8), emulsions are unstable to coalescence because the interfacial tension is so low that very little energy is required to disrupt the membrane. Maximum stability of emulsions is obtained for oil-in-water emulsions using an emulsifier with an HLB number around 10–12 and for water-in-oil emulsions around 3–5. This is because the emulsifiers are sufficiently surface active but do not lower the interfacial tension so much that the droplets are easily disrupted. It is possible to adjust the effective HLB number by using a combination of two or more emulsifiers with different HLB numbers.

One of the major drawbacks of the HLB concept is its failure to account for the significant alterations in the functional properties of an emulsifier molecule that result from changes in temperature or solution conditions, even though the chemical structure of the molecule does not change. Thus, an emulsifier may be capable of stabilizing oil-in-water emulsions at one temperature but water-in-oil emulsions at another temperature. For this reason, a new system referred to as the hydrophile–lipophile deviation (HLD) method has been developed to account for environmental conditions and water-to-oil ratio on surfactant performance.

3. Molecular Geometry and Phase Inversion Temperature

The molecular geometry of an emulsifier molecule is described by a packing parameter p (see Figure 3.3) as follows:

$$p = \frac{\upsilon}{la_0} \tag{3.2}$$

where

υ and l are the volume and length of the hydrophobic tail

a_0 is the cross-sectional area of the hydrophilic head group

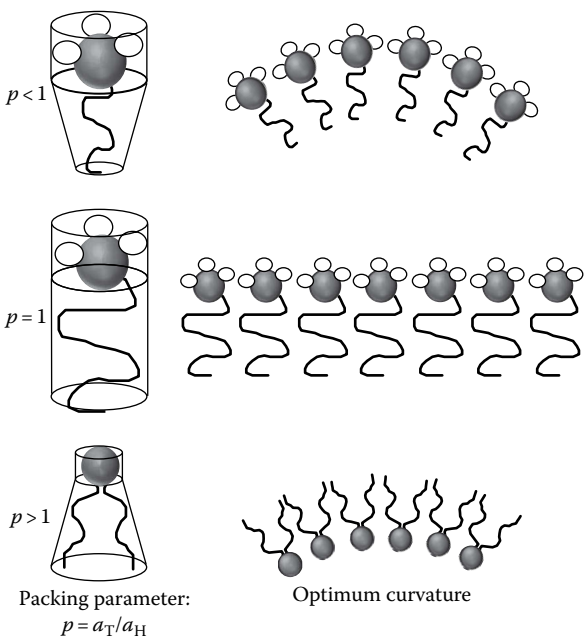

FIGURE 3.3 Relationship between the molecular geometry of surfactant molecules and their optimum curvature.

When surfactant molecules associate with each other, they tend to form monolayers having a curvature that allows the most efficient packing of the molecules. At this *optimum curvature*, the monolayer has its lowest free energy, and any deviation from this curvature requires the expenditure of energy [8,11]. The optimum curvature of a monolayer depends on the packing parameter of the emulsifier: for $p = 1$, monolayers with zero curvature are preferred; for $p < 1$, the optimum curvature is convex; and for $p > 1$, the optimum curvature is concave (Figure 3.3). Simple geometrical considerations indicate that spherical micelles are formed when p is less than 0.33, nonspherical micelles when p is between 0.33 and 0.5, and bilayers when p is between 0.5 and 1 [11]. Above a certain concentration, bilayers join up to form vesicles because energetically unfavorable end effects are eliminated. At values of p greater than 1, reversed micelles are formed, in which the hydrophilic head groups are located in the interior (away from the oil), and the hydrophobic tail groups are located at the exterior (in contact with the oil) (Figure 3.2). The packing parameter therefore gives a useful indication of the type of association colloid that is formed by an emulsifier molecule in solution.

The packing parameter is also useful because it accounts for the temperature dependence of the physicochemical properties of surfactant solutions and emulsions. The temperature at which an emulsifier solution converts from a micellar to a reversed micellar system or an oil-in-water emulsion converts to a water-in-oil emulsion is known as the phase inversion temperature (PIT). Consider what happens when an emulsion that is stabilized by a lipid-based emulsifier is heated (Figure 3.4). At temperatures well below the PIT (≈20°C), the packing parameter is significantly less than unity, and

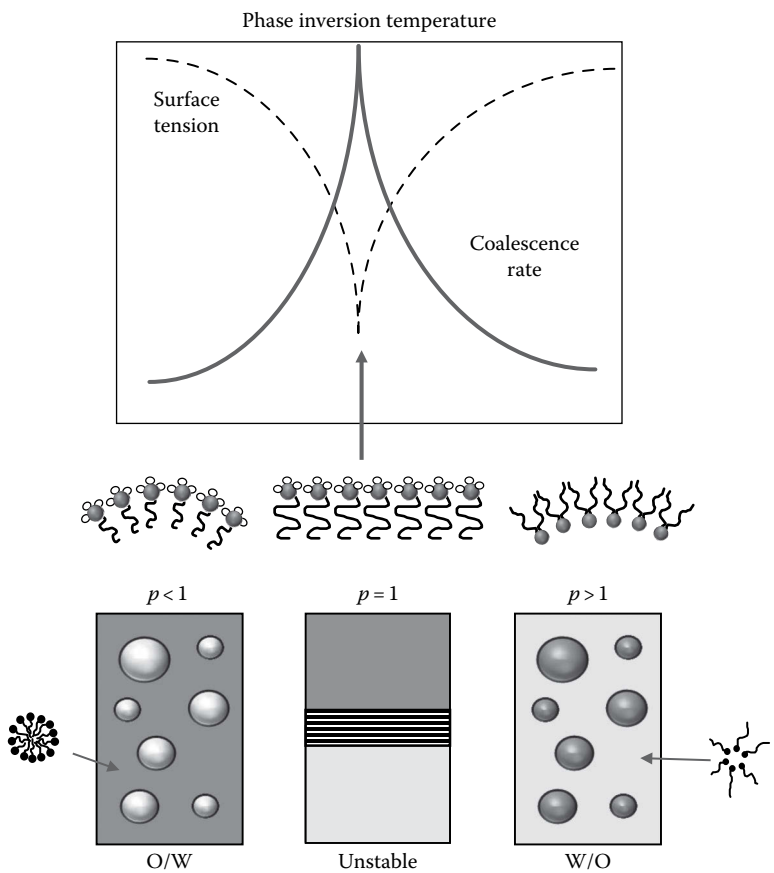

FIGURE 3.4 Phase inversion temperature in emulsions. An oil-in-water emulsion may invert to a water-in-oil emulsion upon heating due to dehydration of the surfactant head groups changing the optimum curvature.

so a system that consists of oil-in-water emulsion in equilibrium with a swollen micellar solution is favored. As the temperature is raised, the hydrophilic head groups of the emulsifier molecules become increasingly dehydrated, which causes p to increase toward unity. Thus, the emulsion droplets become more prone to coalescence and the swollen micelles grow in size. At PIT, $p \approx 1$, and the emulsion breaks down because the droplets have an ultralow interfacial tension and therefore readily coalesce with each other. The resulting system consists of excess oil and excess water (containing some emulsifier monomers), separated by a third phase that contains emulsifier molecules aggregated into bilayer structures. At temperatures sufficiently greater than the PIT, the packing parameter is much larger than unity, and the formation of a system that consists of a water-in-oil emulsion in equilibrium with swollen reversed micelles is favored. A further increase in temperature leads to a decrease in the size of the reversed micelles and in the amount of water solubilized within them. The method of categorizing emulsifier molecules according to their molecular geometry is now widely accepted as the most useful means of determining the types of emulsion they tend to stabilize [17].

4. Other Factors

The classification schemes mentioned earlier provide information about the type of emulsion an emulsifier tends to stabilize (i.e., O/W or W/O), but they do not provide much insight into the size of the droplets that form during homogenization or the stability of the emulsion droplets once formed [1]. In choosing a suitable emulsifier for a particular application, these factors must also be considered. The speed at which an emulsifier adsorbs to the surface of the emulsion droplets produced during homogenization determines the minimum droplet size that can be produced: the faster the adsorption rate, the smaller the size. The magnitude and range of the repulsive forces generated by a membrane, and its viscoelasticity, determine the stability of the droplets to aggregation.

IV. BIOPOLYMERS

Proteins and polysaccharides are the two most important biopolymers used as functional ingredients in food emulsions. These biopolymers are used principally for their ability to stabilize emulsions, enhance viscosity, and form gels.

A. Molecular Characteristics

Molecular characteristics of biopolymers, such as molecular weight, conformation, flexibility, and polarity, ultimately determine the properties of biopolymer solutions. These characteristics are determined by the type, number, and sequence of monomers that make up the polymer. Proteins are polymers of amino acids [19], whereas polysaccharides are polymers of monosaccharides [20]. The 3D structures of biopolymers in aqueous solution can be categorized as globular, fibrous, or random coil (Figure 3.5). Globular biopolymers have fairly rigid compact structures; fibrous

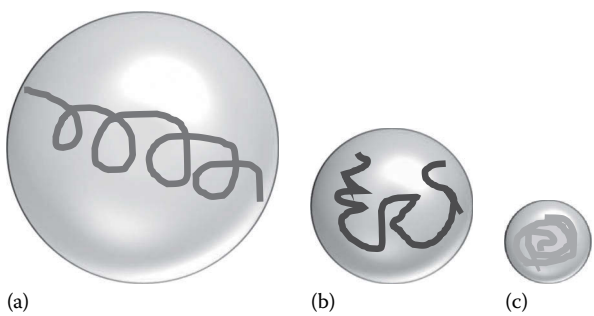

FIGURE 3.5 Typical molecular conformations adopted by biopolymers in aqueous solution: (a) rigid-rod; (b) random coil; (c) globular.

biopolymers have fairly rigid, rodlike structures; and random coil biopolymers have highly dynamic and flexible structures. Biopolymers can also be classified according to the degree of branching of the chain. Most proteins have linear chains, whereas polysaccharides can have either linear (e.g., amylose) or branched (e.g., amylopectin) chains.

The conformation of a biopolymer in solution depends on the relative magnitude of the various types of attractive and repulsive interaction that occur within and between molecules, as well as the configurational entropy of the molecule. Biopolymers that have substantial proportions of nonpolar groups tend to fold into globular structures in which the nonpolar groups are located in the interior (away from the water) and the polar groups are located at the exterior (in contact with the water) because this arrangement minimizes the number of unfavorable contacts between hydrophobic regions and water. However, since stereochemical constraints and the influence of other types of molecular interaction usually make it impossible for all the nonpolar groups to be located in the interior, the surfaces of globular biopolymers have some hydrophobic character. Many kinds of food protein have compact globular structures, including β-lactoglobulin, α-lactalbumin, and bovine serum albumin [6]. Biopolymers that contain a high proportion of polar monomers, distributed fairly evenly along their backbone, often have rodlike conformations with substantial amounts of helical structure stabilized by hydrogen bonding. Such biopolymers (e.g., collagen, cellulose) usually have low water solubilities because they tend to associate strongly with each other rather than with water; consequently, they often have poor functional properties. However, if the chains are branched, the molecules may be prevented from getting close enough together to aggregate, and so they may exist in solution as individual molecules. Predominantly polar biopolymers containing monomers that are incompatible with helix formation (e.g., β-casein) tend to form random coil structures.

In practice, biopolymers may have some regions along their backbone that have one type of conformation and others that have a different conformation. Biopolymers may also exist as isolated molecules or as aggregates in solution, depending on the relative magnitude of the biopolymer–biopolymer, biopolymer–solvent, and solvent–solvent interactions. Biopolymers are also capable of undergoing transitions from one type of conformation to another in response to environmental changes such as alterations in their pH, ionic strength, solvent composition, and temperature. Examples include helix ⇔ random coil and globular ⇔ random coil. In many food biopolymers, this type of transition plays an important role in determining the functional properties (e.g., gelation).

B. Functional Properties

1. Emulsification

Biopolymers that have a high proportion of nonpolar groups tend to be surface active, that is, they can accumulate at oil–water interfaces [1–4]. The major driving force for adsorption is the hydrophobic effect. When the biopolymer is dispersed in an aqueous phase, some of the nonpolar groups are in contact with water, which is a thermodynamically unfavorable condition. By adsorbing to an interface, the biopolymer can adopt a conformation of nonpolar groups in contact with the oil phase (away from the water) and hydrophilic groups located in the aqueous phase (in contact with the water). In addition, adsorption reduces the number of contacts between the oil and water molecules at the interface, thereby reducing the interfacial tension. The conformation a biopolymer adopts at an oil–water interface, and the physicochemical properties of the membrane formed, depend on its molecular structure. Flexible random coil biopolymers adopt an arrangement in which the predominantly nonpolar segments protrude into the oil phase, the predominantly polar segments protrude into the aqueous phase, and the neutral regions lie flat against the interface (Figure 3.6, left). The membranes formed by molecules of these types tend to have relatively open structures, to be relatively thick, and to have low viscoelasticities. Globular biopolymers (usually proteins) adsorb to an interface so that the predominantly nonpolar regions on their surface face the oil phase; thus, they tend to have a definite orientation at an interface (Figure 3.6, right). Once they have adsorbed to an

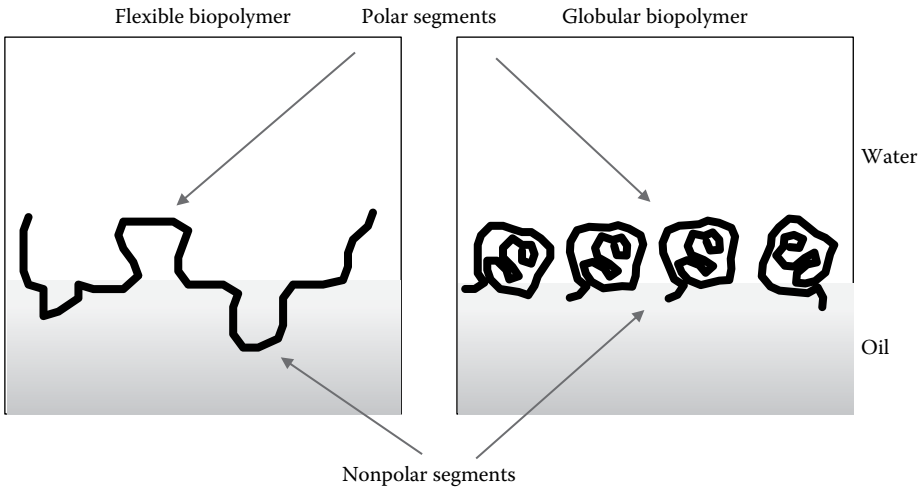

FIGURE 3.6 The conformation of biopolymers at oil–water interfaces depends on their molecular structure and flexibility. Biopolymers adopt a conformation where polar groups extend into water and nonpolar groups extend into oil.

interface, biopolymers often undergo structural rearrangements that permit them to maximize the number of contacts between nonpolar groups and oil [4].

Random coil biopolymers have flexible conformations and therefore rearrange their structures rapidly, whereas globular biopolymers are more rigid and therefore unfold more slowly. The unfolding of a globular protein at an interface often exposes amino acids that were originally located in the hydrophobic interior of the molecule, which can lead to enhanced interactions with neighboring protein molecules through hydrophobic attraction or disulfide bond formation. Consequently, globular proteins tend to form relatively thin and compact membranes, high in viscoelasticity. Thus, membranes formed from globular proteins tend to be more resistant to rupture than those formed from random coil proteins [3].

To be effective emulsifiers, biopolymers must rapidly adsorb to the surface of the emulsion droplets formed during homogenization and provide a membrane that prevents the droplets from aggregating. Biopolymer membranes can stabilize emulsion droplets against aggregation by a number of different physical mechanisms [1]. All biopolymers are capable of providing short-range steric repulsive forces that are usually strong enough to prevent droplets from getting sufficiently close together to coalesce. If the membrane is sufficiently thick, it can also prevent droplets from flocculating. Otherwise, it must be electrically charged so that it can prevent flocculation by electrostatic repulsion. The properties of emulsions stabilized by charged biopolymers are particularly sensitive to the pH and ionic strength of aqueous solutions [1a]. At pH values near the isoelectric point of proteins, or at high ionic strengths, the electrostatic repulsion between droplets may not be large enough to prevent the droplets from aggregating (see Section VI.A.5).

Proteins are commonly used as emulsifiers in foods because many of them naturally have a high proportion of nonpolar groups. Most polysaccharides are so hydrophilic that they are not surface active. However, a small number of naturally occurring polysaccharides have some hydrophobic character (e.g., gum arabic) or have been chemically modified to introduce nonpolar groups (e.g., some hydrophobically modified starches), and these biopolymers can be used as emulsifiers.

2. Thickening and Stabilization

The second major role of biopolymers in food emulsions is to increase the viscosity of the aqueous phase [1a]. This modifies the texture and mouthfeel of the food product ("thickening"), as well as

reducing the rate at which particles sediment or cream ("stabilization"). Both proteins and polysaccharides can be used as thickening agents, but polysaccharides are usually preferred because they can be used at much lower concentrations. The biopolymers used to increase the viscosity of aqueous solutions are usually highly hydrated and extended molecules or molecular aggregates. Their ability to increase the viscosity depends principally on their molecular weight, degree of branching, conformation, and flexibility. The viscosity of a dilute solution of particles increases as the concentration of particles increases [3]:

$$\eta = \eta_0(1 + 2.5\phi) \tag{3.3}$$

where
- η is the viscosity of the solution
- η_0 is the viscosity of the pure solvent
- ϕ is the volume fraction of particles in solution

Biopolymers are able to enhance the viscosity of aqueous solutions at low concentrations because they have an *effective* volume fraction that is much greater than their actual volume fraction [1a]. A biopolymer rapidly rotates in solution because of its thermal energy, and so it sweeps out a spherical volume of water that has a diameter approximately equal to the end-to-end length of the molecule (Figure 3.7). The volume of the biopolymer molecule is only a small fraction of the total volume of the sphere swept out, and so the effective volume fraction of a biopolymer is much greater than its actual volume fraction. Consequently, small concentrations of biopolymer can dramatically increase the viscosity of a solution (Equation 3.3). The effectiveness of a biopolymer at increasing the viscosity increases as the volume fraction it occupies within the sphere it sweeps out decreases. Thus large, highly extended linear biopolymers increase the viscosity more effectively than small compact or branched biopolymers.

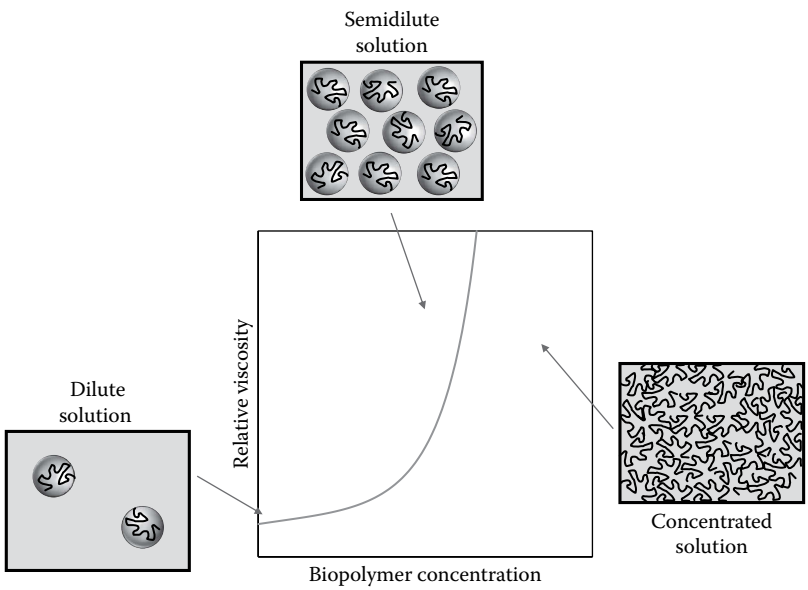

FIGURE 3.7 Extended biopolymers in aqueous solutions sweep out a large volume of water as they rotate, which increases their effective volume fraction and therefore their viscosity. The viscosity of an aqueous biopolymer solution increases with increasing biopolymer concentration.

In a dilute biopolymer solution, the individual molecules (or aggregates) do not interact with each other. When the concentration of biopolymer increases above some critical value c^*, the viscosity increases rapidly because the spheres swept out by the biopolymers overlap with each another. This type of solution is known as a *semidilute* solution, because even though the molecules are interacting with one another, each individual biopolymer is still largely surrounded by solvent molecules. At still higher polymer concentrations, the molecules pack so close together that they become entangled, and the system has more gel-like characteristics. Biopolymers that are used to thicken the aqueous phase of emulsions are often used in the semidilute concentration range [3].

Solutions containing extended biopolymers often exhibit strong shear-thinning behavior; that is, their apparent viscosity decreases with increasing shear stress. Some biopolymer solutions even have a characteristic yield stress. When a stress is applied below the yield stress, the solution acts like an elastic solid, but when it exceeds the yield stress the solution acts like a liquid. Shear thinning tends to occur because the biopolymer molecules become aligned with the shear field, or because the weak physical interactions responsible for biopolymer–biopolymer interactions are disrupted. The characteristic rheological behavior of biopolymer solutions plays an important role in determining their functional properties in food emulsions. For example, a salad dressing must be able to flow when it is poured from a container but must maintain its shape under its own weight after it has been poured onto a salad. The amount and type of biopolymer used must therefore be carefully selected to provide a low viscosity when the salad dressing is poured (high applied stress), but a high viscosity when the salad dressing is allowed to sit under its own weight (low applied stress).

The viscosity of biopolymer solutions is also related to the mouthfeel of a food product. Liquids that do not exhibit extensive shear-thinning behavior at the shear stresses experienced in the mouth are perceived as being "slimy." On the other hand, a certain amount of viscosity is needed to contribute to the "creaminess" of a product.

The shear-thinning behavior of biopolymer solutions is also important for determining the stability of food emulsions to creaming [1a]. As oil droplets move through an emulsion, they exert very small shear stresses on the surrounding liquid. Consequently, they experience a very large viscosity, which greatly slows down the rate at which they cream and therefore enhances stability. Many biopolymer solutions also exhibit thixotropic behavior (i.e., their viscosity decreases with time when they are sheared at a constant rate) as a result of disruption of the weak physical interactions that cause biopolymer molecules to aggregate. A food manufacturer must therefore select an appropriate biopolymer or combination of biopolymers to produce a final product that has a desirable mouthfeel and texture.

3. Gelation

Biopolymers are used as functional ingredients in many food emulsions (e.g., yogurt, cheese, desserts, egg, and meat products) because of their ability to cause the aqueous phase to gel [1a]. Gel formation imparts desirable textural and sensory attributes, as well as preventing the droplets from creaming. A biopolymer gel consists of a 3D network of aggregated or entangled biopolymers that entraps a large volume of water, giving the whole structure some solid-like characteristics. The appearance, texture, water-holding capacity, reversibility, and gelation temperature of biopolymer gels depend on the type, structure, and interactions of the molecules they contain.

Gels may be transparent or opaque, hard or soft, brittle or rubbery, homogeneous or heterogeneous; they may exhibit syneresis or have good water-holding capacity. Gelation may be induced by a variety of different methods, including altering the temperature, pH, ionic strength, or solvent quality; adding enzymes; and increasing the biopolymer concentration. Biopolymers may be cross-linked by covalent and/or noncovalent bonds.

It is convenient to distinguish between two types of gel: particulate and filamentous (Figure 3.8). Particulate gels consist of biopolymer aggregates (particles or clumps) that are assembled together to form a 3D network. This type of gel tends to be formed when there