# 4 Lipids

*D. Julian McClements and Eric A. Decker*

## CONTENTS

4.1 Introduction ... 156
4.2 Major Lipid Components ... 157
4.2.1 Fatty Acids ... 157
4.2.1.1 Nomenclature of Saturated Fatty Acids ... 157
4.2.1.2 Nomenclature of Unsaturated Fatty Acids ... 157
4.2.2 Acylglycerols ... 159
4.2.2.1 Composition of Fats ... 160
4.2.3 Phospholipids ... 162
4.2.4 Sphingolipids ... 163
4.2.5 Sterols ... 163
4.2.6 Waxes ... 164
4.2.7 Miscellaneous Lipids ... 164
4.3 Physicochemical Properties of Lipids ... 164
4.3.1 Physical Properties of Triacylglycerols ... 166
4.3.1.1 Rheological Properties ... 166
4.3.1.2 Density ... 167
4.3.2 Crystallization and Melting of Food Lipids ... 169
4.3.3 Physicochemical Mechanism of Lipid-Phase Transitions ... 171
4.3.3.1 Supercooling ... 172
4.3.3.2 Nucleation ... 172
4.3.3.3 Crystal Growth ... 175
4.3.3.4 Postcrystallization Events ... 176
4.3.4 Crystal Structure ... 176
4.3.4.1 Morphology ... 176
4.3.4.2 Polymorphism ... 176
4.4 Lipid Processing: Isolation, Purification, and Modification ... 178
4.4.1 Lipid Refining ... 178
4.4.1.1 Degumming ... 179
4.4.1.2 Neutralization ... 179
4.4.1.3 Bleaching ... 179
4.4.1.4 Deodorization ... 179
4.4.2 Altering the SFC of Food Lipids ... 179
4.4.2.1 Blending ... 180
4.4.2.2 Dietary Interventions ... 180
4.4.2.3 Genetic Manipulation ... 180
4.4.2.4 Fractionation ... 180
4.4.2.5 Hydrogenation ... 180
4.4.2.6 Interesterification ... 182

4.5 Functionality of Triacylglycerols in Foods ... 184
4.5.1 Texture ... 184
4.5.2 Appearance ... 185
4.5.3 Flavor ... 186
4.6 Chemical Deterioration of Lipids: Hydrolytic Reactions ... 186
4.7 Chemical Deterioration of Lipids: Oxidative Reactions ... 186
4.7.1 Mechanisms of Lipid Oxidation ... 187
4.7.2 Prooxidants ... 190
4.7.2.1 Prooxidants That Promote Formation of Lipid Hydroperoxides ... 191
4.7.2.2 Prooxidants That Promote Formation of Free Radicals ... 194
4.7.2.3 Prooxidants That Promote Decomposition of Hydroperoxides ... 194
4.7.3 Formation of Lipid Oxidation Decomposition Products ... 195
4.7.3.1 $\beta$-Scission Reaction ... 196
4.7.3.2 Additional Reactions of Fatty Acid Decomposition Products ... 198
4.7.3.3 Cholesterol Oxidation ... 198
4.7.4 Antioxidants ... 198
4.7.4.1 Control of Free Radicals ... 198
4.7.4.2 Control of Prooxidants ... 204
4.7.4.3 Control of Oxidation Intermediates ... 205
4.7.4.4 Antioxidant Interactions ... 205
4.7.4.5 Physical Location of Antioxidants ... 206
4.7.5 Other Factors Influencing Lipid Oxidation Rates ... 206
4.7.6 Measurement of Lipid Oxidation ... 207
4.7.6.1 Sensory Analysis ... 207
4.7.6.2 Primary Lipid Oxidation Products ... 207
4.7.6.3 Secondary Lipid Oxidation Products ... 208
4.8 Food Lipids and Health ... 209
4.8.1 Bioactivity of Fatty Acids ... 209
4.8.1.1 *Trans* Fatty Acids ... 210
4.8.1.2 $\omega$-3 Fatty Acids ... 210
4.8.1.3 Conjugated Linoleic Acid ... 211
4.8.1.4 Phytosterols ... 211
4.8.1.5 Carotenoids ... 211
4.8.2 Low Calorie Lipids ... 211
4.9 Summary ... 212
References ... 212

## 4.1 INTRODUCTION

Lipids are a broad group of chemically diverse compounds that are soluble in organic solvents. Food lipids are generally referred to as fats (solid) or oils (liquid) indicating their physical state at ambient temperatures. Food lipids are also classified as nonpolar (e.g., triacylglycerol and cholesterol) and polar lipids (e.g., phospholipids) to indicate differences in their solubility and functional properties. Polar lipids often contain a hydrophilic "head" group that has a high affinity for water attached to a lipophilic "tail" group that has a high affinity for oil [1]. These surface-active lipids may alter the properties of foods through a variety of physicochemical mechanisms, including adsorbing to interfaces, stabilizing particles, interacting with biopolymers, and modifying crystal nucleation, growth, and structure [1–5] (see Chapter 13 for more details).

The total lipid content and the lipid composition of foods can vary tremendously. Since food lipids play an important role in food quality by contributing to attributes such as texture, flavor, nutrition, and caloric density, the manipulation of these important food components has been a major emphasis in food product development research over the past few decades. This research has focused

on the alteration of lipid composition to change texture, alter fatty acid and cholesterol composition, decrease total fat, alter bioavailability, and make lipids more oxidatively stable. In addition, the physical stability of lipids is important in food quality since many lipids exist as dispersions/emulsions that are thermodynamically unstable. In order to make changes in lipid composition while ensuring the production of high quality foods, a fundamental understanding of the chemical and physical properties of lipids is critical. This chapter will focus on the chemical composition of lipids, their physical properties and crystallization behavior, methods to modify the fatty acid and triacylglycerol composition and thus the physicochemical properties of lipids, propensity to undergo oxidative deterioration, and the role of lipids in health and disease. Information on analytical methods for food lipids is provided elsewhere [6,7].

## 4.2 MAJOR LIPID COMPONENTS

The following section is a brief description of the nomenclature of the major classes of food lipids. For more information of lipid nomenclature see O'Keefe [8] or the web page of the International Union of Pure and Applied Chemists (IUPAC), http://www.chem.qmul.ac.uk/iupac/lipid.

### 4.2.1 Fatty Acids

The major components of lipids are fatty acids, compounds that contain an aliphatic chain with a carboxylic acid group. Most natural fatty acids have an even number of carbons in a straight chain because of the biological process of fatty acid elongation where two carbons are added at a time. Exceptions of fatty acids with odd carbon numbers and branched chains can be found in sources such as microorganisms and dairy fats. The majority of fatty acids in nature range from 14 to 24 carbons. While some fats contain fatty acids with $<$14 carbons, significant levels of short-chain fatty acids are mainly found in tropical oils and dairy fats. Fatty acids are generally classified as either saturated or unsaturated, with unsaturated fatty acids containing double bonds. Fatty acids can be described by systematic, common, and abbreviated names.

#### 4.2.1.1 Nomenclature of Saturated Fatty Acids

The IUPAC have standardized systematic descriptions of fatty acids. The IUPAC system names the parent hydrocarbon of the fatty acid on the basis of the number of carbons (e.g., ten carbons would be decane). Since fatty acids contain a carboxylic acid group, the terminal *e* in the hydrocarbon's name is replaced by *oic* (e.g., decanoic; Table 4.1). Common names exist for most of the even number and many of the odd number fatty acids (Table 4.1). Many of the common names originate from the source that the fatty acid was commonly or traditionally isolated (e.g., palmitic acids and palm oil). A numerical system can be used for abbreviated names. The first number in this system designates the number of carbons in the fatty acids while the second number designates the number of double bonds (e.g., hexadecanoic = palmitic = 16:0). Obviously, this second number will always be zero for the saturated fatty acids.

#### 4.2.1.2 Nomenclature of Unsaturated Fatty Acids

Fatty acids that contain double bonds in their aliphatic chain are referred to as unsaturated fatty acids. In the IUPAC system, the *anoic* designation is changed to *enoic* to designate the presence of a double bond (Table 4.1). On the basis of the number of double bonds, the terms *di-*, *tri-*, *tetra-*, and so on are added. Common names also exist for the unsaturated fatty acids (with the exception of some of the long-chain polyunsaturated fatty acids) and the numerical abbreviation system is similar to the saturated fatty acids with the second number indicating the number of double bonds (e.g., octadecadienoic = 18:2). The positions of the double bonds in the IUPAC system are numbered by the delta ($\Delta$) system that indicates the position of the double bonds from the carboxylic acid end of the fatty acid. For example, oleic acid, which has 18 carbons and one double bond, would

**TABLE 4.1**
**Systematic, Common, and Numerical Names for Fatty Acids Found in Foods**

| Systematic Name | Common Name | Numerical Abbreviation |
|---|---|---|
| | *Saturated fatty acids* | |
| Hexanoic | Caproic | 6:0 |
| Octanoic | Caprylic | 8:0 |
| Decanoic | Capric | 10:0 |
| Dodecanoic | Lauric | 12:0 |
| Tetradecanoic | Myristic | 14:0 |
| Hexadecanoic | Palmitic | 16:0 |
| Octadecanoic | Stearic | 18:0 |
| | *Unsaturated fatty acids* | |
| *cis*-9-Octadecenoic | Oleic | 18:1 Δ9 |
| *cis*-9, *cis*-12-Octadecadienoic | Linoleic | 18:2 Δ9 |
| *cis*-9, *cis*-12, *cis*-15-Octadecatrienoic | Linolenic | 18:3 Δ9 |
| *cis*-5, *cis*-8, *cis*-11, *cis*-14-Eicosatetraenoic | Arachidonic | 20:4 Δ5 |
| *cis*-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17-Eicosapentaenoic | EPA | 20:5 Δ5 |
| *cis*-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19-Docosahexaenoic | DHA | 22:6 Δ4 |

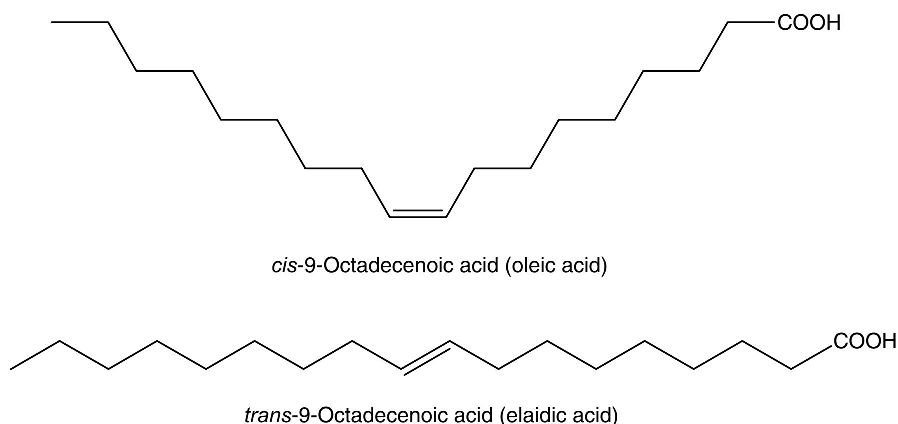



**FIGURE 4.1** Differences between *cis* and *trans* double bonds in unsaturated fatty acids.

be 9-octadecenoic acid and linoleic acid, which has 18 carbons and two double bonds, would be 9, 12-octadecadienoic acid. An alternative numbering system that indicates the position of the double bonds from the methyl end of the fatty acids is known as the omega ($\omega$) system (sometimes given a shorthand notation of "$n$"). The $\omega$ system is sometimes useful because it can group fatty acids on the basis of their biological activity and biosynthetic origin since many enzymes recognize fatty acids from the free methyl end of the molecule when it is esterified to glycerol. For instance, the $\omega$-3 fatty acids often have similar bioactivity in their ability to decrease blood triacylglycerol levels [9].

The natural configuration of double bonds in unsaturated fatty acids is the *cis* configuration. In the *cis* configuration, the carbons of the aliphatic chain are on the same side of the double bond while *trans* double bonds would have the carbons on opposite sides (Figure 4.1). Double bonds in polyunsaturated fatty acids (greater than two double bonds) are most commonly in a methylene-interrupted configuration often termed the pentadiene system. In a pentadiene system, the two double bonds would be at carbons 1 and 4. In other words, the double bonds are not conjugated but instead

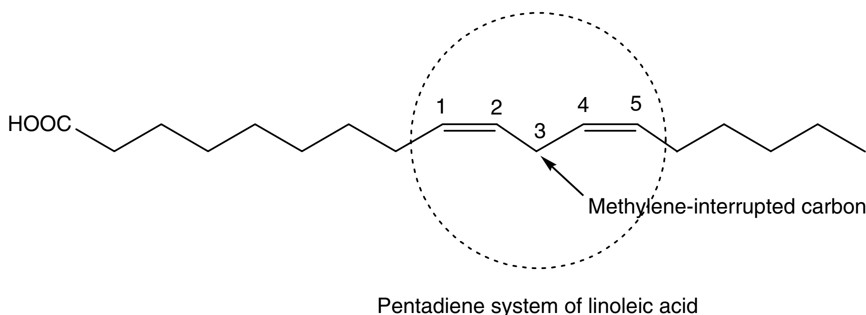



**FIGURE 4.2** The pentadiene systems of the polyunsaturated fatty acid, linoleic acid.

are separated by a methylene-interrupted carbon (Figure 4.2). This means that the double bonds of most unsaturated fatty acids are three carbons apart (e.g., 9, 12, 15 octadecatrienoic). It is therefore possible to predict the position of all the double bonds in most natural unsaturated fatty acids if the location of the first double bond is known. This is why the numerical abbreviation system will sometimes only give the number of double bonds and the position of the first double bond (e.g., 9, 12, 15 octadecatrienoic = 18:3, $\Delta 9$ = 18:3, $\omega 3$).

The presence of double bonds influences the melting point of the fatty acids. Double bonds in the *cis* configuration will cause the fatty acid to arrange in a bent configuration. Thus, unsaturated fatty acids are not linear making it difficult for them to orient themselves into tight packing configurations. Because of stearic hindrance to packing, van der Waals interactions between unsaturated fatty acids are relatively weak; therefore, they exist mainly in the liquid state at room temperature; that is, their melting point/solidification temperature is relatively low. As more double bonds are added, the molecule becomes more bent, the van der Waals interactions decrease further, and the melting point decreases. Fatty acids with double bonds in the *trans* configuration are more linear than unsaturated fatty acids in the *cis* configuration. This results in tighter packing of the molecules and higher melting points. For example, the approximate melting point of stearic acid (octadecanoic) is 70°C, oleic acid (*cis*-9-octadecenoic) is 5°C, and elaidic acid (*trans*-9-octadecenoic) is 44°C [10].

### 4.2.2 Acylglycerols

Over 99% of the fatty acids found in plants and animals are esterified to glycerol. Free fatty acids are not common in living tissues because they are cytotoxic owing to their ability to disrupt cell membrane organization. Once fatty acids are esterified onto glycerol, their surface activity decreases, as does their cytotoxicity.

Acylglycerols can exist as mono-, di-, and triesters known as monoacylglycerols, diacylglycerols, and triacylglycerols, respectively. Triacylglycerols are the most common of the three in foods, although the mono- and diesters are sometimes used as food additives (e.g., emulsifiers). The central carbon of a triacylglycerol exhibits chirality if different fatty acids are present at the terminal carbons of the glycerol. Because of this, the three carbons on the glycerol portion of the triacylglycerol can be differentiated with stereospecific numbering (*sn*). If the triacylglycerol is shown in a planar Fischer projection, the carbons are numbered 1–3 from top to bottom.

Triacylglycerols can be named by several different systems. Triacylglycerols are often named using the common names of the fatty acids. If the triacylglycerol contains only one fatty acid (e.g., stearic acid abbreviated as St), it could be named tristearin, tristearate, glycerol tristearate, tristearoyl glycerol, StStSt, or 18:0–18:0–18:0. Triacylglycerols that contain different fatty acids are named differently depending on whether the stereospecific location of each fatty acid is known. The nomenclature for these heterogeneous triacylglycerols replaces the *-ic* at the end of the fatty acid name with *-oyl*. If the stereospecific location is not known, a triacylglycerol-containing

palmitic acid, oleic acid, and stearic acid would be named palmitoyl-oleoyl-stearoyl-glycerol. Alternatively, this triacylglycerol could be named palmito-oleo-stearin or glycerol-palmito-oleo-stearate. If the stereospecific location of the fatty acids is known, *sn*- is added to the name such as in 1-palmitoyl-2-oleoyl-3-stearoyl-*sn*-glycerol, *sn*-1-palmito-2-oleo-3-stearin, or *sn*-glycerol-1-palmito-2-oleo-3-stearate. If two of the fatty acids are identical, the naming can be shortened as 1,2-dipalmitoyl-3-stearoyl-*sn*-glycerol, *sn*-1,2-dipalmito-3-stearin, or *sn*-glycerol-1,2-dipalmito-3-stearate. Heterogeneous triacylglycerol can also be named using fatty acid abbreviations such as in PStO or 16:0–18:0–18:1 (stereospecific location unknown) or *sn*-PStO or *sn*-16:0–18:0–18:1 (stereospecific location known) for 1-palmitoyl-2-oleoyl-3-stearoyl-*sn*-glycerol.

#### 4.2.2.1 Composition of Fats

Food lipids contain a wide variety of fatty acid compositions as shown in Table 4.2. Several general trends can be seen among lipids. Most vegetable oils, especially those from oilseeds, are highly unsaturated and contain primarily fatty acids in the 18 carbon series. Oils high in oleic acid include olive and canola, oils high in linoleic include soybean and corn, and oils high in linolenic include linseed. Triacylglycerols from plant sources that contain high amounts of saturated fatty acids include cocoa butter and the tropical oils (e.g., coconut). Coconut and palm kernel oils are also unique in that they contain high amounts of the medium-chain fatty acids 8:0 to 14:0 with 12:0 predominating. The level of saturated fatty acids in fats and oils from animals is generally in the order of milk fat > sheep > beef > pig > chicken > turkey > marine fish with palmitic and stearic being the major saturated fatty acids. The fatty acid composition of animal fats are dependent on the digestive system of the animal with fat from nonruminants (e.g., poultry, pigs, and fish) being partially dependent on the fatty acid compositions of their diets. An example of this is pigs, such as Iberian hams, where dietary regimes are manipulated to produce lard with a high oleic acid composition. Among the nonruminants, triacylglycerols from marine animals are unique because they contain high amounts of the $\omega$-3 fatty acids, eicosapentaenoic and docosahexaenoic. In sheep and cows, dietary fatty acids are subject to biohydrogenation by microbial enzymes in the rumen. This results in the conversion of unsaturated fatty acids into saturated fatty acids and can also produce fatty acids with conjugated double bonds such as conjugated linoleic acid (CLA). Since ruminants consume primarily lipids of plant origin where the fatty acids are primarily in the 18 carbon series, the end product of this biohydrogenation pathway is stearic acid. Thus, butter, beef fat, and sheep fat contain higher amounts of stearic acid than fats from nonruminants. Ruminal bacteria are also unique in that they can ferment carbohydrates to acetate and $\beta$-hydroxybutyrate. In the mammary gland, these substrates are converted to fatty acids to give butter fat a high concentration of saturated, short-chain (4:0 and 6:0) fatty acids that are not found in other food triacylglycerols. Ruminal bacteria also promote the formation of keto-, hydroxyl, and branched fatty acids. Because of the impact of ruminal bacteria on fatty acids, butter fat contains hundreds of different fatty acids.

The stereospecific location of fatty acids can also vary in food triacylglycerols. Triacylglycerols in some fats such as tallow (beef fat), olive oil, and peanut oil have most of their fatty acids evenly distributed among all three positions of glycerol. However, some fats can have very specific trends for the stereospecific location of fatty acids. Many triacylglycerols from plants sources have the (poly)unsaturated fatty acids concentrated at the *sn*-2 position. The best example of this is cocoa butter where over 85% of its oleic acid is *sn*-2, with palmitic and stearic acids being evenly distributed at *sn*-1 and *sn*-3. Triacylglycerols from some animal fats tend to have saturated fatty acids concentrated at *sn*-2. For instance, palmitic acid is primarily at the *sn*-2 position in milk fat and lard (pork fat). The stereospecific location of a fatty acid can be an important determinant on their impact in nutrition. When triacylglycerols are digested in the intestine, fatty acids from *sn*-1 to *sn*-3 are released by pancreatic lipase resulting in two free fatty acids and a *sn*-2 monoacylglycerol. If long-chain saturated fatty acids are at *sn*-1 and *sn*-3, their bioavailability is lower because the free fatty acids can form insoluble calcium salts on hydrolysis by pancreatic lipase. Thus, placement of long-chain saturated

**TABLE 4.2**
**Fatty Acid Composition (Weight% of Total Fatty Acids) of Common Foods (Only the Major Fatty Acids in These Products Are Listed)**

| Food Lipid | 4:0 | 6:0 | 8:0 | 10:0 | 12:0 | 14:0 | 16:0 | 16:1$\Delta$9 | 18:0 | 18:1$\Delta$9 | 18:2$\Delta$9 | 18:3$\Delta$9 | 20:5$\Delta$5 | 22:6$\Delta$4 | Total Saturated | Crystal Habit |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Olive | | | | | | | 13.7 | 1.2 | 2.5 | 71.1 | 10.0 | 0.6 | | | 16.2 | $\beta$ |
| Canola | | | | | | | 3.9 | 0.2 | 1.9 | 64.1 | 18.7 | 9.2 | | | 5.5 | $\beta$ |
| Corn | | | | | | | 12.2 | 0.1 | 2.2 | 27.5 | 57.0 | 0.9 | | | 14.4 | $\beta$ |
| Soybean | | | | | | 0.1 | 11.0 | 0.1 | 4.0 | 23.4 | 53.2 | 7.8 | | | 15.0 | $\beta$ |
| Linseed | | | | | | | 4.8 | | 4.7 | 19.9 | 15.9 | 52.7 | | | 9.5 | |
| Coconut | | 0.5 | 8.0 | 6.4 | 48.5 | 17.6 | 8.4 | | 2.5 | 6.5 | 1.5 | | | | 91.9 | $\beta'$ |
| Cocoa | | | | | | 0.1 | 25.8 | 0.3 | 34.5 | 35.3 | 2.9 | | | | 60.4 | $\beta$ |
| Butterfat | 3.8 | 2.3 | 1.1 | 2.0 | 3.1 | 11.7 | 26.2 | 1.9 | 12.5 | 28.2 | 2.9 | 0.5 | | | 62.7 | $\beta'$ |
| Beef fat | | | | 0.1 | 0.1 | 3.3 | 25.5 | 3.4 | 21.6 | 38.7 | 2.2 | 0.6 | | | 50.6 | $\beta'$ |
| Pork fat | | | | 0.1 | 0.1 | 1.5 | 24.8 | 3.1 | 12.3 | 45.1 | 9.9 | 0.1 | | | 38.8 | $\beta$ |
| Chicken | | | | | 0.2 | 1.3 | 23.2 | 6.5 | 6.4 | 41.6 | 18.9 | 1.3 | | | 31.1 | |
| Atlantic Salmon | | | | | | 5.0 | 15.9 | 6.3 | 2.5 | 21.4 | 1.1 | 0.6 | 1.9 | 11.9 | 23.4 | $\beta'$ |
| Chicken Eggs | | | | | | 0.3 | 22.1 | 3.3 | 7.7 | 36.6 | 11.1 | 0.3 | | | 30.1 | |

*Source:* All fatty acid compositions are adapted from White, P.J. (2000). In *Fatty Acids in Foods and Their Health Implications*, 2nd edn. (Chow, C.K., ed.), Marcel Dekker, Inc., New York, NY, pp. 153–174, with the exception of Atlantic Salmon that is adapted from Ackman, R.G. (2000). In *Fatty Acids in Foods and Their Health Implications*, 2nd edn. (Chow, C.K., ed.), Marcel Dekker, Inc., New York, NY, pp. 153–174.

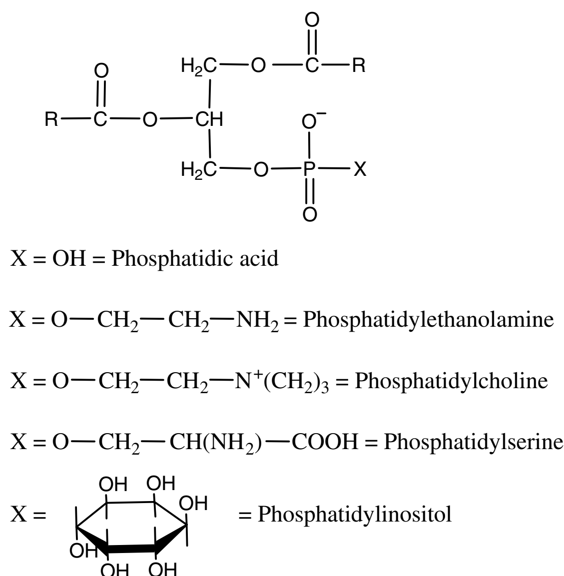



**FIGURE 4.3** Structures of phospholipids commonly found in foods.

fatty acids at *sn*-2 in milk fats may be a mechanism to insure that these fatty acids are absorbed by infant. Since long-chain saturated fatty acids at *sn*-1 and *sn*-3 are absorbed inefficiently, they provide less calories [13] and have less impact on blood lipid profiles. For example, when lard has its fatty acids randomly distributed and thus has more palmitic acid at *sn*-1 and *sn*-3, it increases plasma palmitic acid less than unmodified lard where 65% of palmitic acid is at *sn*-2. Structured triacylglycerols such as Salatrim have lower calories than normal fat because they have a high concentration of stearic acid (18:0) at *sn*-1 and *sn*-3 (see Section 4.8.2).

### 4.2.3 Phospholipids

The phospholipids or phosphoglycerides are modifications of triacylglycerols where phosphate groups are typically found in the *sn*-3 position (see Figure 4.3 for the structures of phospholipids). The simplest phospholipid is phosphatidic acid (PA) where the substitution group on the phosphate at *sn*-3 is an —OH. Other modifications of the substitution group on the phosphate at *sn*-3 result in phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylethanolamine (PE), and phosphatidylinositol (PI) (Figure 4.3). Nomenclature is similar to triacylglycerols with the name and location of the phosphate group coming at the end of the name (e.g., 1-palmitoyl-2-stearoyl-*sn*-glycero-3-phosphoethanolamine). The term "lyso" signifies that a fatty acid has been removed from the phospholipid. In the food industry, lysophospholipids usually refer to a phospholipid where the fatty acid has been removed from the *sn*-2 position. Official nomenclature requires that the stereospecific location of the fatty acid removed should be named (e.g., 2-lysophospholipids, IUPAC). PC is commonly referred to as lecithin in the food industry, however, the lecithin sold as a food additive is not usually pure PC. Instead, it contains a mixture of a variety of different phospholipids, as well as some other components.

The presence of the highly polar phosphate group on phospholipids makes these compounds surface active. This surface activity allows phospholipids to arrange in bilayers that are critical for the properties of biological cell membranes. Since cell membranes need to maintain fluidity, the fatty acids found in phospholipids are often unsaturated to prevent crystallization at ambient temperatures. The fatty acids at the *sn*-2 position are typically more unsaturated than at the *sn*-1

position. The unsaturated fatty acids at the *sn*-2 position can be released by phospholipases so they can be utilized as substrates for enzymes such as cyclooxygenase and lipoxygenase (LOX). The surface activity of phospholipids means that they can be used to modify the physical properties of lipids by acting as emulsifiers and by modifying lipid crystallization behavior.

### 4.2.4 Sphingolipids

The sphingolipids are lipids that commonly contain a sphingosine base. Common sphingolipids include sphingomyelin (a sphingophospholipid; Figure 4.4), ceramides, cerebrosides, and gangliosides. These lipids are most commonly found associated with cell membranes especially in nervous tissue. They are generally not major components of food lipids.

### 4.2.5 Sterols

Sterols are derivatives of steroids. These nonpolar lipids all have three six-carbon rings and a five-carbon ring that is attached to an aliphatic chain (Figure 4.5). Sterols have a hydroxyl group attached to carbon 3 of the A ring. Sterol esters are sterols with a fatty acid esterified onto the hydroxyl group

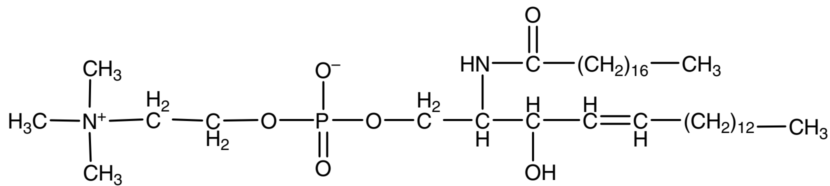


**FIGURE 4.4** Structure of sphingomyelin, a common sphingolipid.

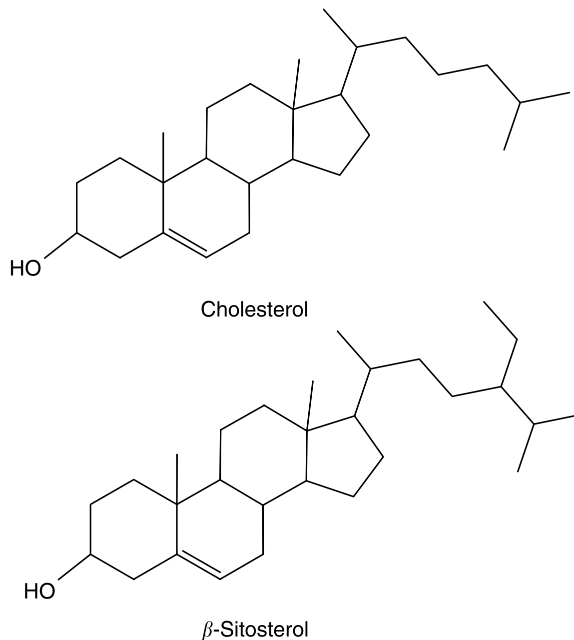



**FIGURE 4.5** Structures of sterols commonly found in foods.

at carbon 3. Sterols are found in both plants (phytosterols) and animals (zoosterols). Cholesterol is the major sterol found in animal lipids. Plant lipids contain numerous sterols with $\beta$-sitosterol and stigmasterol predominating. Cholesterol can be found in plant lipids as a minor sterol component. The hydroxyl group at carbon 3 of sterols makes these compounds surface active. Cholesterol therefore can orient itself into cell membranes where it is important in stabilizing membrane structure. Cholesterol is also important because it is the precursor for the synthesis of bile acids and 7-dehydrocholesterol is the precursor for the production of vitamin D in the skin by ultraviolet (UV) irradiation [14]. High blood cholesterol and in particular high cholesterol in low-density lipoprotein (LDL) has been attributed to increased risk for cardiovascular disease. For this reason, reduced levels of dietary cholesterol are desirable. This can be achieved by reduction of animal fats in the diet and/or by removal of cholesterol from animal fats by supercritical carbon dioxide extraction or molecular distillation. Dietary phytosterols decrease cholesterol absorption in the intestine and therefore have been added to foods to reduce blood cholesterol levels (see Sections 4.8.1.1.1–4.8.1.1.4).

### 4.2.6 Waxes

The strict chemical definition of a wax is an ester of a long-chain acid and a long-chain alcohol. In reality, industrial and food waxes are a combination of chemical classes including wax esters, sterol esters, ketones, aldehydes, alcohols, hydrocarbons, and sterols [14]. Waxes can be classified according to their origin as animal (beeswax), plant (carnauba wax), and mineral (petroleum waxes). Waxes are found on the surface of plant and animal tissues to inhibit water loss or to repel water. Waxes are commonly added to the surface of fruits to slow dehydration during storage.

### 4.2.7 Miscellaneous Lipids

Other food lipids including the fat-soluble vitamins (A, D, E, and K) and carotenoids are covered in other sections of this book.

## 4.3 PHYSICOCHEMICAL PROPERTIES OF LIPIDS

This section will be primarily concerned with the physical properties of lipids and their influence on food properties. In particular, we will focus on how the molecular structure and organization of lipids determine their functional properties (e.g., melting characteristics, crystal morphology, and interactions) and how these functional properties determine the bulk physicochemical and sensory properties of food products (e.g., texture, stability, appearance, and flavor).

While there are a number of different categories of lipids present in food systems, this section will concentrate primarily on triacylglycerols because of their high natural abundance and major importance in food products. As mentioned earlier, triacylglycerols are esters of a glycerol molecule and three fatty acid molecules, and each fatty acid may have different numbers of carbon atoms, degrees of unsaturation, and branching (Section 4.2). The fact that there are many different types of fatty acids, and that these fatty acids can be located at different positions on the glycerol molecule, means that foods may contain a wide variety of different triacylglycerols. Indeed, edible fats and oils always contain a great many different types of triacylglycerol molecules or "species," with the precise type and concentration depending on their origin [15–17].

Triacylglycerol molecules have a "tuning-fork" structure, with the two fatty acids at the ends of the glycerol molecule pointing in one direction and the fatty acid in the *sn*-2 position pointing in the opposite direction (Figure 4.6). They are predominantly nonpolar molecules and so the most important types of molecular interaction that are responsible for their structural organization are van der Waals attraction and steric repulsion [18]. The interactions between two molecules can be described by the intermolecular pair potential $w(s)$, which is a measure of the strength of the attraction or repulsion between the molecules at a particular separation $s$ (Figure 4.7). At a certain

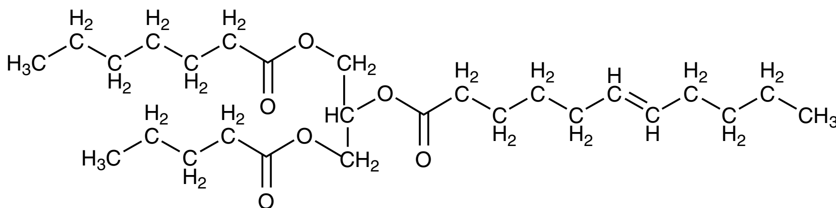



**FIGURE 4.6** Chemical structure of a triacylglycerol molecule, which is assembled from three fatty acids and a glycerol molecule.

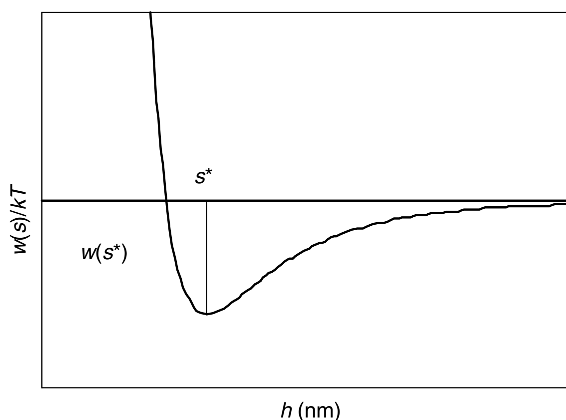



**FIGURE 4.7** The strength of the attractive interactions between lipid molecules depends on the depth of the minimum in the overall molecular interaction potential.

molecular separation ($s^*$), there is a minimum in the intermolecular pair potential, which indicates that this is the most stable state. The value of $s^*$ provides a measure of the average distance between triacylglycerols, while the depth of the pair potential at this value ($w(s^*)$) provides a measure of the strength of the attractive forces that hold the molecules together in the solid and liquid states (Figure 4.7). The structural organization of the molecules in triacylglycerols is primarily determined by their physical state, which depends on a balance between the attractive molecular interactions and the disorganizing influence of the thermal energy. Lipids exist as liquids above their melting point and as solids at temperatures that are sufficiently below their melting point to overcome supercooling effects (see below).

Lipid molecules may adopt a variety of different structural organizations in both the solid and liquid states depending on their precise molecular characteristics (e.g., chain length, degree of unsaturation, polarity) [19,20]. In the solid state, the organization of the lipid molecules may vary in several ways, including the overall organization of the triacylglycerol molecules relative to one another, the angle of tilt of the molecules within the crystal lattice, and the packing of the hydrocarbon chains. These differences mean that fat crystals can exist in a number of different polymorphic crystal forms (discussed later), which have different physical properties and melting behavior. Even in the liquid state, triacylglycerols are not randomly orientated but have some order owing to self-organization of the lipid molecules into structural entities (e.g., lamellar structures) [19,21]. The size and number of these structural entities is believed to decrease as the temperature is increased.

It should be noted that the term *fat* is conventionally used to refer to a lipid that is solid-like at room temperature, whereas the term *oil* is used to refer to a lipid that is liquid, although these terms are often used interchangeably [22,23].

**TABLE 4.3**
**Comparison of Some Bulk Physicochemical Properties of a Liquid Oil (Triolein) and Water at 20°C**

| | Oil | Water |
|---|---|---|
| Molecular weight | 885 | 18 |
| Melting point (°C) | 5 | 0 |
| Density (kg $m^{-3}$) | 910 | 998 |
| Compressibility (m $s^2$ $kg^{-1}$) | $5.03 \times 10^{-10}$ | $4.55 \times 10^{-10}$ |
| Viscosity (mPa s) | ≈50 | 1.002 |
| Thermal conductivity (W $m^{-1}$ $K^{-1}$) | 0.170 | 0.598 |
| Specific heat capacity (J $kg^{-1}$ $K^{-1}$) | 1980 | 4182 |
| Thermal expansion coefficient (°$C^{-1}$) | $7.1 \times 10^{-4}$ | $2.1 \times 10^{-4}$ |
| Dielectric constant | 3 | 80.2 |
| Surface tension (mN $m^{-1}$) | ≈35 | 72.8 |
| Refractive index | 1.46 | 1.333 |

### 4.3.1 Physical Properties of Triacylglycerols

The physical properties of edible fats and oils depend primarily on the molecular structure, interactions, and organization of the triacylglycerol molecules that they contain [20,23–28]. In particular, the strength of the attractive interactions between the molecules and the effectiveness of their packing within a condensed phase largely determine their thermal behavior, density, and rheological properties (Table 4.3).

#### 4.3.1.1 Rheological Properties

Most liquid oils are Newtonian liquids with intermediate viscosities, typically between 30 and 60 mPa s at room temperature [24,29]. Nevertheless, castor oil tends to have a much higher viscosity than most other oils because it contains an appreciable fraction of fatty acids with an alcohol group along their hydrocarbon backbones (i.e., ricinolenic acid), which is capable of forming relatively strong hydrogen bonds with neighboring molecules [24]. The viscosity of liquid oils tends to decrease steeply with increasing temperature and can be conveniently described by a logarithmic relationship [29].

Most "solid fats" actually consist of a mixture of fat crystals dispersed in a liquid oil matrix. The rheological properties of these solid fats are highly dependent on the concentration, morphology, interactions, and organization of the fat crystals present in the system [20,23]. Solid fats normally exhibit a type of rheological behavior known as "plasticity." A plastic material behaves like a solid below a critical applied stress, known as the yield stress ($\tau_0$), but behaves like a liquid above this stress. The rheological behavior of an ideal plastic material, known as a *Bingham Plastic*, is shown in Figure 4.8. For an applied *shear* stress, the rheological characteristics of this type of material can be described by the following equation [23]:

$$\tau = G\gamma \quad (\text{for } \tau < \tau_0) \tag{4.1}$$

$$\tau - \tau_0 = \eta\dot{\gamma} \quad (\text{for } \tau \geq \tau_0) \tag{4.2}$$

where $\tau$ is the applied shear stress, $\gamma$ is the resultant shear strain, $\dot{\gamma}$ is the rate of shear strain, $G$ is the shear modulus (related to the strength or rigidity of material in response to shear strain), $\eta$ is the

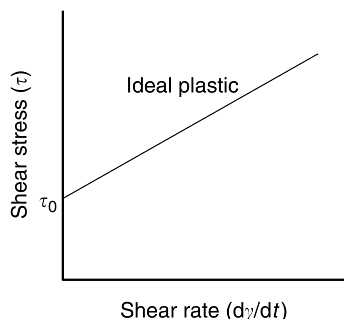



**FIGURE 4.8** An ideal plastic material (*Bingham Plastic*) behaves like a solid below a critical applied stress, known as the yield stress ($\tau_0$), but behaves like a liquid above this stress.

shear viscosity, and $\tau_0$ is the yield stress (the point where the material starts to flow). In practice, solid fats tend to exhibit nonideal plastic behavior. For example, above the yield stress the fat may not flow like an ideal liquid and may exhibit non-Newtonian behavior (e.g., shear thinning). Below the yield stress, the fat might not behave as an ideal solid and exhibits some flow characteristics (e.g., viscoelasticity). In addition, the yield stress may not occur at a well-defined value, but may occur over a range of applied stresses because there is a gradual break down of the fat crystal network structure [30]. The yield stress of a fat tends to increase with increasing solid-fat content (SFC) and tends to be higher for crystal morphologies that are able to form three-dimensional networks that extend throughout the volume of the system more easily (i.e., small needle shaped crystals). A detailed discussion of the characteristics of plastic fats has recently been given elsewhere [23].

The structural origin of the plastic behavior of solid fats can be attributed to their ability to form a three-dimensional network of tiny fat crystals dispersed in a liquid oil matrix [23,31]. Below a certain applied stress there is a small deformation of the sample, but the weak bonds between the fat crystals are not disrupted. When the critical yield stress is exceeded, the weak bonds are broken and the fat crystals slide past one another leading to flow of the sample. Once the force is removed the flow stops, and the fat crystals begin to form bonds with their neighbors again. The rate at which this process occurs may have important implications for the functionality of the product. The influence of the rheological characteristics of triacylglycerols on the physicochemical and sensory properties of foods is described later.

#### 4.3.1.2 Density

The density of a lipid is defined as the mass of material required to occupy a given volume [32]. This information is often important when designing food processing operations, since it determines the amount of material that can be stored in a tank or flow through a pipe of a given volume. The density of lipids is also important in certain food applications because it influences the overall properties of the system, for example, the creaming rate of oil droplets in oil-in-water (O/W) emulsions depends on the density difference between the oil and aqueous phases [33]. The densities of liquid oils tend to be around 910–930 kg $m^{-3}$ at room temperature and tend to decrease with increasing temperature [24]. The densities of completely solidified fats tend to be around 1000–1060 kg $m^{-3}$, and they too decrease with increasing temperature [24]. In many foods, the fat is partially crystalline and so the density depends on the SFC, that is, the fraction of the total fat phase that is solidified. The density of a partially crystalline fat tends to increase as the SFC increases, for example, after cooling below the crystallization temperature. Measurements of the density of a partially crystalline fat can therefore sometimes be used to determine its SFC.

The density of a particular lipid depends primarily on the efficiency of the packing of the triacylglycerol molecules within it: the more efficient the packing, the higher the density.

Thus, triacylglycerols that contain linear saturated fatty acids are able to pack more efficiently than those that contain branched or unsaturated fatty acids, and so they tend to have higher densities [22,23]. The reason that solid fats tend to have higher densities than liquid oils is also because the molecules tend to be packed more efficiently. Nevertheless, this is not always the case [34]. For example, in lipid systems containing high concentrations of pure triacylglycerols that crystallize over a narrow temperature range, it has been shown that the density of the overall lipid system actually decreases on crystallization because of void formation.

*Thermal properties:* The most important thermal properties of lipids from a practical standpoint are the specific heat capacity ($C_P$), thermal conductivity ($\kappa$), melting point ($T_{mp}$), and enthalpy of fusion ($\Delta H_f$) [24]. These thermal characteristics determine the total amount of heat that must be supplied (or removed) from a lipid system to change its temperature from one value to another, as well as the rate at which this process can be achieved. The specific heat capacities of most liquid oils and solid fats are around 2 J $g^{-1}$, and increase with increasing temperature [24]. Lipids are relatively poor conductors of heat and tend to have appreciably lower thermal conductivities (~0.165 W $m^{-1}$ $s^{-1}$) than water (~0.595 W $m^{-1}$ $s^{-1}$). Detailed information about the thermal properties of different kinds of liquid and solid lipids have been tabulated elsewhere [24,29]. Representative values are included in Table 4.3.

The melting point and heat of fusion of a lipid depend on the packing of the triacylglycerol molecules within the crystals formed: the more effective the packing, the higher the melting point and the enthalpy of fusion [18,23]. Thus, the melting points and heats of fusions of pure triacylglycerols tend to increase with increasing chain length. They are higher (1) for saturated fatty acids than for unsaturated fatty acids; (2) for straight-chained fatty acids than for branched fatty acids; (3) for triacylglycerols with a more symmetrical distribution of fatty acids on the glycerol molecule; (4) for *trans* than for *cis* unsaturated forms (Table 4.4); and (5) for more stable polymorphic forms (discussed later). The crystallization of lipids is one of the most important factors determining their influence on the bulk physicochemical and sensory properties of foods and therefore it will be treated in some detail in a later section.

**TABLE 4.4**
**Melting Points and Heats of Fusion of the Most Stable Polymorphic Forms of Selected Triacylglycerol Molecules**

| Triacylglycerol | Melting Point (°C) | $\Delta H_f$ (J $g^{-1}$) |
|---|---|---|
| LLL | 46 | 186 |
| MMM | 58 | 197 |
| PPP | 66 | 205 |
| SSS | 73 | 212 |
| OOO | 5 | 113 |
| LiLiLi | −13 | 85 |
| LnLnLn | −24 | — |
| SOS | 43 | 194 |
| SOO | 23 | — |

L = lauric acid (C12:0), M = myristic acid (C14:0), P = palmitic acid (C16:0), S = stearic acid (C16:0), O = oleic acid (C18:1), Li = linoleic (C18:2), Ln = linolenic (C18:3).

*Source:* Adapted from Walstra, P. (2003). *Physical Chemistry of Foods*, Marcel Dekker, Inc., New York, NY.

For some applications, knowledge of the temperature where a lipid starts to breakdown owing to thermal degradation is important (e.g., frying or baking). The thermal stability of lipids can be characterized by their smoke, flash, and fire points [32]. The *smoke point* is the temperature at which the sample begins to smoke when tested under specified conditions. The *flash point* is the temperature at which the volatile products generated by the lipid are being produced at a rate where they can be temporarily ignited by application of a flame, but cannot sustain combustion. The *fire point* is the temperature at which the evolution of volatiles because of thermal decomposition occurs so quickly that continuous combustion can be sustained after application of a flame. Measurements of these temperatures are particularly important when selecting lipids that are going to be used at high temperatures (e.g., during baking or frying). The thermal stability of triacylglycerols is much better than that of free fatty acids, hence the propensity of lipids to breakdown during heating is largely determined by the amount of volatile organic material that they contain, such as free fatty acids [32].

*Optical properties:* Knowledge of the optical properties of lipids is important to food chemists for a number of reasons. First, the optical properties of lipids influence the overall appearance of many food materials [35]. Second, certain optical properties of lipids (e.g., refractive index and absorption spectrum) can be used to provide valuable information about their composition or quality [24,32]. The most important optical properties of lipids are their refractive index and absorption spectra. The refractive indices of liquid oils typically fall between 1.43 and 1.45 at room temperature [24]. The refractive index of a particular oil is mainly determined by the molecular structure of the fatty acids that it contains. The refractive index tends to increase with increasing chain length, increasing number of double bonds, and increasing conjugation of double bonds [24]. Empirical equations have been developed to relate the molecular structure of lipids to their refractive indices [24]. Hence, measurements of the refractive index of liquid oils can be used to provide some information about the average molecular weight or degree of unsaturation of the fatty acids that they contain. Measurements of the UV-visible absorption spectra of oils can also provide valuable information about their composition, quality, or molecular properties (e.g., presence of conjugated double bonds, carotenoids, or chlorophyll) [32]. For example, conjugated dienes adsorb UV light around 232 nm, whereas conjugated trienes adsorb around 270 nm.

The absorption spectrum of an oil can also have a pronounced influence on the final appearance of a food product. Pure triacylglycerols have little inherent color because they do not contain groups that adsorb light in the visible region of the electromagnetic spectrum. Nevertheless, commercial oils tend to be colored because they contain appreciable amounts of pigments that do absorb light (e.g., carotenoids and chlorophyll). For this reason, edible oils often undergo a decolorization step during their refinement. In emulsified foods, lipids also contribute to the opacity of the product because of their ability to scatter light, which is a direct result of the difference in refractive index between the lipid and aqueous phases.

*Electrical properties:* Knowledge of the electrical properties of lipids is sometimes important because several analytical techniques used to analyze fatty foods are based on measurements of their electrical characteristics, for example, electrical conductivity measurements of fat concentration or electrical pulse counting of fat droplet size [33]. Lipids tend to have fairly low relative dielectric constants ($\varepsilon_R \approx 2$–4) because of the low polarity of triacylglycerol molecules (Table 4.3). The dielectric constant of pure triacylglycerols tends to increase with increasing polarity (e.g., owing to the presence of —OH groups or owing to oxidation) and decreasing temperature [24]. Lipids also tend to be poor conductors of electricity, having relatively high electrical resistances.

### 4.3.2 Crystallization and Melting of Food Lipids

The physical state (solid or liquid) of the lipids in many food products plays an important role in their production and in determining their final quality attributes [20]. For example, the overall physicochemical and sensory properties of products such as margarine, butter, ice cream, whipped cream,

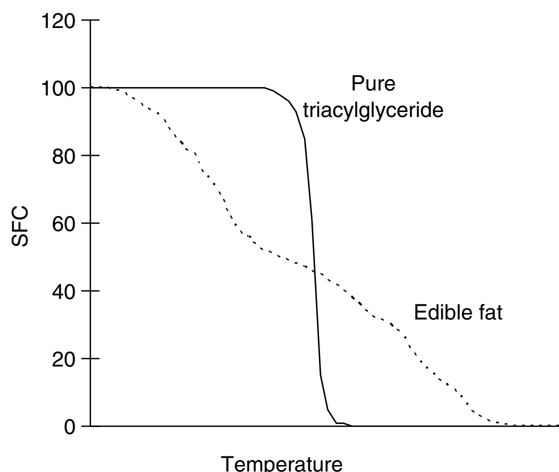



**FIGURE 4.9** Comparison of the melting profile of a pure triacylglycerol and a typical edible fat. The edible fat melts over a much wider range of temperatures because it consists of a mixture of many different pure triacylglycerol molecules each with different melting points.

and baked goods are strongly influenced by the crystallization behavior of the lipids that they contain. The creation of food products with desirable properties therefore depends on an understanding of the major factors that influence the crystallization and melting of lipids in foods [19,23,26].

*Solid fat content:* The physical state of the lipids in a food is usually characterized in terms of the "SFC," which is the fraction (0–1) or percentage (0–100%) of lipid that is solid at a particular temperature. The temperature dependence of the SFC is one of the most important criteria influencing the selection of lipids for particular food applications, because it strongly influences the efficiency of the production process and the final properties of many fatty foods. The melting behavior of a pure triacylglycerol is shown schematically in Figure 4.9. The SFC falls from 100 to 0% when the temperature is increased from below to above the melting point (Figure 4.9). For a pure triacylglycerol the transition from solid-to-liquid occurs over a narrow range of temperatures close to the melting point ($T_{mp}$). The melting point of a pure triacylglycerol depends on the chain length, branching, and degree of unsaturation of its constituent fatty acids, as well as their relative positions along the glycerol molecule (Table 4.4). Edible fats contain a complex mixture of many different types of triacylglycerol molecules, each with a different melting point, and so they usually melt over a wide range of temperatures, rather than at a distinct temperature as would be the case for a pure triacylglycerol (Figure 4.9). As mentioned earlier, the desirable "plastic" rheological properties of edible fats usually occur over the range of temperatures where the lipids are partially crystalline (the "plastic range").

The melting profile of an edible fat is not simply the weighted sum of the melting profiles of its constituent triacylglycerols, because high melting point triacylglycerols are soluble in lower melting point ones [27]. For example, in a 50:50 mixture of tristearin and triolein it is possible to dissolve 10% of solid tristearin in liquid triolein at 60°C [22,28]. The solubility of a solid component in a liquid component can be predicted assuming they have widely differing melting points (>20°C):

$$\ln x = \frac{\Delta H_{fus}}{R}\left[\frac{1}{T_{mp}} - \frac{1}{T}\right] \tag{4.3}$$

Here $x$ is the solubility, expressed as a mole fraction, of the higher melting point component in the lower melting point component, and $\Delta H_{fus}$ is the molar heat of fusion [22]. In addition, the melting

characteristics of food lipids depend on the nature of the fat crystals present (e.g., solid solution vs. mixed crystals, crystal morphology, and crystal polymorphic form [see later]).

The SFC of fatty foods is usually measured by calorimetry, changes in volume (dilatometry) or nuclear magnetic resonance (NMR). NMR is the preferred method to measure SFC since it requires little sample preparation and can be carried out quickly and simply [36]. SFC is an important parameter in food lipids because it provides information on important quality properties. Examples include crystallization behavior at refrigeration temperatures that will impact cloud point and emulsion stability, melting behavior at different temperatures that influences mouthfeel, baking properties, and spreadability of a lipid at refrigeration (tub margarine) or room temperature (stick margarine).

As mentioned earlier, the SFC-temperature profile of edible fats plays a major role in determining the functional and sensory properties of many fatty foods [16,22,27,28,37]. For example, it is important that margarines are "hard" enough to retain their shape when stored in a refrigerator or brought to room temperature, but that they are "soft" enough to be spread with a knife [38]. In addition, it is important that the fat crystals melt during mastication to provide a desirable mouthfeel. For this reason, it is important to use lipids that have SFC– and rheology–temperature profiles that are appropriate for specific applications [23].

### 4.3.3 Physicochemical Mechanism of Lipid-Phase Transitions

The arrangement of triacylglycerol molecules in the solid and liquid state is shown schematically in Figure 4.10. The physical state of a triacylglycerol at a particular temperature depends on its free energy, which is made up of contributions from both enthalpy and entropy terms: $\Delta G_{S\rightarrow L} = \Delta H_{S\rightarrow L} - T\Delta S_{S\rightarrow L}$ [39]. The enthalpy term ($\Delta H_{S\rightarrow L}$) represents the change in the overall strength of the molecular interactions between the triacylglycerols when they are converted from a solid to a liquid, whereas the entropy term ($\Delta S_{S\rightarrow L}$) represents the change in the organization of the molecules that is brought about by the melting process. The strength of the bonds between the lipid molecules is greater in the solid state than in the liquid state because the molecules are able to pack more efficiently, and so $\Delta H_{S\rightarrow L}$ is positive (unfavorable), which favors the solid state. On the other hand, the entropy of the lipid molecules in the liquid state is greater than that in the solid state, and therefore $\Delta S_{S\rightarrow L}$ is positive (favorable), which favors the liquid state. At low temperatures, the enthalpy term dominates the entropy term ($\Delta H_{S\rightarrow L} > T\Delta S_{S\rightarrow L}$), and therefore the solid state has the lowest free energy [19,23,39]. As the temperature increases, the entropy contribution becomes increasingly important. Above a certain temperature, known as the *melting point*, the entropy term dominates the enthalpy term ($T\Delta S_{S\rightarrow L} > \Delta H_{S\rightarrow L}$) and so the liquid state has the lowest free energy.

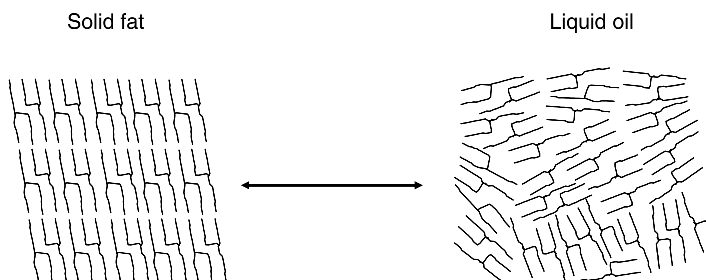



**FIGURE 4.10** The arrangement of triacylglycerols in the solid and liquid states depends on a balance between the organizing influence of the attractive interactions between the molecules and the disorganizing influence of the thermal energy.

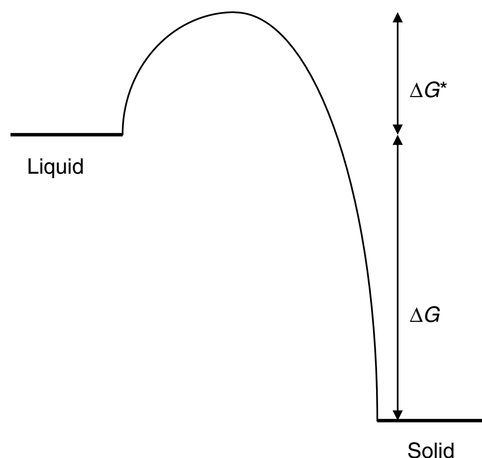



**FIGURE 4.11** When the activation energy associated with nuclei formation is sufficiently high then a liquid oil can persist in a metastable state below the melting point of a fat.

A material therefore changes from a solid to a liquid when its temperature is raised above the melting point. A solid-to-liquid transition (melting) is endothermic because energy must be supplied to the system to pull the molecules further apart. Conversely, a liquid-to-solid transition (crystallization) is exothermic because energy is released as the molecules come closer together. Even though the free energy of the solid state is lowest below the melting point, solid crystals may not appear until a liquid oil has been cooled well below the melting point because of a free energy penalty associated with nuclei formation (see below).

Overall, the crystallization of fats can be conveniently divided into a number of stages: supercooling, nucleation, crystal growth, and postcrystallization events [19,20,23,40].

#### 4.3.3.1 Supercooling

Although the solid form of a lipid is thermodynamically favorable at temperatures below its melting point, the lipid can persist in the liquid form below the melting point for a considerable period before any crystallization is observed. This is because of an activation energy associated with nuclei formation ($\Delta G^*$) that must be overcome before the liquid–solid phase transition can occur (Figure 4.11). If the magnitude of this activation energy is sufficiently high compared to the thermal energy, crystallization will not occur on an observable timescale, and the system exists in a *metastable* state. The height of the activation energy depends on the ability of crystal nuclei to be formed in the liquid oil that are stable enough to grow into crystals (see below). The degree of supercooling of a liquid can be defined as $\Delta T = T - T_{\mathrm{mp}}$, where $T$ is the temperature and $T_{\mathrm{mp}}$ is the melting point. The value of $\Delta T$ at which crystallization is first observed depends on the chemical structure of the lipid, the presence of any contaminating materials, the cooling rate, the microstructure of the lipid phase (e.g., bulk vs. emulsified oil), and the application of external forces [19,23]. Pure oils containing no impurities can often be supercooled by more than 10°C before any crystallization is observed [41].

#### 4.3.3.2 Nucleation

Crystal growth can only occur after stable nuclei have been formed in a liquid. These nuclei are believed to be clusters of oil molecules that form small-ordered crystallites, and are created when a number of lipid molecules collide and become associated with each other [19]. There is a free energy

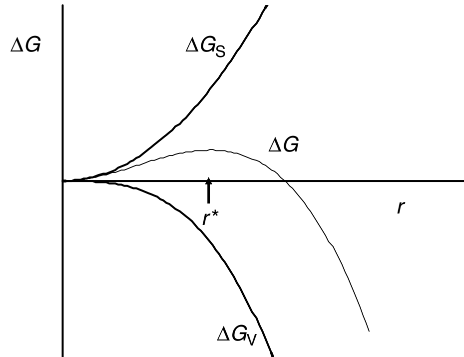



**FIGURE 4.12** The critical size of a nucleus required for crystal growth depends on a balance between the volume and surface contributions to the free energy of nuclei formation. Nuclei that are spontaneously formed with radii below $r^*$ grow, whereas those formed with radii below this value dissociate.

change associated with the formation of one of these nuclei (Figure 4.12). There is a negative free energy ($\Delta G_V$) change that is proportional to the volume of the nucleus formed, which is because of the enthalpy and entropy changes that occur in the interior of the nucleus owing to the phase transition. On the other hand, the formation of a nucleus leads to the creation of a new interface between the solid and liquid phases, and this process involves an increase in free energy to overcome the interfacial tension. This positive free energy ($\Delta G_S$) change is proportional to the surface area of the nucleus formed. The total free energy change associated with the formation of a nucleus is therefore a combination of a volume and a surface term [19,23]:

$$\Delta G = \Delta G_V + \Delta G_S = \frac{4}{3}\pi r^3 \frac{\Delta H_{fus} \Delta T}{T_{mp}} + 4\pi r^2 \gamma_i \tag{4.4}$$

where $r$ is the radius of the nuclei, $\Delta H_{fus}$ is the enthalpy change per unit volume associated with the liquid–solid transition (which is negative), and $\gamma_i$ is the solid–liquid interfacial tension. The volume contribution becomes increasingly negative as the size of the nuclei increases, whereas the surface contribution becomes increasingly positive (Figure 4.12). Since the surface area to volume ratio decreases with increasing size, the surface contribution tends to dominate for small nuclei, while the volume contribution tends to dominate for large nuclei. As a result, the overall free energy change associated with nuclei formation has a maximum value at a critical nucleus radius ($r^*$):

$$r^* = \frac{2\gamma_i T_{mp}}{\Delta H_{fus} \Delta T} \tag{4.5}$$

If a nucleus is spontaneously formed that has a radius that is below this critical size, then it will tend to dissociate so as to reduce the free energy of the system. On the other hand, if a nucleus is formed that has a radius that is above this critical value, then it will tend to grow into a crystal. This equation indicates that the critical size of nuclei required for crystal growth decreases as the degree of supercooling increases, which accounts for the increase in nucleation rate that is observed experimentally when the temperature is decreased. Practically, this means that liquid oils must be cooled appreciably below their thermodynamic melting points before crystal formation is observed.

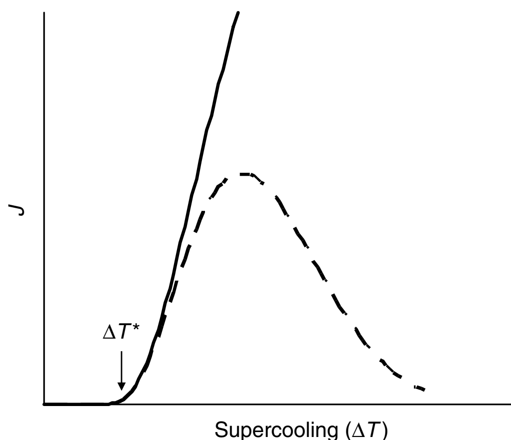



**FIGURE 4.13** Theoretically, the rate of the formation of stable nuclei increases with supercooling (solid line), but in practice, the nucleation rate decreases below a particular temperature because the diffusion of oil molecules is retarded by the increase in oil viscosity (broken line).

The rate at which nucleation occurs can be mathematically related to the activation energy $\Delta G^*$ that must be overcome before stable nuclei are formed [19]:

$$J = A\exp(-\Delta G^*/kT) \tag{4.6}$$

where $J$ is the nucleation rate, which is equal to the number of stable nuclei formed per second per unit volume of material, $A$ is a preexponential factor, $k$ is Boltzmann's constant, and $T$ is the absolute temperature. The value of $\Delta G^*$ is calculated by replacing $r$ in Equation 4.4 with the critical radius given in Equation 4.5. The variation of the nucleation rate predicted by Equation 4.5 with the degree of supercooling ($\Delta T$) is shown in Figure 4.13. The formation of stable nuclei is negligibly slow at temperatures just below the melting point, but increases dramatically when the liquid is cooled below a certain temperature, $T^*$. In reality, the nucleation rate is observed to increase with the degree of cooling down to a certain temperature, after which it decreases on further cooling. This is because the increase in viscosity of the oil that occurs as the temperature is decreased slows down the diffusion of lipid molecules toward the liquid–nucleus interface [19,42]. Consequently, there is a maximum in the nucleation rate at a particular temperature (Figure 4.13).

The type of nucleation described above occurs when there are no impurities present in the oil, and is usually referred to as *homogeneous nucleation* [19]. If the liquid oil is in contact with foreign surfaces, such as the surfaces of dust particles, fat crystals, oil droplets, air bubbles, reverse micelles, or the vessel containing the oil, then nucleation can be induced at a higher temperature than expected for a pure system [19,23,43]. Nucleation owing to the presence of these foreign surfaces is referred to as *heterogeneous nucleation*, and can be divided into two types: primary and secondary. Primary heterogeneous nucleation occurs when the foreign surfaces have a different chemical structure to that of the oil, whereas secondary heterogeneous nucleation occurs when the foreign surfaces are crystals with the same chemical structure as the liquid oil. Secondary heterogeneous nucleation is the basis for "seeding" nucleation in supercooled lipids [19]. This process involves adding preformed triacylglycerol crystals to a supercooled liquid comprising the same triacylglycerol so as to promote nucleation at a higher temperature than would otherwise be possible.

Heterogeneous nucleation occurs when the impurities provide a surface where the formation of stable nuclei is more thermodynamically favorable than in the pure oil. As a result, the degree of supercooling required to initiate fat crystallization is reduced. On the other hand, certain types of impurities are capable of decreasing the nucleation rate of oils because they are incorporated into

the surface of the growing nuclei and prevent any further oil molecules from being incorporated [19]. Whether an impurity acts as a catalyst or an inhibitor of nucleation depends on its molecular structure and interactions with the nuclei [42,44]. It should be noted that there is still considerable debate about the mathematical modeling of nucleation, since existing theories often give predictions of nucleation rates that are greatly different from experimental measurements [23]. Nevertheless, the general form of the dependence of nucleation rates on temperature are predicted fairly well by existing theories (see Figure 4.13).

#### 4.3.3.3 Crystal Growth

Once stable nuclei have formed they grow into crystals by incorporating molecules from the liquid oil at the solid–liquid interface [19,23,42]. Lipid crystals have a number of different faces, and each face may grow at an appreciably different rate, which partially accounts for the wide variety of different crystal morphologies that can be formed by food lipids. The overall crystal growth rate depends on several factors, including mass transfer of the molecules from the liquid phase to the solid–liquid interface, incorporation of the molecules within the crystal lattice, and removal of the heat generated by the crystallization process from the interface [19]. Environmental or system conditions such as viscosity, thermal conductivity, crystal structure, temperature profile, and mechanical agitation can influence the heat and mass transfer processes and therefore the rate of crystal growth. The crystal growth rate tends to increase initially with increasing degree of supercooling until it reaches a maximum rate, after which it decreases [19]. The dependence of the growth rate on temperature therefore shows a similar trend to the nucleation rate, however, the maximum rate of nuclei formation usually occurs at a different temperature to the maximum rate of crystal growth (Figure 4.14). This difference accounts for the dependence of the number and size of crystals produced on the cooling rate and holding temperature. If a liquid oil is cooled rapidly to a temperature where the nucleation rate is slower than the growth rate, then there will be a small number of large crystals formed. On the other hand, if a liquid oil is cooled to a temperature where the growth rate is slower than the nucleation rate, then there will be a large number of small crystals formed.

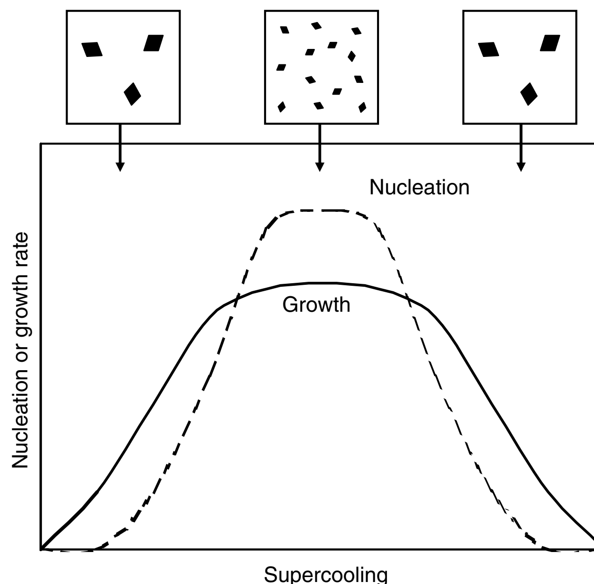



**FIGURE 4.14** The nucleation and crystal growth rates have different temperature-dependencies, which account for differences in the number and size of fat crystals produced under different cooling regimes.

#### 4.3.3.4 Postcrystallization Events

Once the crystals have been formed in a lipid system further changes in their packing, size, composition, and interactions can occur, even though the overall SFC may remain constant [19,23]. Postcrystallization may involve a change from a less stable to a more stable polymorphic form because of the rearrangement of the triacylglycerol molecules within the crystals. If a lipid forms mixed crystals (i.e., crystals that contain a mixture of different types of triacylglycerols), then there may be a change in the composition of the crystals during storage because of the diffusion of triacylglycerol molecules between the crystals. There may also be a net growth in the average size of the crystals within a lipid with time owing to Ostwald ripening, which is the growth of the large crystals at the expense of the smaller ones because of diffusion of lipid molecules between the crystals [19]. Finally, the bonds between fat crystals may strengthen over time during storage owing to a sintering mechanism (i.e., fusion of the crystals together) [23,27,28]. These postcrystallization changes can have pronounced influences on the bulk physicochemical and sensory properties of foods and therefore it is important to understand and control them. For example, postcrystallization events often lead to an increase in the size of the crystals in a lipid, which is undesirable in many cases because it leads to a gritty perception during consumption [43].

### 4.3.4 Crystal Structure

#### 4.3.4.1 Morphology

The term "morphology" refers to the size, shape, and location of the crystals formed when a lipid crystallizes. The morphology of the crystals depends on a number of internal (e.g., molecular structure, composition, packing, and interactions) and external factors (e.g., temperature–time profile, mechanical agitation, and impurities). In general, when a liquid oil is cooled rapidly to a temperature well below its melting point a large number of small crystals are formed, but when it is cooled slowly to a temperature just below its melting point a smaller number of larger crystals are formed [19,23]. This is because of differences in the temperature dependence of the nucleation and crystallization rates (Figure 4.14). The nucleation rate tends to increase more rapidly with decreasing temperature than the crystallization rate up to a certain maximum value, and then it tends to decrease more rapidly with a further decrease in temperature. Thus, rapid cooling tends to produce many nuclei simultaneously that subsequently grow into small crystals, whereas slow cooling tends to produce a smaller number of nuclei that have time to grow into larger crystals before further nuclei are formed (Figure 4.14).

The structure and physical properties of crystals produced by cooling a complex mixture of triacylglycerols is also strongly influenced by the cooling rate and temperature [19,23,31]. If an oil is cooled rapidly, all the triacylglycerols crystallize at approximately the same time and a *solid solution* is formed, which consists of homogeneous crystals in which the triacylglycerols are intimately mixed with each other [22,23]. On the other hand, if the oil is cooled slowly, the higher melting point triacylglycerols crystallize first, while the low melting point triacylglycerols crystallize later, and so *mixed crystals* are formed. These crystals are heterogeneous and consist of some regions that are rich in high melting point triacylglycerols and other regions that are depleted in these triacylglycerols. Whether a fat forms mixed crystals or a solid solution influences many of its physicochemical properties, such as density, rheology, and melting profile [22,23], that could have an important influence on the properties of a food product.

#### 4.3.4.2 Polymorphism

Triacylglycerols exhibit a phenomenon known as (monotropic) *polymorphism*, which is the ability of a material to exist in a number of crystalline structures with different molecular packing [19,23]. The three most commonly occurring types of packing in triacylglycerols are hexagonal,

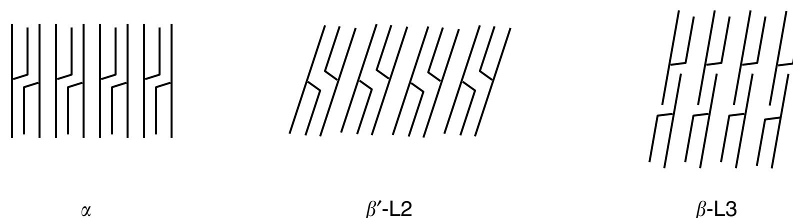



**FIGURE 4.15** Common types of overall molecular organization of triacylglycerols within crystalline phases. (Adapted from Walstra, P. (2003). *Physical Chemistry of Foods*, Marcel Dekker, Inc., New York, NY.)

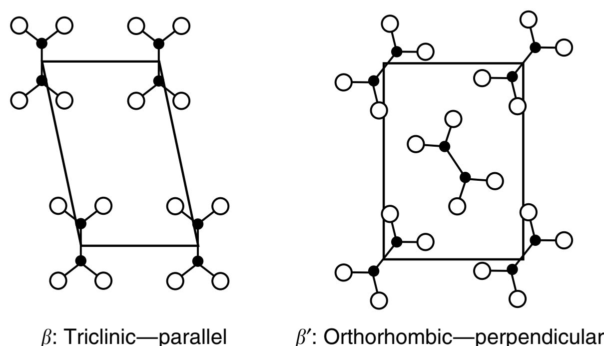



**FIGURE 4.16** Two most common packing types of hydrocarbon chains: Triclinic (parallel) and orthorhombic (perpendicular). The black circles represent carbon atoms and the white circles represent hydrogen atoms. The hydrocarbon chains are viewed from the top. (Adapted from Larsson, K. (2004). In *Food Emulsions*, 4th edn. (Friberg, S., Larsson, K., and Sjoblom, J., eds.), Marcel Dekker, Inc., New York, NY, chap. 3.)

orthorhombic, and triclinic, which are usually designated as $\alpha$, $\beta'$, and $\beta$ polymorphic forms, respectively (Figures 4.15 and 4.16). The type of crystalline form adopted depends on the molecular structure and composition of the lipids, as well as the environmental conditions during crystallization (cooling rate, holding temperature, shearing). The thermodynamic stability, and thus the melting point, of the three forms decreases in the order: $\beta > \beta' > \alpha$. Greater stability derives from greater packing density of fatty acyl groups and this is favored by homogeneity among the constitutive fatty acids and symmetry among the triacylglycerol species. Compatibility and segregation of fatty acids into the crystal lattice may give rise to unit cells with long spacings equivalent to either two or three fatty acid lengths in dimension (L2 and L3 in Figure 4.15). Even though the $\beta$ form is the most thermodynamically stable, triacylglycerols often crystallize in the $\alpha$ form initially because it has the lowest activation energy for nuclei formation (Figure 4.17). With time the crystals transform to the most stable polymorphic form at a rate that depends on environmental conditions, such as temperature, pressure, and the presence of impurities [27]. The time taken for this type of crystal transformation to occur is strongly influenced by the homogeneity of the triacylglycerol composition [23]. The transition from the $\alpha$ form tends to occur fairly rapidly for relatively homogeneous compositions where the triacylglycerols all have fairly similar molecular structures. On the other hand, the transition is relatively slow for multicomponent fats where the triacylglycerols have diverse molecular structures. The different types of polymorphic forms of lipids can be distinguished from each other using a variety of methods, including x-ray diffraction, DSC, IR, NMR, and Raman spectroscopy [19]. These methods are largely based on the fact that the crystals are organized differently in different polymorphic forms that alter their physicochemical and structural properties (Figures 4.15, 4.16, and 4.18). Knowledge of the polymorphic form of the crystals in lipids is often important because it can

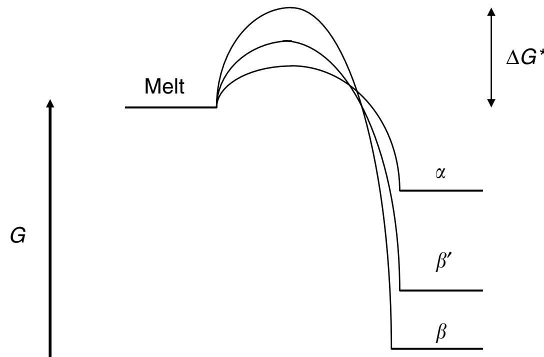



**FIGURE 4.17** The polymorphic state that is initially formed when an oil crystallizes depends on the relative magnitude of the activation energies associated with nuclei formation.

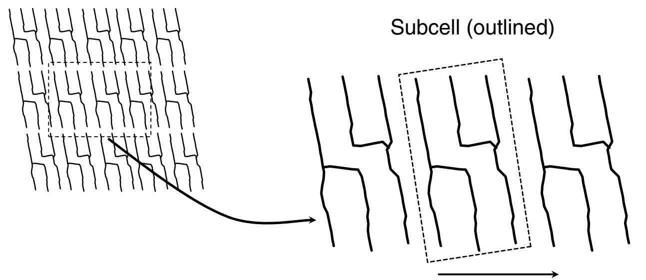



**FIGURE 4.18** The unit cells in crystalline lipids can be characterized by their dimensions.

have a large impact on the thermal behavior and morphology of the crystals formed, and therefore on the physicochemical and sensory properties of foods. For example, the desirable textural characteristics and appearance of products such as margarine, spreads, baked goods, and chocolate depend on ensuring that the fat crystals are produced and maintained in the appropriate polymorphic form [19,38,43]. Finer $\beta'$ crystals are preferred in margarines and spreads, where smoothness, gloss, and a high degree of surface coverage of dispersed water is required. Larger $\beta$ polymorphic forms are often preferred in bakery shortening (e.g., lard) to create "flakiness" and cocoa butter stability in chocolate. Blending of lipids may also be used to control whether the $\beta'$ or $\beta$ polymorphs are the predominate crystal habits formed. Table 4.2 shows which edible lipids tend to form $\beta'$ or $\beta$ crystals as the most stable polymorph.

## 4.4 LIPID PROCESSING: ISOLATION, PURIFICATION, AND MODIFICATION

### 4.4.1 Lipid Refining

Triacylglycerols are extracted from both plant and animal sources. *Rendering* is a thermal processing operation that breaks down cellular structures to release the triacylglycerols from animal byproducts and underutilized fish species. Plant triacylglycerols can be isolated by pressing (olives) or solvent extraction (oilseeds) or a combination of the two (for detailed discussion of fat and oil extraction see Reference 45). The resulting crude oils and fats from these processes will not only contain triacylglycerols but also lipids (such as free fatty acids, phospholipids, lipid-soluble off-flavors, and carotenoids) as well as nonlipid materials (such as proteins and carbohydrates). These components

must be removed to produce oils and fats with the desired color, flavor, and shelf-life. The major refining steps are described below.

#### 4.4.1.1 Degumming

The presence of phospholipids will cause the formation of water-in-oil (W/O) emulsions in fats and oils. These emulsions will make the oil cloudy and the water can present a hazard when the oils are heated to temperatures above 100°C (spattering and foaming). Degumming is a process that removes phospholipids by the addition of 1–3% water at 60–80°C for 30–60 min. Small amounts of acid are often added to the water to increase the hydrogen of the phospholipids in it. Settling, filtering, or centrifugation is then used to remove the coalesced "gums" formed by the phospholipids and water. With oils such as soybean, the phospholipids are recovered and sold as lecithin.

#### 4.4.1.2 Neutralization

Free fatty acids must be removed from crude oils because they can cause off-flavors, accelerate lipid oxidation, cause foaming, and interfere with hydrogenation and interesterification operations. Neutralization is accomplished by mixing a solution of caustic soda with the crude oil, which causes the free fatty acids to form soluble soaps that can be removed by separating the oil phase from the water phase containing the soaps. The amount of caustic soda used is dependent on the free fatty acid concentrations in the crude oil. The resulting soap stock can be used as animal feed or to produce surfactants and detergents.

#### 4.4.1.3 Bleaching

Crude oils often contain pigments that produce undesirable colors (carotenoids, gossypol, etc.) or promote lipid oxidation (chlorophyll). Pigments are removed by mixing the hot oil (80–110°C) with absorbents such as neutral clays, synthetic silicates, activated carbon, or activated earths. The absorbent is then removed by filtration. This process is usually done under vacuum since absorbents can accelerate lipid oxidation. An added benefit of bleaching is the removal of residual free fatty acids and phospholipids and the breakdown of lipid hydroperoxides.

#### 4.4.1.4 Deodorization

Crude lipids contain undesirable aroma compounds such as aldehydes, ketones, and alcohols that occur naturally in the oil or are produced from lipid oxidation reactions that occur during extraction and refining. These volatile compounds are removed by subjecting the oil to steam distillation at high temperatures (180–270°C) and low pressures. Deodorization processes can also breakdown lipid hydroperoxides to increase the oxidative stability of the oil but may result in the formation of *trans* fatty acids. After deodorization is complete, citric acid (0.005–0.01%) is added to chelate and inactivate prooxidant metals. Deodorizer distillate will contain tocopherols and sterols that can be recovered and used as antioxidants and functional food ingredients (phytosterols).

### 4.4.2 Altering the SFC of Food Lipids

Natural fats with desirable plastic ranges are not always available and are sometimes expensive. In addition, alteration of fatty acid profiles is often desirable to make the fat less susceptible to oxidation (decrease unsaturation) or more nutritionally desirable (increase unsaturation). Therefore, several technologies have been developed to alter the SFC of food lipids.

#### 4.4.2.1 Blending

The simplest method to alter fatty acid composition and melting profile is by blending fats with different triacylglycerol compositions. This practice is performed in products such as frying oils and margarines.

#### 4.4.2.2 Dietary Interventions

The fatty acid composition of animal fats can be altered by manipulation of the type of fats in the diet. This practice is effective in nonruminants such as pigs, poultry, and fish. Increasing the levels of unsaturated fatty acids in fats from ruminants (cows and sheep) is not very efficient because bacteria in the rumen will biohydrogenate the fatty acids before they reach the small intestine where they can be absorbed into the blood.

#### 4.4.2.3 Genetic Manipulation

The fatty acid composition of fats can be manipulated genetically by altering the enzyme pathways that produce unsaturated fatty acids. Genetic manipulation has been done successfully by both traditional breeding programs and by genetic modification technologies. Several oils that have been obtained from genetically altered plants such as sunflowers are commercially available. Most of these oils contain elevated levels of oleic acid.

#### 4.4.2.4 Fractionation

The fatty acid and triacylglycerol composition of fats can also be altered by holding the fat at a temperature where the most saturated or long-chain triacylglycerols will crystallize and then collecting either the solid (more saturated or long-chain) or liquid (more unsaturated or short-chain) phases. This is commonly done to vegetable oils in a process called *winterization*. Winterization is necessary for oils used in products that are refrigerated to prevent the triacylglycerols from crystallizing and becoming cloudy. Winterization is also necessary for oils used in mayonnaise or salad dressings where crystallization would destabilize the emulsion.

#### 4.4.2.5 Hydrogenation

Hydrogenation is a chemical process that adds hydrogen to double bonds. The process is used to alter lipids so they are more solid at room temperature, exhibit different crystallization behavior (by making the triacylglycerol composition more homogenous), and/or are more oxidatively stable. These goals are accomplished by the removal of double bonds to make the fatty acids more saturated. An additional use of hydrogenation is to bleach oils since the destruction of double bonds in compounds such as carotenoids will cause them to lose color. Products produced by hydrogenation include margarines, shortenings, and partially hydrogenated oils that have improved oxidative stability.

The hydrogenation reaction requires a catalyst to speed up the reaction, hydrogen gas to provide the substrate, temperature control to initially heat up the oil to make it liquid and then cool the oil once the exothermic reaction is started, and agitation to mix the catalyst and substrates [45]. The oil used in hydrogenation must first be refined since contaminants will reduce the effectiveness of, or "poison," the catalysts. Hydrogenation is done as a batch or continuous process at temperatures ranging from 250°C to 300°C. Reduced nickel is the most common catalyst that is added at 0.01–0.02%. The nickel is incorporated onto a porous support to provide a catalyst with high surface area that can be recovered by filtration. Continuous mixing is a critical parameter since mass transfer of the reactants limits the reaction. The reaction takes 40–60 min during which progress is monitored by change in

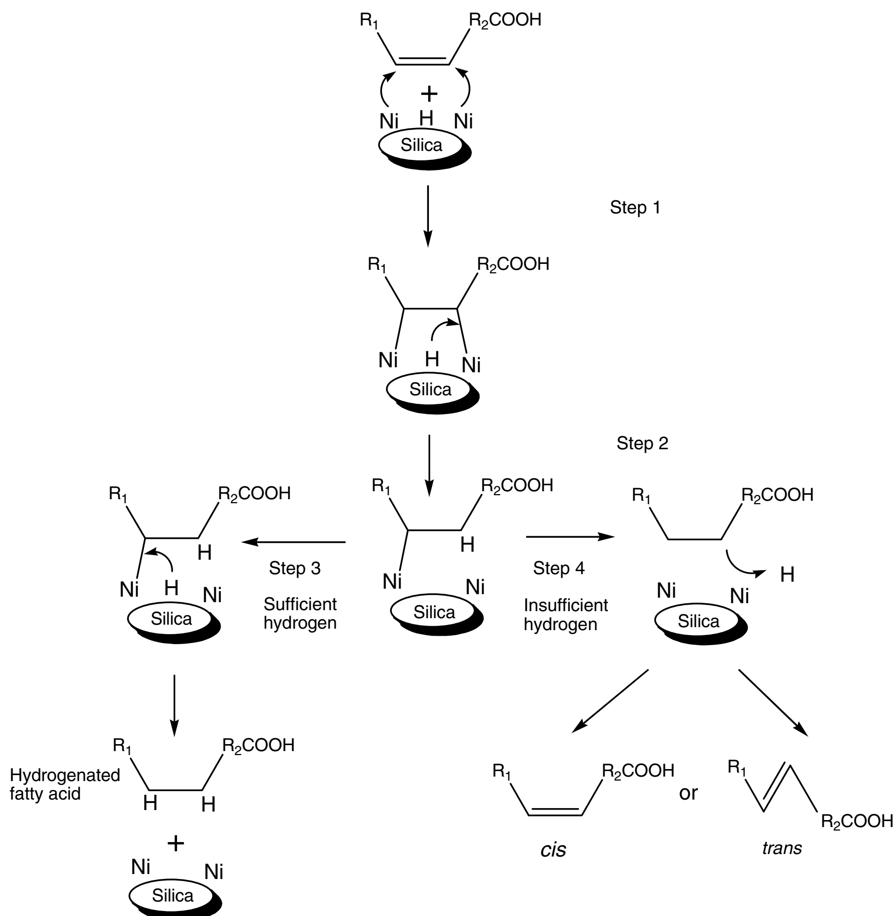



**FIGURE 4.19** The pathways involved in hydrogenation that led to formation of saturated fatty acids and *cis* and *trans* unsaturated fatty acids.

refractive index. Upon completion, catalysts are recovered by filtration so that they can be used in another reaction.

The mechanism of hydrogenation involves initial complexation of the unsaturated fatty acid with the catalyst at each end of the double bond (Figure 4.19, step 1). Hydrogen that is absorbed to the catalysts can then break one of the carbon–metal complexes to form a half-hydrogenated state with the other carbon remaining linked to the catalyst (step 2). To complete hydrogenation, the half-hydrogenated state interacts with another hydrogen to break the remaining carbon–catalyst bond to produce a hydrogenated fatty acid (step 3). However, if hydrogen is not available, the reverse reaction can occur and the fatty acid is released from the catalyst and the double bond reforms (step 4). The double bond that reforms can be in the *cis* or *trans* configuration (geometric isomers) and can be at the same carbon number or it can migrate to the adjacent carbon (e.g., a fatty acid with a double bond originally between carbons 9 and 10 can migrate to carbons 8 and 9 or 10 and 11; positional isomers). The propensity of the double bond to reform is related to the concentration of hydrogen associated with the catalyst. Thus, conditions such as low hydrogen pressure, low agitation, high temperature (reaction is faster than rate of hydrogen diffusion to the catalyst), and high catalyst concentrations (difficult to saturate catalyst with hydrogen) result in high levels of geometric and positional isomers. This can be problematic since dietary *trans* fatty acids is associated with increased cardiovascular disease risk.

Hydrogenation often proceeds in a selective and sequential manner. The rate of hydrogenation of polyunsaturated fatty acids is faster than monounsaturated fatty acids. This is partially due to the higher catalyst affinity for pentadiene double bond systems in polyunsaturated fatty acids than for monounsaturated fatty acids. Preferential hydrogenation of the most unsaturated fatty acid is especially prevalent when hydrogen concentration at the catalyst is low. From a stability standpoint, hydrogenation of the most unsaturated fatty acids first is often desirable since this increases the oxidative stability of the oil with minimal formation of high temperature melting saturated triacylglycerols that cause problems with crystallization and texture. However, low hydrogen concentrations can also lead to high production of geometric and positional isomers meaning that the lipid can contain high amounts of *trans* fatty acids, which is nutritionally undesirable.

#### 4.4.2.6 Interesterification

Interesterification is a process that involves the rearranging of acyl groups in triacylglycerols. Generally, this is a random process that results in production of a triacylglycerol profile different from that of the original lipid. This results in significant changes in the melting profiles of lipids without changing fatty acid composition [46]. Interesterification also alters crystallization behavior of the fat by making it more difficult for the lipids to form the most stable crystal type ($\beta$, triclinic) since the triacylglycerol composition becomes more heterogeneous. Interesterification is performed by acidolysis, alcoholysis, glycerolysis, and transesterification [46]. Transesterification is the most common method used to alter the properties of food lipids. In this process, alkylates of sodium (e.g., sodium ethylate) are commonly used to accelerate transesterification since they are inexpensive and active at low temperatures. The real catalyst for the reaction is thought to be a carbonyl anion of a diacylglycerol (Figure 4.20). The negative diacylglycerol can attack the slightly positive carbonyl group of a fatty acid on a triacylglycerol to form a transition complex. Upon transesterification the transition complex decomposes in such a way that the fatty acid is transferred to the diacylglycerols and the anion migrates to the site of the transferred fatty acid. The transesterification process can occur within the same (intraesterification) or a different (interesterification) triacylglycerol. For interesterification to take place the reaction medium must have, low levels of water, free fatty acids, and peroxides (that deactivate the catalyst). Random transesterification is performed at 100–150°C and is complete in 30–60 min. The reaction is stopped by the addition of water to inactivate the catalyst.

Interesterification can be performed on mixtures of lipids such as a fat with a high temperature melting range and an oil with a low temperature melting range. If these two lipid sources were simply blended, their melting profile could have a discontinuous, stair-stepped SFC curve (cf. Figure 4.9) as the blend is progressively heated. Interesterification of these two lipids would create new triacylglycerols containing combinations of both saturated and unsaturated fatty acids and having gradual melting throughout the plastic range. Another application would be to interesterify a fat with a very homogeneous triacylglycerol composition to produce heterogeneous triacylglycerols; this would widen the plastic range and prompt $\beta'$ (orthorhombic) crystals as the most stable polymorphs.

Interesterification is not always random. In directed interesterification, the reaction temperature is held low enough so that when highly saturated triacylglycerols are produced, they crystallize and are removed from participation in the reaction. This process would produce a liquid phase that is more unsaturated and a solid phase that is more saturated than the parent lipid. Interesterification can also be performed by using lipases as catalysts [47]. The advantage of lipases is that they can have specificity for different stereospecific locations on the triacylglycerol or specificity for different fatty acids. This means that structured triacylglycerols can be produced with changes in fatty acid composition or triacylglycerol type (e.g., changes at *sn*-2 position). By altering fatty acid and/or triacylglycerol composition these fats may have superior nutritional or physical properties. Unfortunately, enzymatic interesterification is limited by its high cost and its application is limited to products of high value such as cocoa butter substitutes and infant formula lipids.

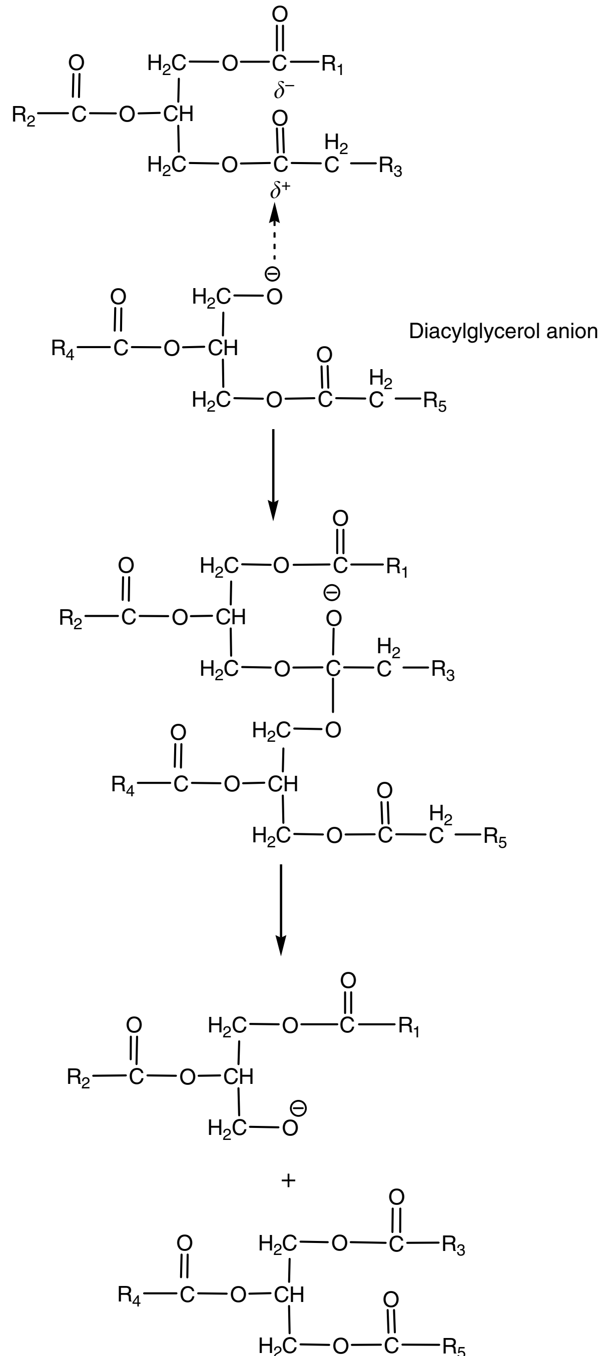



**FIGURE 4.20** The proposed mechanism of the interesterification reaction involving catalysis by the carbonyl anion of a diacylglycerol. (Adapted from Rousseau, D. and Marangoni, A.G. (2002). In *Food Lipids, Chemistry, Nutrition and Biotechnology* (Akoh, C.C. and Min, D.B., eds.), Marcel Dekker, Inc., New York, NY, pp. 301–334.)

## 4.5 FUNCTIONALITY OF TRIACYLGLYCEROLS IN FOODS

The ability of food scientists to improve the quality of food products depends on an improved understanding of the multiple roles that fats and oils play in determining their properties. This section highlights some of the most important roles that lipids play in determining the texture, appearance, and flavor of food products, using specific examples to highlight important aspects of triacylglycerol functionality.

### 4.5.1 TEXTURE

The influence of lipids on the texture of foods is largely determined by the physical state of the lipid and the nature of the food matrix (e.g., bulk fat, emulsified fat, or structural fat). For bulk liquid oils, such as cooking or salad oils, the texture is determined primarily by the viscosity of the oil over the temperature range of utilization. For partially crystalline fats, such as in chocolate, baked products, shortenings, butter, and margarine, the texture is mainly determined by the concentration, morphology, and interactions of the fat crystals [19,23,43]. In particular, the melting profile of the fat crystals plays a major role in determining properties such as texture, stability, spreadability, and mouthfeel. The characteristic creamy texture of many O/W food emulsions is determined by the presence of fat droplets (e.g., creams, desserts, dressings, and mayonnaise). In these systems, the viscosity of the overall system is determined mainly by the concentration of oil droplets present rather than by the viscosity of the oil within the droplets [33]. For example, whole milk (~4% fat) has a relatively low viscosity, heavy cream (~40% fat) is highly viscous, and mayonnaise (~80% fat) is semisolid, even though the viscosity of the oil phase within the droplets may be fairly similar among these products. In W/O food emulsions, the overall rheology of the system is largely determined by the rheology of the oil phase. In most food W/O emulsions, such as margarine, butter, and spreads, the oil phase is partially crystalline and has plastic-like properties. The rheology of these products is therefore determined by the SFC and the morphology and interactions of the fat crystals present, which in turn is governed by the crystallization and storage conditions (see below). For example, the "spreadability" of these products is determined by the formation of a three-dimensional network of aggregated fat crystals in the continuous phase that provides the product with mechanical rigidity [31,48]. In many foods, the lipids form an integral part of a solid matrix that also contains various other components (e.g., chocolate, cakes, cookies, biscuits, cheese). The physical state of the lipids in these systems impacts their texture by forming a network of interacting fat crystals that gives the final product desirable rheological properties, such as firmness or snap. The presence of the fat phase in these products may also influence the overall texture in a variety of other ways and a few examples of food products where lipids play a major role on their texture are given below.

Margarine production is a good example of the importance of lipid crystallization on determining the overall texture of food products. Initially, the manufacturer must select a lipid phase that contains a blend of triacylglycerols that will provide the appropriate SFC–temperature profile and crystal morphology in the final product. This lipid phase is then homogenized in its liquid state with an aqueous phase to form an O/W emulsion. This emulsion is then processed under carefully controlled time–temperature–shear conditions to obtain the desired extent of crystallization, crystal size, polymorphic form, and degree of crystal interaction [38]. Ideally, the final product should contain a three-dimensional network of small aggregated crystals in the $\beta'$ polymorphic form, as this provides the desired textural and stability characteristics. Margarine production is usually carried out by passing the O/W emulsion through a "scraped surface heat exchanger" followed by a "crystallizer." In the scraped surface heat exchanger the emulsion is rapidly cooled and exposed to high shear rates, which promotes the rapid formation of fat crystals in the lipid phase. The presence of fat crystals in the oil droplets promotes the conversion of the O/W emulsion into a W/O emulsion owing to partial coalescence [23]. The W/O emulsion consists of water droplets embedded in a lipid

phase that contains a network of aggregated crystals. The fat crystals formed are initially in the $\alpha$ polymorphic form but are converted to the more stable $\beta'$ polymorphic form during the crystallization step. It is important to control the extent of this transformation during the manufacturing process since this determines the number and strength of the bonds formed between the fat crystals and therefore the rheology of the final product. On the other hand, it is also important to prevent the polymorphic transition from the $\beta'$ form to the more stable $\beta$ form during storage, since this leads to the formation of large crystals (>30 μm) that are perceived as "grainy" or "gritty" in the mouth. This conversion can often be prevented by adding surfactants that interfere with the polymorphic transition [1], choosing lipids that will not form $\beta$ crystals, or by appropriate blending of lipids to favor the $\beta'$ crystal habit.

Another example of the importance of fat crystallization on the texture of food products is provided by shortenings. Shortenings are fats that are used to provide characteristic functional properties to a variety of different food products, including cakes, breads, pastry, fried products, and baked products [43]. These functional properties include tenderness, texture, mouthfeel, structural integrity, lubrication, incorporation of air, heat transfer, and extended shelf-life. Various physicochemical mechanisms underlie these functional properties. Shortenings are named as such because they help to prevent interactions between proteins or starch molecules, which serve to "tenderize" the product by reducing gluten cohesion and "shortening" the texture [43]. They provide textural characteristics in other foods because of their ability to form a three-dimensional fat crystal network. To obtain the desired functional characteristics in a particular product, it is important to choose a blend of fats and oils that gives the appropriate melting profile and polymorphic characteristics, then to process the fat using controlled cooling and shearing conditions to obtain the desired crystal type and structure [43]. It is usually important that the lipid is partially crystalline at storage temperatures so that it maintains its structural integrity, but melts during consumption to give a desirable mouthfeel.

### 4.5.2 Appearance

The characteristic appearance of many food products is strongly influenced by the presence of lipids. The color of bulk oils, such as cooking or salad oils, is mainly determined by the presence of pigment impurities that adsorb light, such as chlorophyll and carotenoids. Solid fats are usually optically opaque because of scattering of light by the fat crystals present, whereas liquid oils are usually optically clear. The opacity of the fat depends on the concentration, size, and shape of the fat crystals present. The turbid, cloudy, or opaque appearance of food emulsions is a direct result of the immiscibility of oil and water, since this leads to a system where the droplets of one phase are dispersed in the other phase. Food emulsions usually appear optically opaque because the light passing through them is scattered by the droplets [49]. The intensity of the scattering depends on the concentration, size, and refractive index of the droplets present, so that both the color and opacity of food emulsions are strongly influenced by the presence of the lipid phase. The reason that whole milk (~4% fat) has a much whiter appearance that skim milk (<0.1% fat) is because it contains milk fat globules that scatter light strongly, whereas skim milk only contains casein micelles that scatter light more weakly.

An interesting example of the importance of fat crystallization on the appearance of food products is "bloom," which is a quality defect sometimes observed in chocolates and coatings [50]. Bloom manifests itself as large white spots or a dull-whitish gray appearance on the surface of the product. A variety of mechanisms have been proposed to account for bloom in different products, all of which are due to some stability problem associated with fat crystallization (e.g., poor tempering, incompatibility of fat blends, fat migration, and fat recrystallization). The physicochemical basis of the various mechanisms have been discussed in detail elsewhere [50]. Bloom is a defect that occurs in some chocolate products that have been exposed to fluctuating temperatures during their storage, since this causes the fat phase to melt and recrystallize [1]. It has been proposed that the fat crystals

at the surface change their morphology from flat smooth crystals that specularly reflect light and give a smooth appearance to spiky crystals that diffusely reflect light and cause a cloudy appearance. Bloom can often be retarded or prevented by using surfactants that limit the crystal transition or by carefully controlling the storage temperature to avoid fat polymorphic phase transitions [1].

### 4.5.3 Flavor

Triacylglycerols are relatively large molecules that have a low volatility and hence little inherent flavor. Nevertheless, edible fats and oils from different natural sources have distinctive flavor profiles because of the presence of characteristic volatile compounds, such as lipid oxidation products and natural impurities. Minor fatty acid constituents can also contribute subtle flavor notes, especially in animal fats (Chapter 10). The flavor of many food products is indirectly influenced by the lipid phase because flavor compounds can partition between oil, water, and gaseous regions within the food matrix according to their polarities and volatilities [33]. For this reason, the perceived aroma and taste of foods are often strongly influenced by the type and concentration of lipids present.

Lipids also influence the mouthfeel of many food products [23]. Liquid oils may coat the tongue during mastication, which provides a characteristic oily mouthfeel. Fat crystals also confer a "grainy" or "gritty" mouthfeel if they are large, and "smoothness in texture" if they are small [38]. The melting of fat crystals in the mouth causes a cooling sensation, which is an important sensory attribute of many fatty foods [22].

## 4.6 CHEMICAL DETERIORATION OF LIPIDS: HYDROLYTIC REACTIONS

Free fatty acids cause problems in foods because they produce off-flavor, reduce oxidative stability, cause foaming, and reduce smoke point (the temperature at which an oil begins to smoke). If the liberation of free fatty acids from the glycerol backbone results in the development of off-flavors (e.g., volatile short-chain free fatty acids that form off-aromas or long-chain fatty acids that form soapy tastes), this is known as *hydrolytic rancidity*. However, short-chain free fatty acids are sometimes desirable in products, such as cheeses, where they contribute to flavor profiles.

Free fatty acids can be liberated from triacylglycerols by enzymes called lipases. In living tissues, the activity of (phospho)lipases is strictly controlled since fatty acids can be cytotoxic by disrupting cellular membrane integrity. During processing and storage of the biological tissues used as raw materials for foods, cellular structures and biochemical control mechanisms may be destroyed and lipases can become active (e.g., can come in contact with lipid substrates). A good example of this is seen in the production of olive oil where the oil from the first pressing has low free fatty acid concentrations. Oils from subsequent pressing and oil extracted from the pomace have higher free fatty acid concentrations as the cellular matrix is further disrupted and the lipases have time to hydrolyze triacylglycerols. Triacylglycerol hydrolysis also occurs in frying oils owing to the high processing temperatures and the introduction of water from the fried food. As the free fatty acid content of the frying oil increases, smoke point and oxidative stability decrease and the tendency for foaming increases. Commercial frying oils are filtered on a regular basis with absorbents that are capable of binding and removing free fatty acids to increase the shelf-life of the oil. Triacylglycerol hydrolysis will also occur at extreme pH values.

## 4.7 CHEMICAL DETERIORATION OF LIPIDS: OXIDATIVE REACTIONS

"Lipid oxidation" is a general term that is used to describe a complex sequence of chemical changes that result from the interaction of lipids with oxygen [51,52]. Triacylglycerols and phospholipids

have low volatility and thus do not directly contribute to the aroma of foods. During lipid oxidation reactions, the fatty acids esterified to triacylglycerols and phospholipids will decompose to form small, volatile molecules that produce the off-aromas known as *oxidative rancidity*. In general, these volatile compounds are detrimental to food quality although there are some food products, such as fried foods, dried cereal, and cheeses, for which small amounts of lipid oxidation products are important positive components for their flavor profile.

### 4.7.1 Mechanisms of Lipid Oxidation

The centerpiece of these reactions is the molecular species known as free radicals. Free radicals are molecules or atoms that have unpaired electrons. Free radical species can vary greatly in their energy. Radicals such as the hydroxyl radical ($^{\bullet}OH$) have very high energy and can oxidize virtually any molecule by causing hydrogen abstraction. Other molecules such as the antioxidant, $\alpha$-tocopherol, can form free radicals with low energy that are less capable of attacking molecules such as unsaturated fatty acids.

The kinetics of lipid oxidation in foods often has a lag phase followed by an exponential increase in oxidation rate (Figure 4.21). The length of the lag phase is very important to food processors since this is the period where rancidity is not detected and the quality of the food is high. Once the exponential phase is reached, lipid oxidation proceeds quickly and off-aroma development occurs rapidly. The length of the lag phase of oxidation will increase with decreasing temperature, oxygen concentrations, degree of fatty acid unsaturation, activity of prooxidants, and increasing concentrations of antioxidants. Figure 4.21 shows how gamma-tocopherol can increase the lag phase of the oxidation of a corn O/W emulsion [53].

Oxidation can occur in both free fatty acids and fatty acyl groups. The pathway of fatty acid oxidation can be described by three general steps: initiation, propagation, and termination.

*Initiation:* This step describes the abstraction of a hydrogen from a fatty acid to form a fatty acid radical known as the alkyl radical ($L^{\bullet}$). Once the alkyl radical forms, the free radical is stabilized by delocalization over the double bond(s) resulting in double bond shifting, and in the case of polyunsaturated fatty acids by the formation of conjugated double bonds. This shift in location can produce double bonds in either the *cis* or *trans* configuration with *trans* predominating because of their greater stability. Figure 4.22 shows the initiation steps for hydrogen abstraction from the

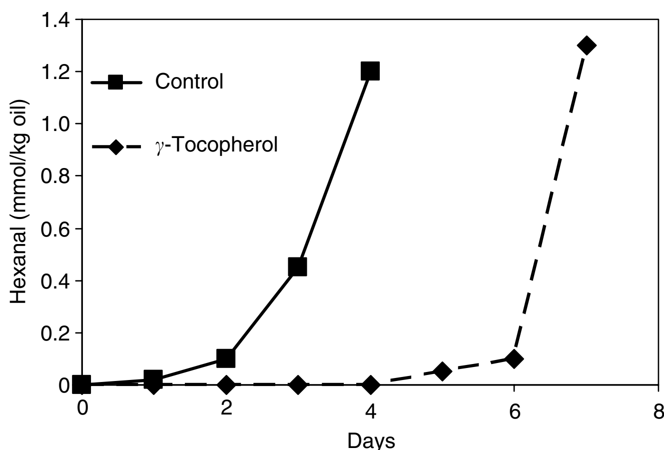



**FIGURE 4.21** The impact of $\gamma$-tocopherol on the lag phase of the oxidation of a corn O/W emulsion. (Adapted from Huang, S.W., Frankel, E.N., and German, J.B. (1994). *J. Agric. Food Chem.* 42: 2108–2114.)

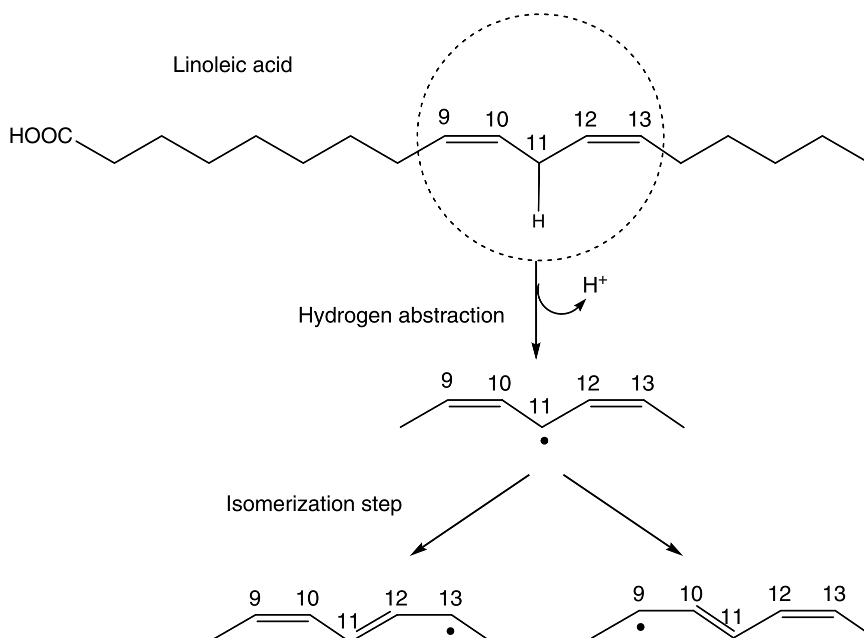



**FIGURE 4.22** The initiation step of lipid oxidation for linoleic acid.

methylene-interrupted carbon of linoleic acid with double bond rearrangement producing two isomers. When hydrogen is abstracted from oleic acid, the alkyl radical can exist at four different locations (Figure 4.23).

The ease of formation of fatty acid radicals increases with increasing unsaturation. The bond dissociation energy for the carbon–hydrogen covalent bond in an aliphatic chain is 98 kcal $mol^{-1}$. If a carbon atom is adjacent to an electron-rich double bond, the carbon–hydrogen covalent bond becomes weaker with the bond dissociation energy decreasing to 89 kcal $mol^{-1}$. In polyunsaturated fatty acids, the double bonds are in a pentadiene configuration with a methylene-interrupted carbon (Figure 4.24). Since the carbon–hydrogen covalent bond of the methylene-interrupted carbon is weakened by two double bonds, its bond dissociation energy is even lower at 80 kcal $mol^{-1}$. As the bond dissociation energy of the carbon–hydrogen bond decreases, hydrogen abstraction becomes easier and lipid oxidation is faster. Linoleic acid (18:2) has been estimated to be 10–40 times more susceptible to oxidation than oleic acid (18:1). As additional double bonds are added onto polyunsaturated fatty acids, an additional methylene-interrupted carbon is added producing another site for hydrogen abstraction. For example, linoleic (18:2) has one methylene-interrupted carbon while linolenic (18:3) has two and arachidonic (20:4) has three (Figure 4.24). In most cases, oxidation rates double with the addition of a methylene-interrupted carbon. Thus, linolenic oxidizes twice as fast as linoleic and arachidonic oxidizes twice as fast as linolenic (four times faster than linoleic).

*Propagation:* The first step of propagation involves the addition of oxygen to the alkyl radical. Atmospheric or triplet oxygen is a biradical because it contains two electrons with the same spin direction that cannot exist in the same spin orbital. The free radicals on triplet oxygen are low energy and will not directly cause hydrogen abstraction. However, the free radicals on oxygen can react with the alkyl radical at a diffusion-limited rate. The combination of the alkyl radical with one of the radicals on triplet oxygen results in the formation of a covalent bond. The other radical on the oxygen remains free. The resulting radical is known as a peroxyl radical ($LOO^{\bullet}$). The high energy of peroxyl radicals allows them to promote the abstraction of a hydrogen from another molecule. Since the carbon–hydrogen covalent bond of unsaturated fatty acids is weak, they are susceptible

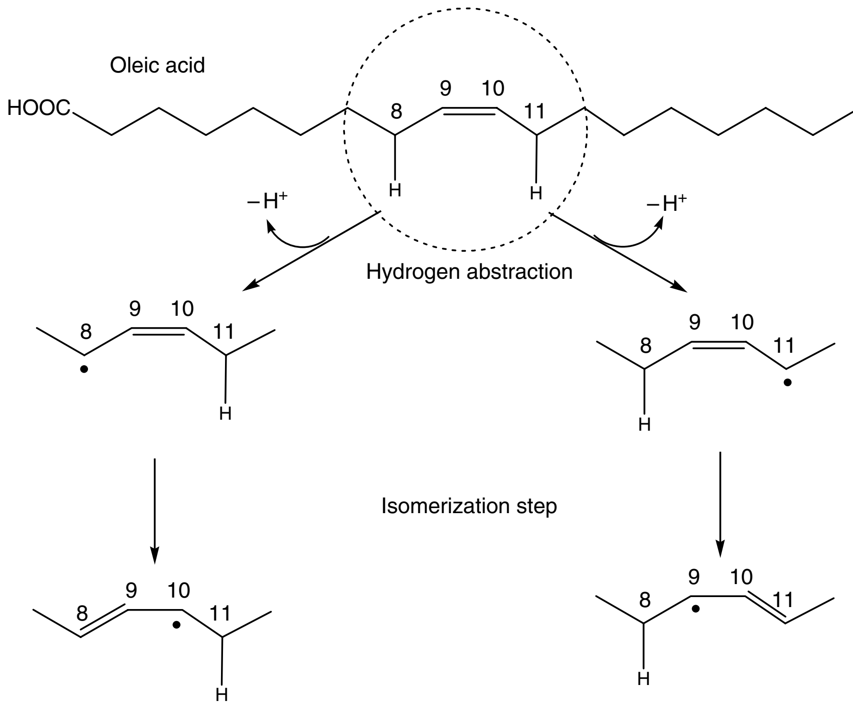



**FIGURE 4.23** The initiation step of lipid oxidation for oleic acid.

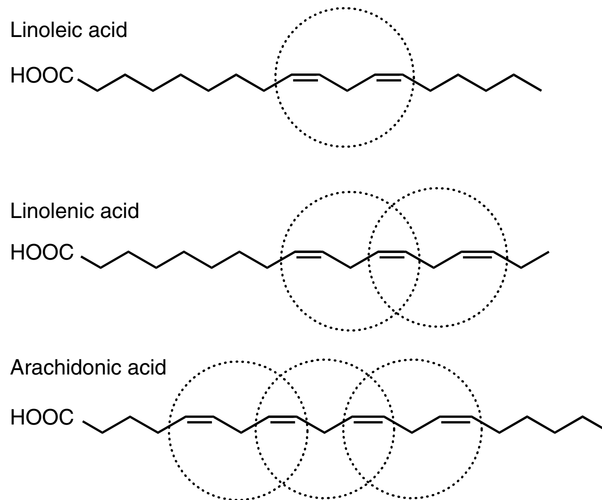



**FIGURE 4.24** The pentadienes of linoleic, linolenic, and arachidonic acids.

to attack from peroxyl radicals. Hydrogen addition to the peroxyl radical results in the formation of a fatty acid hydroperoxide (LOOH) and the formation of a new alkyl radical on another fatty acid. Thus the reaction is propagated from one fatty acid to another. A schematic of this pathway for two linoleic molecules is shown in Figure 4.25. The location of the lipid hydroperoxide will correspond to the location of alkyl radicals (shown in Figures 4.22 and 4.23). Thus, oleate will produce four hydroperoxides, and linoleate will form two.

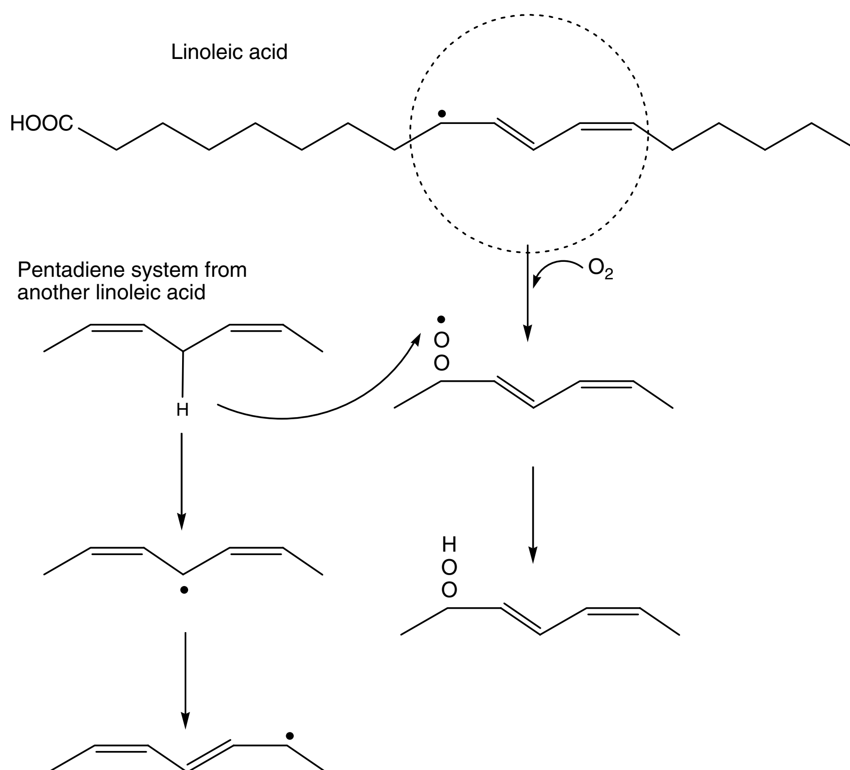



**FIGURE 4.25** The propagation step of lipid oxidation for linoleic acid.

*Termination:* This reaction describes the combination of two radicals to form nonradical species. In the presence of oxygen, the predominant free radical is the peroxyl radical since oxygen will be added onto alkyl radicals at diffusion-limited rates. Thus, under atmospheric conditions, termination reactions may occur between peroxyl and alkoxyl radicals. In low oxygen environments (e.g., frying oils), termination reactions can occur between alkyl radicals to form fatty acid dimers (Figure 4.26). Fatty acid polymers have been used as an indication of frying oil quality [54].

### 4.7.2 Prooxidants

Lipid oxidation is often referred to as autooxidation. The prefix "auto" means "self-acting," thus the term "autooxidation" has been used to describe the self-perpetuating generation of free radicals from unsaturated fatty acids in the presence of oxygen that occurs during lipid oxidation. In the initiation step, abstraction of hydrogen from unsaturated fatty acids results in the production of a single free radical. The addition of oxygen to the alkyl radical to form a peroxyl radical and subsequent abstraction of hydrogen from another fatty acid or antioxidant to form a lipid hydroperoxide in the propagation step does not result in a net increase in free radicals. Thus, if "autooxidation" was the only reaction in lipid oxidation, the formation of oxidation products would increase linearly from time zero. However, in most foods, the lag phase is followed by a rapid exponential increase in oxidation. This indicates that there are other reactions in lipid oxidation that produce additional free radicals.

Prooxidants, which are found in virtually all food systems, are compounds or factors that cause or accelerate lipid oxidation. Many prooxidants are not true catalysts since they are altered during the reaction (e.g., singlet oxygen is converted to a hydroperoxide and ferrous iron is converted to the ferric state). Prooxidants can accelerate lipid oxidation by direct interactions with unsaturated fatty

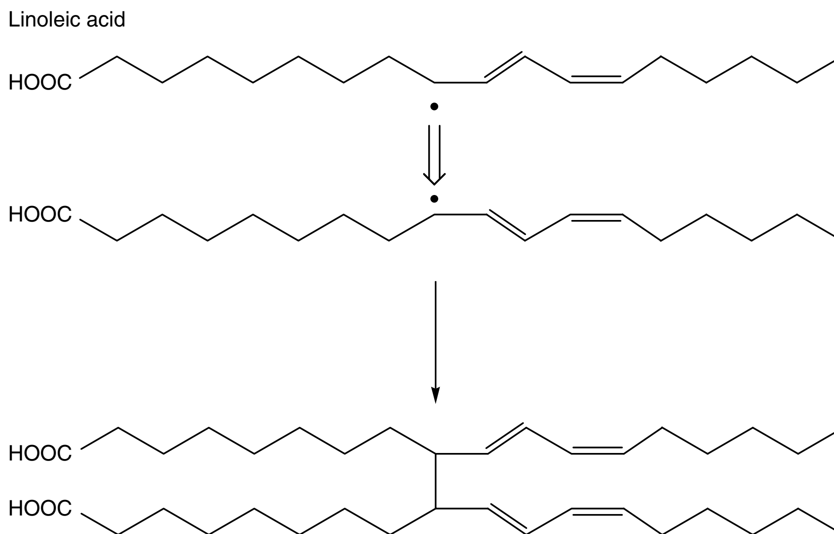



**FIGURE 4.26** An example of a termination step of lipid oxidation under conditions of low oxygen concentrations.

acids to form lipid hydroperoxides (e.g., LOXs and singlet oxygen) or by promoting formation of free radicals (e.g., transition metal or UV-light-promoted hydroperoxide decomposition). It should be noted that lipid hydroperoxides do not contribute to off-aromas and thus do not directly cause rancidity. However, hydroperoxides are important substrates for rancidity since their decomposition often results in the scission of the fatty acid to produce low molecular weight volatile compounds responsible for off-aromas. The major prooxidants in foods are discussed below.

#### 4.7.2.1 Prooxidants That Promote Formation of Lipid Hydroperoxides

*Singlet oxygen:* As mentioned earlier, triplet oxygen ($^3O_2$) is a biradical because its two electrons in the antibonding 2p orbital have the same (parallel or antiparallel) spin direction (Figure 4.27). The Pauli exclusion principle states that two electrons with the same spin direction cannot exist in the same electron orbital. If the electrons in the antibonding 2p orbital have opposite spin directions, oxygen is referred to as singlet oxygen ($^1O_2$). Singlet oxygen can exist in five different configurations with the most common in foods being the $^1\Delta$ state, where the electrons exist in the same orbital (for detailed description see Reference 51). Because singlet oxygen is more electrophilic than triplet oxygen, it can react directly with high electron density double bonds. Since the electrons in singlet oxygen match the spin direction of the electron in double bonds, it can react with an unsaturated fatty acid to directly form lipid hydroperoxides 1500 times faster than triplet oxygen. Singlet oxygen can react with either carbon at the end of a double bond with the double bond then shifting to form a *trans* double bond. This means that oxidation of linoleate by singlet oxygen can produce four different hydroperoxides (Figure 4.27) compared with the typical two hydroperoxides produced in the initiation step of lipid oxidation (Figure 4.22). These different hydroperoxide locations will lead to the formation of several unique fatty acid decomposition products as will be discussed later.

Singlet oxygen is most commonly produced by photosensitization. Chlorophyll, riboflavin, and myoglobin are photosensitizers in foods that can absorb energy from light to form an excited singlet state, which is then converted to an excited triplet state. The excited triplet state can react directly with substrates such as unsaturated fatty acids and abstract a hydrogen to cause initiation of lipid oxidation. This pathway is known as type 1 and will produce the same lipid hydroperoxides seen in

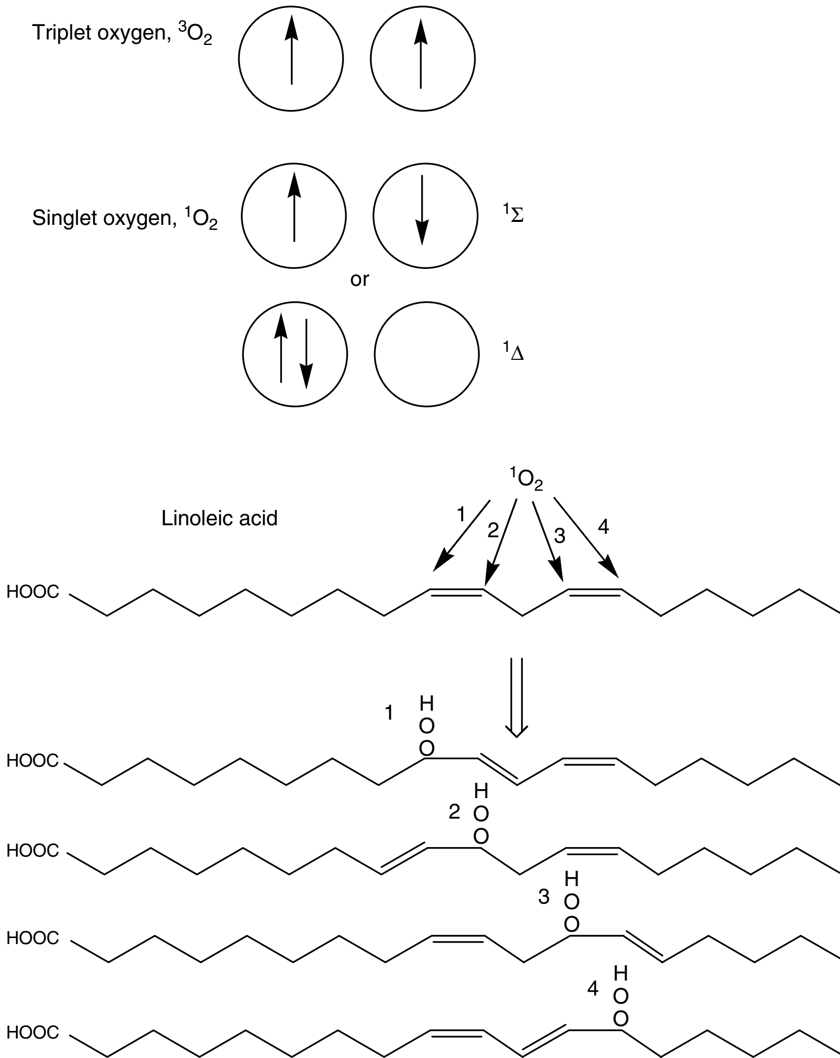



**FIGURE 4.27** Singlet oxygen and singlet oxygen-promoted hydroperoxide formation on linoleic acid. (Adapted from Min, D.B. and Boff, J.M. (2002). In *Food Lipids, Chemistry, Nutrition and Biotechnology* (Akoh, C.C. and Min, D.B., eds.), Marcel Dekker, Inc., New York, NY, pp. 335–364.)

the initiation step described in Figure 4.22. The excited triplet state of the photosensitizer can also react with triplet oxygen to form singlet oxygen and singlet state of the photosensitizer in the type 2 pathway. Types 1 and 2 pathways are dependent on oxygen concentrations, with type 2 favored in high oxygen environments. Singlet oxygen can also be formed chemically, enzymatically, and by the decomposition of hydroperoxides. However, production by photosensitization is believed to be the major pathway of singlet oxygen formation in foods.

*Lipoxygenase:* Numerous plant tissues and animal tissues contain enzymes, known as LOXs that produce lipid hydroperoxides. LOX from plant seeds such as soybeans and peas exist as several isoforms (for review see Reference 55). In soybeans, isoform L-1 primarily reacts with free fatty acids and produces hydroperoxides at carbon 13 in both linoleic and linolenic acid. Isoform L-2 produces hydroperoxides at positions 9 and 13 and is active on both free and esterified linoleic and linolenic acid. Plant LOXs are cytoplasmic enzymes that contain a nonheme iron. The iron in inactive

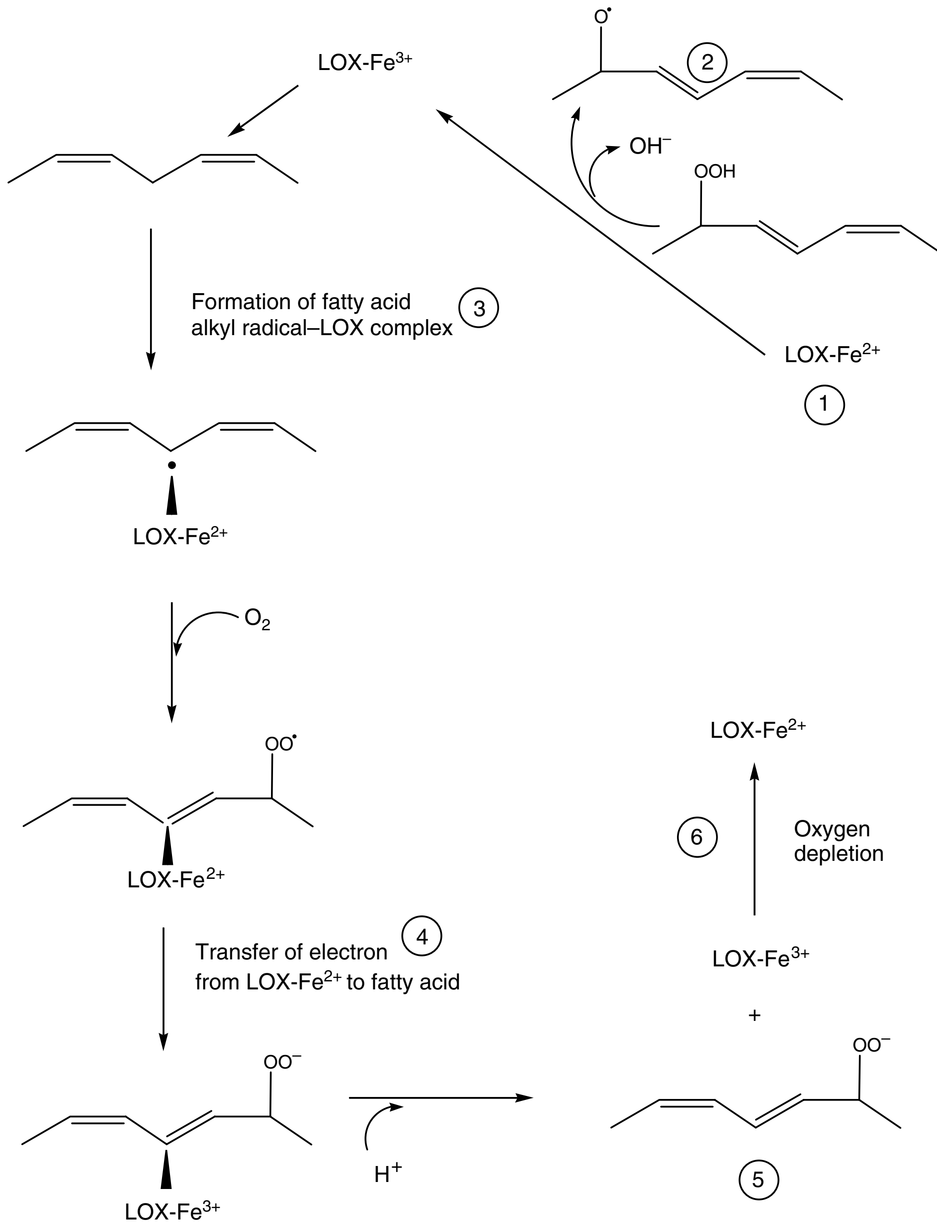



**FIGURE 4.28** Mechanism of LOX-promoted hydroperoxide formation on linoleic acid. (Adapted from Zhuang, H., Barth, M.M., and Hildebrand, D. (2002). In *Food Lipids, Chemistry, Nutrition and Biotechnology* (Akoh, C.C. and Min, D.B., eds.), Marcel Dekker, Inc., New York, NY, pp. 413–464.)

LOX is in the ferrous state (Figure 4.28; [1]). Activation occurs by the oxidation of the iron to the ferric state, a process that is usually promoted by a peroxide (2). LOX then catalyzes the abstraction of hydrogen from the methylene-interrupted carbon to form the alkyl radical and the conversion of the LOX iron back to the ferrous state resulting in the formation of a fatty acid alkyl radical–LOX complex (3). An electron from the ferrous iron is then donated to the peroxyl radical to form a peroxyl anion (4). When the peroxyl anion reacts with hydrogen to form the hydroperoxide, the fatty acid is released from the enzyme (5). Once oxygen is depleted from the system, the enzyme abstracts a

hydrogen from a fatty acid and the iron is converted to ferrous (6). Since no oxygen is present, the alkyl radical is released and LOX is returned to its inactive form. LOXs have also been reported in animal tissues especially those highly associated with the circulatory system (e.g., fish gills) [56].

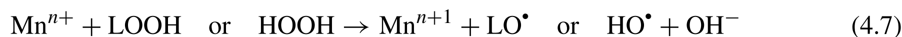
#### 4.7.2.2 Prooxidants That Promote Formation of Free Radicals

*Ionizing radiation:* Foods are sometimes subjected to ionizing radiation to destroy pathogens and extend shelf-life. However, ionizing radiation can convert molecules to excited states that produce free radicals. Ionizing radiation produces the hydroxyl radical ($^{\bullet}OH$) from water. The hydroxyl radical is the most reactive radical known so it is capable of abstracting hydrogen from lipids as well as molecules such as proteins and DNA. Therefore, it is not surprising that irradiation of foods, especially muscle foods that are high in lipids and prooxidants, can increase oxidative rancidity.

#### 4.7.2.3 Prooxidants That Promote Decomposition of Hydroperoxides

Lipid hydroperoxides are found in essentially all lipid-containing foods. Hydrogen peroxide is also found in food when it is utilized as a processing aid and when it is produced by enzymes such as superoxide dismutase (SOD). Food triacylglycerols typically contain 1–100 nmol lipid hydroperoxide per gram lipid. This is 400–1000 times greater than the estimated lipid hydroperoxide concentrations found *in vivo* (e.g., plasma lipids) suggesting that oxidation occurs during the extraction and refining of fats and oils [57]. Lipid hydroperoxides can be decomposed by high temperatures during thermal processing or by a variety of prooxidants. Upon decomposition they produce additional radicals, a factor that could be responsible for the exponential increase in oxidation that is seen after the lag phase or induction period observed in many foods. The decomposition of lipid hydroperoxides also leads to the formation of alkoxyl radicals that can enter into $\beta$-scission reactions. The $\beta$-scission reaction is the main pathway responsible for decomposing fatty acids into low molecular weight compounds that are volatile enough to be perceived as oxidative rancidity (discussed below).

*Transition metals:* Transition metals are found in all foods since they are common constituents of biological materials, water, ingredients, and packaging materials. Transition metals are one of the major food prooxidants that decrease the oxidative stability of foods and biological tissues through their ability to decompose hydroperoxides into free radicals [58,59]. These reactive metals decompose hydrogen and lipid peroxides through the following redox cycling pathway:



$$Mn^{n+} + LOOH \quad \text{or} \quad HOOH \rightarrow Mn^{n+1} + LO^{\bullet} \quad \text{or} \quad HO^{\bullet} + OH^{-} \qquad (4.7)$$

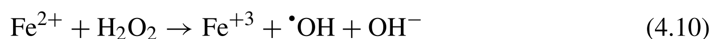
$$Mn^{n+1} + LOOH \rightarrow Mn^{n+} + LOO^{\bullet} + H^{+} \qquad (4.8)$$

$Mn^{n+}$ and $Mn^{n+1}$ are transition metals in their reduced and oxidized states; LOOH and HOOH are lipid and hydrogen peroxides; and $LO^{\bullet}$, $HO^{\bullet}$, and $LOO^{\bullet}$ are alkoxyl, hydroxyl, and peroxyl radicals, respectively. Hydroxyl radical is produced from hydrogen peroxide while alkoxyl radicals are produced from lipid hydroperoxide. When iron and hydroperoxide are involved in this pathway, it is known as the Fenton reaction. The concentration, chemical state, and type of the metal will influence the rate of hydroperoxide decomposition. Copper and iron are the most common transition metals in foods capable of participating in these reactions with iron generally being found at greater concentrations than copper. Copper is more reactive with the cuprous state ($Cu^{1+}$) decomposing hydrogen peroxide over 50-fold faster than ferrous ions ($Fe^{2+}$). Redox state is also important with $Fe^{2+}$ decomposing hydrogen peroxide over $10^5$ times faster than $Fe^{3+}$. In addition, $Fe^{2+}$ is more water soluble than $Fe^{3+}$, meaning that it is more available to promote hydroperoxide decomposition

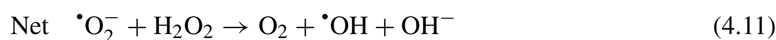
in water-based foods. Peroxide type is also important with $Fe^{2+}$ decomposing lipid hydroperoxides about 10 times faster than hydrogen peroxide [58,59].

Since the reduced state of transition metals is more efficient at decomposing hydroperoxides, reducing compounds capable of promoting the redox cycling of transition metals can promote lipid oxidation. Examples of prooxidative reductants include superoxide anion ($^{\bullet}O_2^-$) and ascorbic acid. Superoxide anion is produced by the addition of an electron to triplet oxygen. The added electron in superoxide anion can then be transferred to a transition metal to cause its reduction. Superoxide anion is produced by enzymes, the release of oxygen from oxymyoglobin to produce metmyoglobin, or by cells such as phagocytes. The redox cycling of iron by superoxide anion to promote lipid oxidation is shown in the following pathways. This pathway is known as the Haber–Weiss reaction.

$$Fe^{3+} + {}^{\bullet}O_2^- \rightarrow Fe^{+2} + O_2 \tag{4.9}$$



$$Fe^{2+} + H_2O_2 \rightarrow Fe^{+3} + {}^{\bullet}OH + OH^- \tag{4.10}$$



$$\text{Net} \quad {}^{\bullet}O_2^- + H_2O_2 \rightarrow O_2 + {}^{\bullet}OH + OH^- \tag{4.11}$$

Ascorbic acid can also participate in Haber–Weiss-like reactions, however, unlike superoxide anions, ascorbic acid can also act as an antioxidant. At high ascorbate concentration its antioxidant activity dominates its ability to accelerate metal-promoted oxidation resulting in a net antioxidant effect.

Transition metals associated with proteins can also promote hydroperoxide decomposition. The heme proteins are the best studied of this group with the iron in myoglobin, hemoglobin, peroxidases, and catalase being able to promote both hydrogen and lipid hydroperoxide decomposition. In some cases, heme proteins have been suggested to cause homolytic scission of lipid hydroperoxides, meaning that the breakdown of the hydroperoxide will produce two free radicals (hydroxyl and alkoxyl). Thermal denaturation of these proteins can increase their prooxidant activity presumably by increased exposure of the heme iron that is able to more effectively interact with hydroperoxides. Denaturation of myoglobin is one of the factors that accelerate lipid oxidation in cooked meats, a problem known as warmed-over flavor.

*Light and elevated temperatures:* UV and visible light can promote the decomposition of hydroperoxides to produce free radicals. Thus, packaging to decrease light exposure can attenuate lipid oxidation rates. Elevated temperatures will also decompose lipid hydroperoxides. In fact, lipid hydroperoxide accumulation is often not seen in rancid frying oils since the hydroperoxides breakdown rapidly after formation.

### 4.7.3 Formation of Lipid Oxidation Decomposition Products

Once lipid hydroperoxides are decomposed into alkoxyl radicals, a number of different reaction schemes can occur. The products of these reaction schemes will depend on the fatty acid type as well as the location of the hydroperoxide on the fatty acid. In addition, decomposition products can be unsaturated and have intact pentadiene structures meaning that the oxidation products can be further oxidized. This results in literally hundreds of different fatty acid decomposition products. Since the type of fatty acid decomposition products will depend on the fatty acid composition of the food, lipid oxidation can have different effects on sensory properties. For example, oxidation of vegetable oils that have predominately $\omega$-6 fatty acids will produce “grassy” and “beany” odors while oxidation of the long-chain $\omega$-3 fatty acids in marine oils will produce “fishy” aromas.

One of the reasons that lipid hydroperoxide decomposition leads to the cleavage of the aliphatic chain of fatty acids is that hydroperoxide decomposition produces the alkoxyl radical ($LO^{\bullet}$). The alkoxyl radical is more energetic than either the alkyl ($L^{\bullet}$) or peroxyl ($LOO^{\bullet}$) radicals. Thus, when

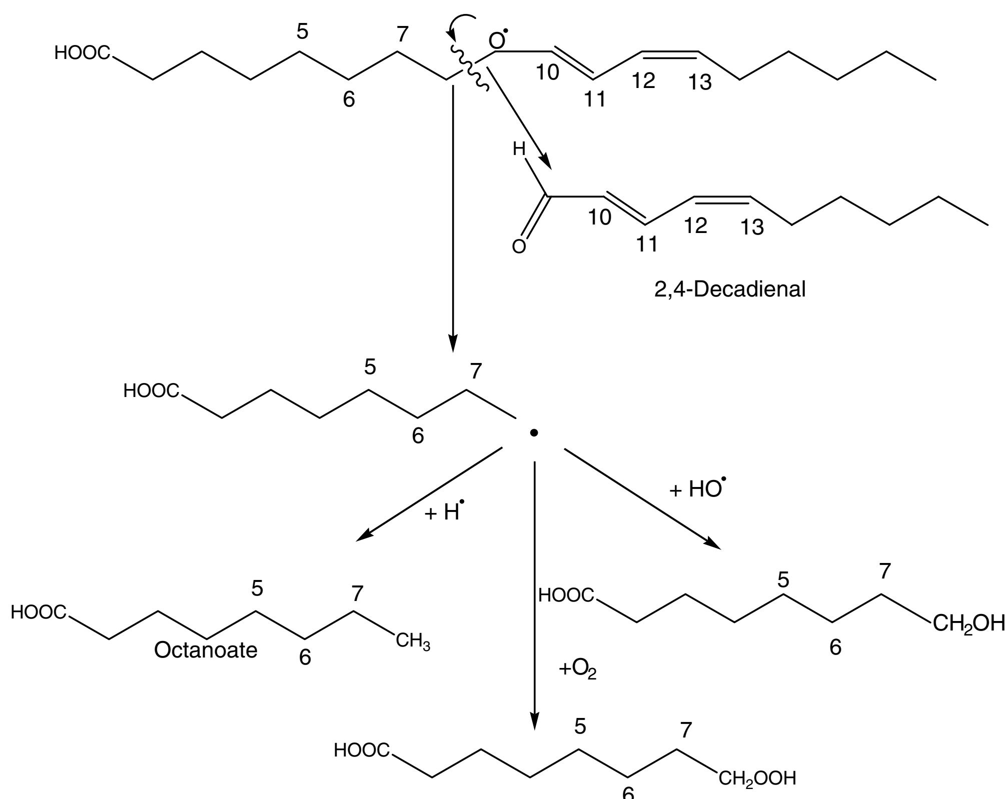



**FIGURE 4.29** Possible reaction pathways for a lipid free radical produced by $\beta$-scission reactions. (Adapted from Frankel, E.N. (1998). *Lipid Oxidation*, Oily Press, Scotland.)

the alkoxyl radical is produced, it has enough energy to abstract an electron from the covalent bonds adjacent to the alkoxyl radical causing a cleavage of the aliphatic chain of fatty acid. This last reaction, known as the $\beta$-scission reaction, is important to food quality since it causes fatty acids to decompose into low molecular weight compounds that are perceived as rancidity.

#### 4.7.3.1 $\beta$-Scission Reaction

Decomposition of lipid hydroperoxides to an alkoxyl radicals ($LO^{\bullet}$) is generally accompanied by the $\beta$-scission reaction. This reaction breaks the aliphatic chain of the fatty acid to produce aldehydes plus a radical on the aliphatic chain (e.g., an alkyl radical). The alkyl radical can then react with a hydrogen radical to form a hydrocarbon, a hydroxyl radical to form an alcohol or oxygen to form a hydroperoxide. Examples of these reactions are shown in Figure 4.29 and more details of these reactions can be found elsewhere [52]. Since hydroperoxide can form at numerous locations on unsaturated fatty acids, a large number of different products are produced from $\beta$-scission reactions. Unesterified linoleic acid will be used to demonstrate the types of products that are produced by $\beta$-scission reactions. One should remember that the decomposition product on the carboxylic acid end of the fatty acid would usually be esterified to the glycerol of a triacylglycerol or phospholipid. Thus, this decomposition product would not be volatile and thus, would not contribute to rancidity unless it undergoes further decomposition reactions to form low molecular weight compounds.

Figure 4.30 shows the formation of linoleic acid decomposition products when the hydroperoxide is located at carbon 9 and $\beta$-scission occurs on the methyl end side of the molecule. In step 1, the hydroperoxide decomposes into the alkoxyl radical. Step 2 shows the $\beta$-scission reaction cleaving the

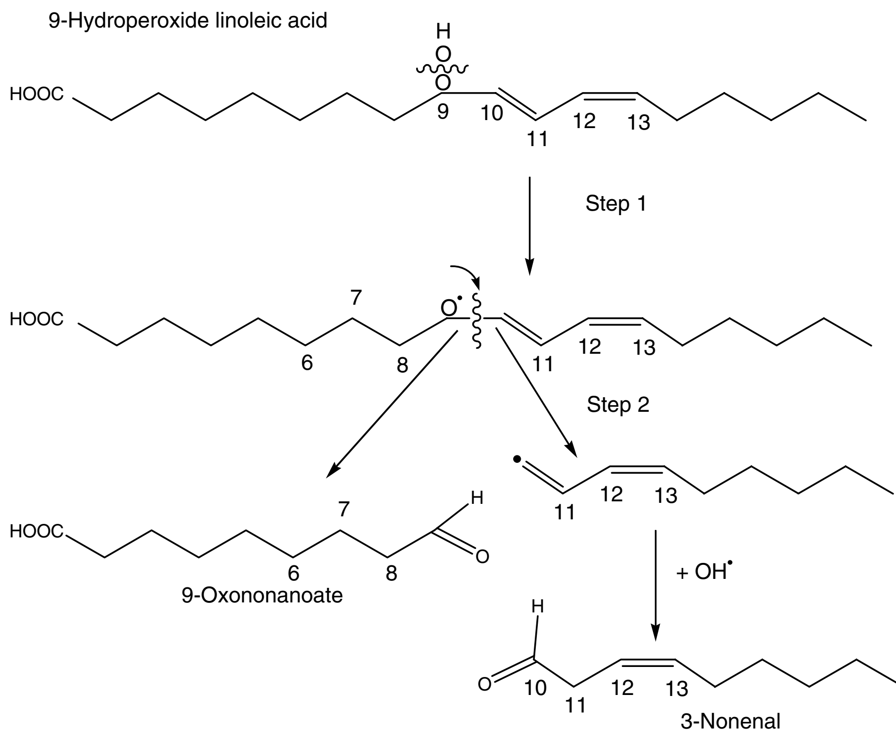



**FIGURE 4.30** $\beta$-Scission decomposition products produced from 9-linoleic acid hydroperoxide when fatty acid cleavage occurs on the methyl end side of the hydroperoxide. (Adapted from Frankel, E.N. (1998). *Lipid Oxidation*, Oily Press, Scotland.)

adjacent carbon–carbon bonds to form two products. This cleavage (step 2) produces 9-oxononanoate and a nine carbon vinyl radical (an olefinic radical). Vinyl radicals often interact with hydroxyl radicals to form aldehydes, thus producing 3-nonenal. Similar pathways will occur if the hydroperoxide is on carbon 13. Cleavage on the carboxylic acid end will produce 12-oxo-9-dodecenoate and hexanal. Cleavage on the methyl end of the fatty acid will produce 13-oxo-9,11-tridecadienoate and pentane. The 9-linoleic acid hydroperoxide can also undergo $\beta$-scission on the carboxylic acid end of the fatty acids, after formation of the alkoxyl radical, as shown in Figure 4.29, to form octanoate and 2,4-decadienal.

When singlet oxygen attacks linoleic acid, it will form hydroperoxides at all of the carbons associated with double bonds (Figure 4.27). This means that it will form hydroperoxides at carbons 9 and 13 as in free radical initiated oxidation plus hydroperoxides at carbons 10 and 12. Typical products from the $\beta$-scission reaction from an alkoxyl radical at carbon 10 will produce 9-oxononanoate and 3-nonenal from cleavage on the carboxylic acid end and 10-oxo-8-decenoate and 2-octene from cleavage at the methyl end of the fatty acid. Typical products from the $\beta$-scission reaction from an alkoxyl radical at carbon 12 will produce 9-undecenoate and 2-heptenal from cleavage on the carboxylic acid end and 12-oxo-9-dodecenoate and hexanal from cleavage at the methyl end of the fatty acid.

As one can see from the above discussion on the $\beta$-scission products and other free radical reactions of linoleic acid, numerous products can be formed. For a detailed discussion on $\beta$-scission decomposition products see Reference 52. Pathways similar to this will occur with other unsaturated fatty acids producing additional unique compounds. The decomposition products often contain double bonds and in some cases intact pentadiene systems. These double bond systems can undergo hydrogen abstraction or singlet oxygen attack that will result in the formation of additional

decomposition products. While the above discussion shows the theoretical decomposition products of linoleic acid, in reality, not all of these products have been detected. This is likely owing to the ability of these compounds to undergo additional decomposition reactions.

#### 4.7.3.2 Additional Reactions of Fatty Acid Decomposition Products

In addition to the fatty acid hydroperoxide products described above, fatty acid radicals can undergo a series of other reactions to form compounds such as olefins, alcohols, carboxylic acids, ketones, epoxides, and cyclic products (for review see Reference 52). Alkyl radicals will react with hydrogen and hydroxyl radicals to produce olefins and alcohols. As mentioned earlier, alkoxyl radicals are high energy radicals. Thus, they can abstract hydrogen from other molecules such as unsaturated fatty acids or antioxidants to produce fatty acid alcohols. Alkoxyl radicals can also lose an electron and be converted to a ketone or bond to an adjacent carbon to form an epoxide. Peroxyl radicals can react with double bonds within the same fatty acid to produce cyclic products such as bicyclic endoperoxides.

Aldehydes produced from the oxidative decomposition of fatty acids are important because of their impact on off-flavor development. However, these aldehydes can react with nucleophilic food components. In particular, they interact with sulfhydryls and amines in proteins that may alter the functionality of the protein. One example is the ability of unsaturated aldehydes to react with histidine in myoglobin via a Michael addition-type reaction [60]. This reaction is thought to contribute to the conversion of myoglobin to metmyoglobin to produce meat discoloration.

#### 4.7.3.3 Cholesterol Oxidation

Cholesterol contains a double bond between carbons 5 and 6. As with fatty acids, this double bond is susceptible to free radical attack and can undergo decomposition reactions to produce alcohols, ketones, and epoxides [61]. The most notable of the cholesterol oxidation pathway begins with the formation of a hydroperoxide at carbon 7. This hydroperoxide can decompose into an alkoxyl radical that can in turn undergo rearrangements to 5,6 epoxides, 7-hydroxylcholesterol, and 7-ketocholesterol. These cholesterol oxidation products are potentially cytotoxic and have been linked to the development of atherosclerosis. Cholesterol oxidation products have primarily been found in animal food products that have undergone thermal processing such as cooked meats, tallow, lard, and butter, as well as dried dairy and egg products.

### 4.7.4 Antioxidants

Oxidative stress occurs in all organisms in an oxygenated environment. Thus biological systems have developed a variety of antioxidant defenses to protect against oxidation. There is no a uniform definition of an antioxidant because there are numerous chemical mechanisms by which oxidation can be inhibited. The biological tissue from which foods is obtained, generally contain several endogenous antioxidant systems. Unfortunately, food processing operations can remove antioxidants or cause oxidative stress that can overcome the endogenous antioxidants systems in the food. Therefore, it is common to incorporate additional antioxidant protection into processed foods. Antioxidant mechanisms of compounds that are used to increase the oxidative stability of foods include control of free radicals, prooxidants, and oxidation intermediates.

#### 4.7.4.1 Control of Free Radicals

Many antioxidants slow lipid oxidation by scavenging free radicals, thus inhibiting initiation, propagation, and $\beta$-scission reactions. Free radical scavengers (FRSs) or chain-breaking antioxidants

can interact with peroxyl ($LOO^\bullet$) and alkoxyl ($LO^\bullet$) radicals by the following reactions:

$$LOO^\bullet \quad \text{or} \quad LO^\bullet + FRS \rightarrow LOOH \quad \text{or} \quad LOH + FRS^\bullet \tag{4.12}$$

Free radical scavengers inhibit lipid oxidation by reacting faster with free radicals than unsaturated fatty acids. FRSs are thought to interact mainly with peroxyl radicals because their lower energy state means they have a longer lifetime (because they are less reactive) and thus, have a greater likelihood of reacting with the low energy hydrogen FRS. This is contrary to high energy free radicals (e.g., $^\bullet OH$) that are so reactive they interact with the molecules closest to their site of production. Since FRSs are generally found at low concentrations they would be less likely to react with the high energy free radicals [62].

Antioxidant efficiency is dependent on the ability of the FRS to donate hydrogen to a free radical. As bond energy of a hydrogen in a FRS decreases, the transfer of the hydrogen to the free radical is more energetically favorable and thus more rapid. The ability of a FRS to donate its hydrogen to a free radical can be predicted with the help of standard one-electron reduction potentials [63]. Any compound that has a reduction potential lower than the reduction potential of a free radical (or oxidized species) is capable of donating its hydrogen to that free radical unless the reaction is kinetically unfeasible. For example, FRS including $\alpha$-tocopherol ($E^{\circ\prime} = 500$ mV), catechol ($E^{\circ\prime} = 530$ mV), and ascorbate ($E^{\circ\prime} = 282$ mV), all have reduction potentials below peroxyl radicals ($E^{\circ\prime} = 1000$ mV) and are therefore capable of donating their hydrogen to the peroxyl radical to form a hydroperoxide.

The efficiency of the FRS is also dependent on the energy of the resulting FRS radical ($FRS^\bullet$). If the $FRS^\bullet$ is a low energy radical then the likelihood of the $FRS^\bullet$ catalyzing the oxidation of unsaturated fatty acids decreases. Effective FRS forms low energy radicals owing to resonance delocalization (as shown in Figure 4.31). Effective FRS also produce radicals that do not react rapidly with oxygen to form hydroperoxides. If a radical scavengers form a hydroperoxide, it could undergo decomposition reactions that produce additional radicals that could cause oxidation of unsaturated fatty acids. $FRS^\bullet$ may participate in termination reactions with other $FRS^\bullet$ or lipid radicals to form nonradical species. This means that each FRS is capable of inactivating at least two free radicals, the first being inactivated when the FRS interacts with peroxyl or alkoxyl radicals and the second when the $FRS^\bullet$ enters a termination reactions with another $FRS^\bullet$ or lipid radical (Figure 4.32).

Phenolic compounds possess many of the properties of an efficient FRS. Phenolic compounds donate a hydrogen from their hydroxyl groups and the subsequent phenolic radical can have low energy as the radical is delocalized throughout the phenolic ring structure. The effectiveness of phenolic FRS is often increased by substitution groups on the phenolic ring that increase the ability of the FRS to donate hydrogen to lipid radicals and/or increase the stability of the $FRS^\bullet$ [64]. In foods, the efficiency of phenolic FRS is also dependent on their volatility, pH sensitivity, and polarity. Below are examples of the most common FRS in foods.

*Tocopherols:* Tocopherols are a group of compounds that have a hydroxylated ring system (chromanol ring) with a phytol chain (Figure 4.33). Differences in tocopherol homologs are because of differences in methylation on the chromanol ring with $\alpha$ being trimethylated (positions 5, 7, and 8), $\beta$ (positions 5 and 8) and $\gamma$ (positions 7 and 8) being dimethylated, and $\delta$ being monomethylated (position 8). Tocotrienols differ from tocopherols in that they have three double bonds in their phytol chain at positions 3′, 7′, and 11′. Tocopherols have three asymmetric carbons and thus each homolog can have eight possible stereoisomers. Natural tocopherols are found in the all *rac* or *RRR* configuration. Synthetic tocopherols have stereoisomer with combinations of *R* and *S* configurations. The stereoisomer configuration of $\alpha$-tocopherol is important because only the *RRR* and 2*R*-stereoisomers (*RSR*, *RRS*, and *SRR*) have significant vitamin E activity and can be used for establishment of the Dietary Reference Intake of vitamin E in the United States [65]. $\alpha$-Tocopherol is commonly sold as a methyl ester when used as a nutritional supplement. The methyl ester is hydrolyzed in the gastrointestinal

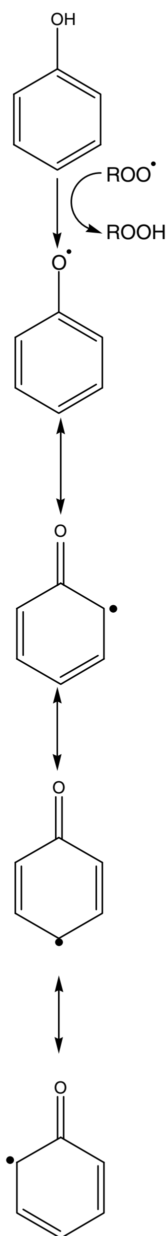



**FIGURE 4.31** Resonance delocalization of phenol radical. (Adapted from Shahidi, F. and Wanasundara, J.P.K. (1992). *Crit. Rev. Food Sci. Nutr.* 32: 67–103.)

tract to regenerate $\alpha$-tocopherol. The methyl ester form of tocopherols blocks the hydroxyl group and decreases the molecule's susceptibility to oxidative degradation until digested. It should be noted that the blocking of the hydroxyl group by the methyl ester removes the antioxidant activity of the tocopherol. Therefore, methyl esters of tocopherols would not be effective antioxidants in foods.

Reaction between tocopherols and lipid peroxyl radicals leads to the formation of a lipid hydroperoxide and several resonance structures of tocopheroxyl radicals. These tocopheroxyl radicals can interact with other lipid radicals or with each other to form a variety of termination products. The types and amounts of these products are dependent on oxidation rates, radical species, physical location

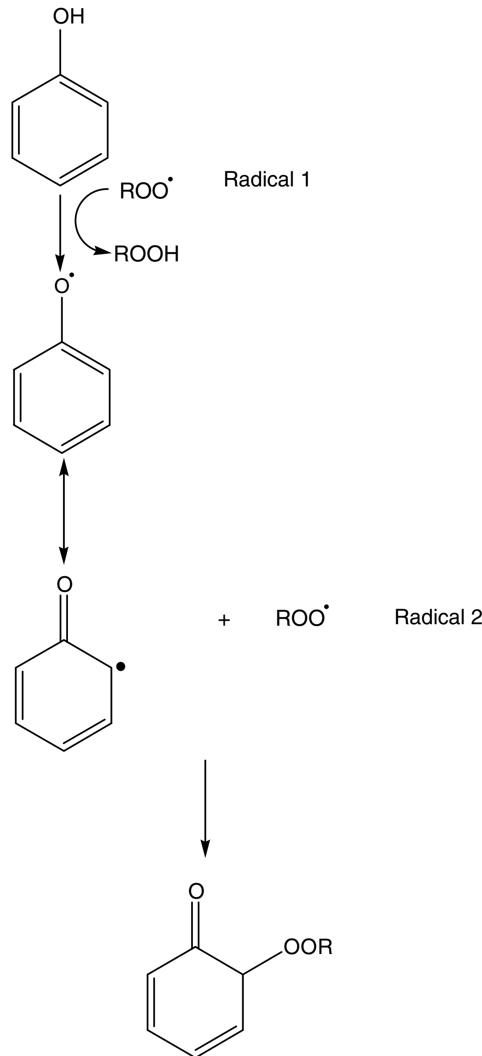



**FIGURE 4.32** A termination reaction between an antioxidant radical and a lipid peroxyl radical (ROO•).

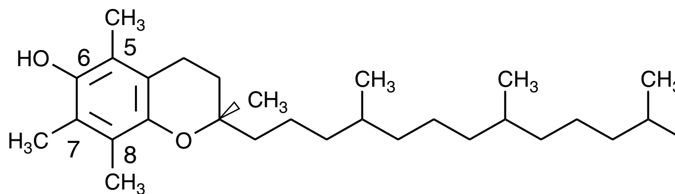



**FIGURE 4.33** The structure of $\alpha$-tocopherol.

(e.g., bulk vs. membrane lipids), and tocopherol concentration (see Reference 62 for more details). Tocopherols are generally insoluble in water. However, they do vary in polarity, with $\alpha$-tocopherol (trimethylated) being the most nonpolar and $\delta$-tocopherol (monomethylated) being the most polar. These differences in polarity alter the surface activity of the tocopherols, a factor that may impact their antioxidant activity (see Section 4.7.4.5).

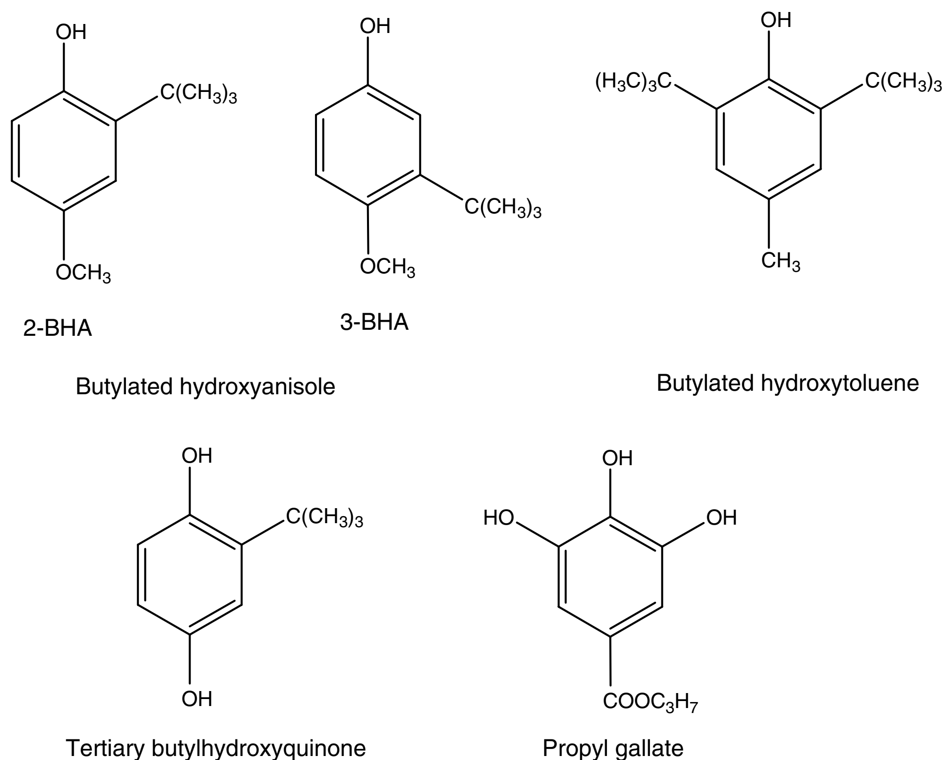



**FIGURE 4.34** Structures of synthetic antioxidants used in foods.

*Synthetic phenolics:* Phenol is not a good antioxidant but addition of substitution groups onto the phenolic ring can enhance antioxidant activity. Thus, the majority of synthetic antioxidants are substituted monophenolic compounds. The most common synthetic FRSs used in foods include butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiary butylhydroquinone (TBHQ), and propyl gallate (Figure 4.34). These synthetic FRSs vary in polarity in the order of BHT (most nonpolar) $>$ BHA $>$ TBHQ $>$ propyl gallate (see Section 4.7.4.5 for an explanation of the importance of antioxidant polarity). As with other FRSs, interactions between the synthetic antioxidants and lipid radicals result in the formation of a low energy resonance-stabilized phenolic radical. The low energy of the synthetic antioxidant radicals means that they do no rapidly catalyze the oxidation of unsaturated fatty acids. In addition, synthetic antioxidant radicals do not react readily with oxygen to form unstable antioxidant hydroperoxides, which decompose into high energy free radicals that can promote oxidation. Instead, they tend to react in radical–radical termination reactions, as shown in Figure 4.32. Synthetic phenolics are effective in numerous food systems; however, their use in the food industry has recently declined owing to safety concerns and consumer demand for all natural products.

*Plant phenolics:* Plants contain a diverse group of phenolic compounds including simple phenolics, phenolic acids, anthocyanins, hydroxycinnamic acid derivatives, and flavonoids. These phenolics are widely distributed in fruits, spices, tea, coffee, seeds, and grains. All of the phenolic classes have the structural requirements of FRSs although their activity varies greatly. Factors influencing the FRS activity of plant phenolics include position and degree of hydroxylation, polarity, solubility, reducing potential, stability of the phenolic to food processing operations, and stability of the phenolic radical. Rosemary extracts are the most commercially important source of natural phenolics used as a food additive to inhibit lipid oxidation by FRS. Carnosic acid, carnosol, and rosmarinic acid are the major

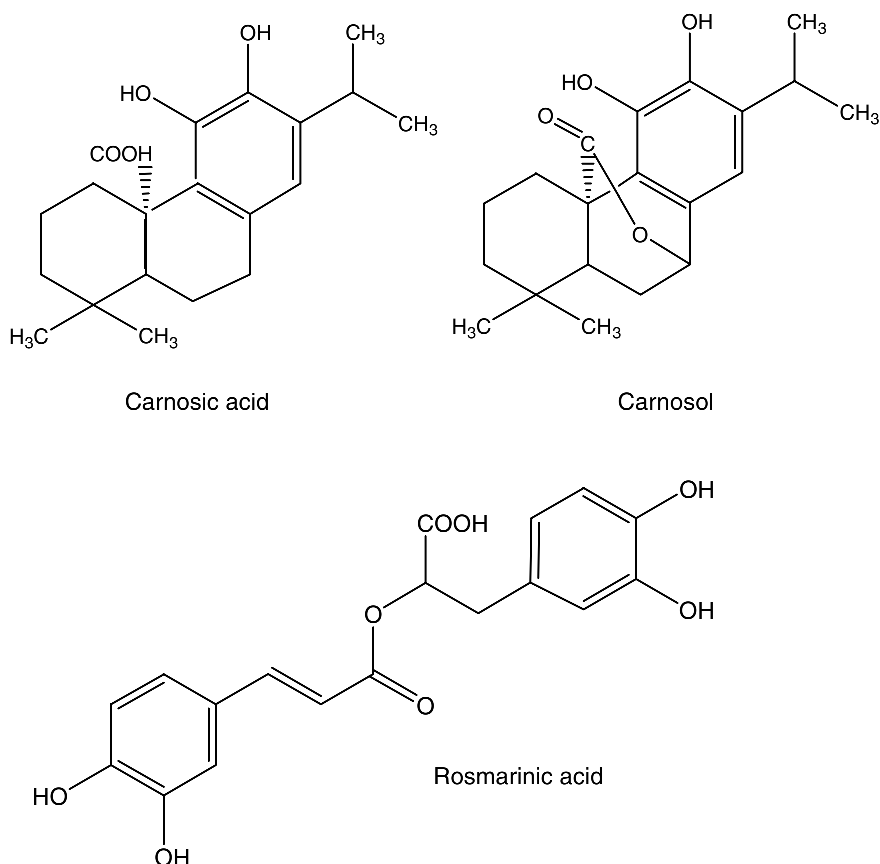



**FIGURE 4.35** Structures of phenolic antioxidants found in rosemary extracts.

FRSs in rosemary extracts (Figure 4.35). Rosemary extracts can inhibit lipid oxidation in a wide variety of food products including meats, bulk oils, and lipid emulsions [66–68]. Utilization of phenolic antioxidants from crude herb extracts, such as rosemary, is often limited by the presence of flavor compounds such as monoterpenes. Phenolics found naturally in plant foods and oils are important to the endogenous oxidative stability of foods. Phenolic levels in plants can vary as a function of plant maturity, variety, tissue type, growing conditions, and postharvest age and storage conditions [69–71].

*Ascorbic acid and thiols:* Free radicals are often generated in the water phase of foods by processes such as the Fenton reaction that produces hydroxyl radicals from hydrogen peroxide. Free radicals may also be surface active, meaning that they could migrate or partition at interface between the lipid phase and water phase in lipid dispersions. To protect against free radicals derived in the aqueous phase, biological systems contain water-soluble compounds capable of free radical scavenging. Ascorbic acid and thiols scavenge free radicals resulting in the formation of low energy radicals (for review see Reference 72). Thiols such as cysteine and glutathione may contribute to the oxidative stability of plant and muscle foods but they are rarely added to foods as antioxidants. One exception to this is the thiols found in proteins that can inhibit lipid oxidation in food products [72]. Ascorbate and its isomer erythorbic acid can both scavenge free radicals. Both have similar activity but erythorbic acid is more cost effective. Ascorbic acid is also available as a conjugate with palmitic acid. The conjugate is lipid soluble and surface active, and this makes it an effective antioxidant in bulk oils

and emulsions. In the gastrointestinal tract ascorbyl palmitate is hydrolyzed to ascorbic and palmitic acid, thus there are no restrictions on its usage levels.

#### 4.7.4.2 Control of Prooxidants

The rate by which lipids oxidize in foods is very dependent on prooxidant concentrations and activity (e.g., transition metals, singlet oxygen, and enzymes). Control of prooxidants is therefore a very effective strategy to increase the oxidative stability of foods. Both endogenous and exogenous antioxidants will impact the activity of transition metals and singlet oxygen.

*Control of prooxidant metals:* Iron and copper are examples of important prooxidant transition metals that accelerate lipid oxidation by promoting hydroperoxide decomposition. The prooxidative activity of metals is altered by chelators or sequestering agents. Chelators inhibit the activity of prooxidant metals by one or more of the following mechanisms: prevention of metal redox cycling; occupation of all metal coordination sites; formation of insoluble metal complexes; and/or steric hindrance of interactions between metals and lipids or oxidation intermediates (e.g., hydroperoxides) [73]. Some metal chelators can increase oxidative reactions by increasing metal solubility and/or altering the redox potential. The tendency of a chelator to accelerate or inhibit prooxidant activity depends on metal-to-chelator ratio. For instance, EDTA (ethylenediamine tetraacetic acid) is ineffective or prooxidative when EDTA:iron ratios are $\leq 1$ and antioxidative when EDTA:iron is $>1$ [74]. The prooxidative behavior of chelators is thought to be because of their ability to increase transition metal solubility.

The main metal chelators found in foods contain multiple carboxylic acid (e.g., EDTA and citric acid) or phosphate groups (e.g., polyphosphates and phytate). Chelators must be ionized to be active; therefore their activity decreases at pH values below the $pK_a$ of the ionizable groups. The most common chelators used as food additives are citric acid, EDTA, and polyphosphates. The effectiveness of phosphates increases with increasing number of phosphate groups; thus, tripolyphosphate and hexametaphosphate are more effective than phosphoric acid [75]. Prooxidant metals can also be controlled by metal binding proteins, such as transferrin, phosvitin, lactoferrin, ferritin, and casein (reviewed in Reference 73).

*Control of singlet oxygen:* As mentioned earlier, singlet oxygen is an excited state of oxygen that can promote the formation of lipid hydroperoxides. Carotenoids are a diverse group ($>$600 different compounds) of yellow to red colored polyenes. The activity of singlet oxygen can be controlled by carotenoids by both chemical and physical quenching mechanisms [76,77]. Carotenoids chemically quench singlet oxygen when singlet oxygen attacks the double bonds of the carotenoid. This reaction leads to the formation of oxygenated carotenoid breakdown products such as aldehydes, ketones, and endoperoxides. These reactions cause carotenoid decomposition, leading to loss of color. The more effective mechanism of singlet oxygen inactivation by carotenoids is physical quenching. Carotenoids physically quench singlet oxygen by a transfer of energy from singlet oxygen to the carotenoid to produce an excited state carotenoid and ground state triplet oxygen. Energy is dissipated from the excited carotenoid by vibrational and rotational interactions with the surrounding solvent to return the carotenoid to the ground state. The nine or more conjugated double bonds in a carotenoid are necessary for physical quenching. Carotenoids that have oxygenated $\beta$-ionone ring structures at the ends are often more effective at physically quenching singlet oxygen. Carotenoids can also physically absorb the energy of photoactivated sensitizers such as riboflavin preventing the photosensitizer from promoting the formation of singlet oxygen.

*Control of LOXs:* Lipoxygenases are active lipid oxidation catalysts found in plants and some animal tissues. LOX activity can be controlled by heat inactivation and plant breeding programs that decrease the concentration of these enzymes in edible tissues.

#### 4.7.4.3 Control of Oxidation Intermediates

Compounds are found in foods that indirectly influence lipid oxidation rates by interacting with prooxidant metals or oxygen to form reactive species. Examples of such compounds include superoxide anion and hydroperoxides.

*Superoxide anion:* Superoxide participates in oxidative reactions by reducing transition metals to a more active state or by promoting the release of iron bound to protein. In addition, at pH values below its $pK_a$ (i.e., 4.8), superoxide will form the perhydroxyl radical ($HOO^{\bullet}$) that can directly catalyze lipid oxidation [78]. Owing to the prooxidant nature of superoxide anion in oxidative reactions, biological systems contain SOD. SOD catalyzes the conversion of superoxide anion to hydrogen peroxide by the following reaction:

$$2^{\bullet}O_2^- + 2H^+ \rightarrow O_2 + H_2O_2 \quad (4.13)$$

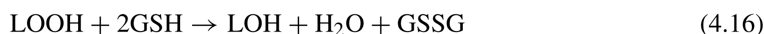
*Peroxides:* Peroxides are important intermediates of oxidative reactions since they decompose via transition metals, irradiation, and elevated temperatures to form free radicals. Hydrogen peroxide exists in foods owing to direct addition (e.g., aseptic processing operations) and formation in biological tissues by mechanisms including the dismutation of superoxide by SOD and the activity of peroxisomes and leukocytes. The inactivation of hydrogen peroxide is catalyzed by catalase, a heme-containing enzyme, by the following reaction [78]:

$$2H_2O_2 \rightarrow 2H_2O + O_2 \quad (4.14)$$

Glutathione peroxidase is a selenium-containing enzyme that can decompose both lipid hydroperoxides and hydrogen peroxide using reduced glutathione (GSH) as a cosubstrate [78]:

$$H_2O_2 + 2GSH \rightarrow 2H_2O + GSSG \quad (4.15)$$

or



$$LOOH + 2GSH \rightarrow LOH + H_2O + GSSG \quad (4.16)$$

where GSSG is oxidized glutathione and LOH is a fatty acid alcohol.

#### 4.7.4.4 Antioxidant Interactions

Food systems usually contain endogenous multicomponent antioxidant systems. In addition, exogenous antioxidants can be added to processed foods. The presence of multiple antioxidants will enhance the oxidative stability of the product owing to interactions between antioxidants. Synergism is often used to describe antioxidant interactions. For antioxidant interactions to be synergistic, the effect of the antioxidant combination must be greater than the sum of the two individual antioxidants. However, in most cases the effectiveness of antioxidant combinations often is equal to or less than their additive effect. While antioxidant combinations can be used to effectively increase the shelf-life of foods, caution should be used in claiming synergistic activity.

Enhanced antioxidant activity can be observed in the presence of two or more different FRSs. In the presence of multiple FRSs, it is possible that one FRS (the primary FRS) will react more rapidly with lipid free radicals than the others owing to lower bond dissociation energies or owing to the fact that its physical location is closer to the site where free radicals are being generated. In the presence of multiple FRSs, the primary FRS, which is rapidly oxidized, can sometimes be regenerated by a secondary FRS with the free radical being transferred from the primary to the secondary FRS. This process is seen with $\alpha$-tocopherol and ascorbic acid. In this system, $\alpha$-tocopherol is the primary FRS owing to its presence in the lipid phase. Ascorbic acid then regenerates the tocopheroxyl

radical or possibly the tocopherylquinone back to $\alpha$-tocopherol, resulting in the formation of the dehydroascorbate [63]. The net result is that the primary FRS ($\alpha$-tocopherol) is maintained in an active state where it can continue to scavenge free radicals in the lipid phase of the food.

Chelator and FRS combinations can result in improved inhibition of lipid oxidation [6]. These enhanced interactions occur by a "sparing" effect provided by the chelator. That is, the chelator decreases the amount of free radicals formed in the food by inhibiting metal-catalyzed oxidation; this slows down inactivation of the FRS through reactions such as termination or autooxidation.

Since multicomponent antioxidant systems can inhibit oxidation by many different mechanisms (e.g., FRS, metal chelation, and singlet oxygen quenching), the use of multiple antioxidants can greatly enhance the oxidative stability of foods. Thus, when designing antioxidant systems, the antioxidants used should have different mechanisms of action and/or physical properties. Determining which antioxidants would be most effective depends on factors such as type of oxidation catalysts, physical state of the food, and factors that influence the activity of the antioxidants themselves (e.g., pH, temperature, and ability to interact with other compounds/antioxidants in the foods).

#### 4.7.4.5 Physical Location of Antioxidants

Antioxidants can show a wide range of effectiveness depending on the physical nature of the lipid [52,79]. For example, hydrophilic antioxidants are often less effective in O/W emulsions than lipophilic antioxidants, whereas lipophilic antioxidants are less effective in bulk oils than hydrophilic antioxidants. This observation has been coined the "polar paradox." Differences in the effectiveness of the antioxidants in bulk oils and W/O emulsions are due to their physical location in the two systems. Polar antioxidants are more effective in bulk oils presumably because they can accumulate at the air–oil interface or in reverse micelles within the oil, the locations where lipid oxidation reactions would be greatest owing to high concentrations of oxygen and prooxidants. In contrast, predominantly, nonpolar antioxidants are more effective in O/W emulsions because they are retained in the oil droplets and/or may accumulate at the oil–water interface, the location where interactions between hydroperoxides at the droplet surface and prooxidants in the aqueous phase occur. Conversely, in O/W emulsions, polar antioxidants would tend to partition into the continuous aqueous phase where they would be less able to protect the lipid.

### 4.7.5 Other Factors Influencing Lipid Oxidation Rates

*Oxygen concentration:* Reduction of oxygen concentration is a common method used to inhibit lipid oxidation. However, the addition of oxygen to the alkyl radical is a diffusion-limited (fast) reaction; therefore, to effectively inhibit lipid oxidation, the majority of oxygen must be removed from the system. Since oxygen solubility is higher in oil than in water, removal of oxygen to stop lipid oxidation can be difficult unless vacuum conditions are used or oxygen is completely replaced by an inert gas (e.g., nitrogen).

*Temperature:* Increasing temperature generally increases lipid oxidation rates. However, increasing temperature also decreases oxygen solubility so in some cases high temperatures can slow oxidation. This can happen in heated bulk oil. However, if food is fried in heated oil, aeration of the oil occurs leading to acceleration of oxidation. Elevated temperatures can also cause antioxidants to degrade, volatilize, and, in the cases of antioxidant enzymes, become inactivated through denaturation.

*Surface area:* Increasing the surface area of lipids can increase lipid oxidation rates since this can lead to increased exposure to oxygen and prooxidants.

*Water activity:* As water is removed from a food system, lipid oxidation rates generally decrease. This is likely because of a decrease in the mobility of reactants such as transition metals and oxygen. In some foods, continued removal of water will result in an acceleration of lipid oxidation. This

acceleration of lipid oxidation at low water activity ($a_w \leq 0.3$) is thought to be due to the loss of a protective water solvation layer surrounding lipid hydroperoxides [80].

### 4.7.6 Measurement of Lipid Oxidation

As one can glean from the above discussion on lipid oxidation pathways, numerous oxidation products can be formed from a single fatty acid. In addition, these decomposition products often contain double bonds and in some cases intact pentadiene systems. These double bond systems can undergo further hydrogen abstraction or singlet oxygen attack that will result in the formation of additional decomposition products. Since food lipids can contain many different unsaturated fatty acids and can be exposed to several different prooxidants, hundreds of decomposition products can be formed. The complexity of these pathways makes analysis of lipid oxidation very challenging. Below is a summary of the most common analytical techniques to monitor the oxidation products in food lipids.

#### 4.7.6.1 Sensory Analysis

The gold standard of lipid oxidation measurements is sensory analysis since this is the only technique that directly monitors the off-aromas and off-flavors generated by oxidative reactions. In addition, sensory analysis can be highly sensitive since humans can detect certain aroma compounds at levels below or close to detection levels for chemical and instrumental techniques. Sensory analysis of oxidized lipids must be done with a panel that is trained in the identification of oxidation products. This training is usually product specific since the oxidation products from different fatty acids can produce different sensory profiles. Owing to the necessity for extensive training, sensory analysis is often time consuming and cost prohibitive and obviously is not suitable for the rapid and extensive analysis required for quality control operations. Thus, many chemical and instrumental techniques have been developed. In the best-case scenario, these chemical and instrumental techniques are most useful when correlated with sensory analysis. Numerous tests exist for measurement of oxidative deterioration of foods. The most common methods and their advantages and disadvantages are discussed below.

#### 4.7.6.2 Primary Lipid Oxidation Products

Primary lipid oxidation products are compounds that are produced by the initiation and propagation steps of lipid oxidation. Since these are the first oxidation products produced, they can appear early in the oxidative deterioration of lipids. However, during the latter stages of oxidation, the concentrations of these compounds decrease as their formation rates become slower than their decomposition rates. A disadvantage of using primary products to measure oxidation is that primary products are not volatile and, thus, do not directly contribute to off-flavors and off-aromas. In addition, under certain conditions (such as high temperatures [frying oils] or high amounts of reactive transition metals), the concentration of primary products may show little net increase since their decomposition rates are relatively high. This would produce misleading results since a very rancid oil could have very low concentrations of primary lipid oxidation products.

*Conjugated double bonds:* Conjugated double bonds are rapidly formed in polyunsaturated fatty acids upon the abstraction of hydrogen in the initiation step. Conjugated dienes have an absorption maximum at 234 nm with a molar extinction coefficient of $2.5 \times 10^4$ $M^{-1}$ $cm^{-1}$ [81]. This extinction coefficient gives an intermediate level of sensitivity compared to other techniques. Conjugated dienes can be useful for simple oil systems, however, it is often ineffective in complex foods where many compounds exist that also absorb at similar wavelengths and thus cause interference. Sometimes, conjugated diene values are used interchangeably with lipid hydroperoxides since many lipid hydroperoxides will contain a conjugated diene system. However, this equivalence should be

discouraged since fatty acid breakdown products can also contain conjugated double bonds and since monounsaturated fatty acids (e.g., oleic) will form hydroperoxides that do not have a conjugated diene system. Conjugated trienes can also be measured in foods at 270 nm. This technique is only useful with lipids that have $\geq$3 double bonds and, thus, is limited to highly unsaturated oils such as those from linseed and fish.

*Lipid hydroperoxides:* A very common method to measure the oxidative quality of lipids is to measure fatty acid hydroperoxides. Most methods that measure lipid hydroperoxides rely on the ability of the hydroperoxides to oxidize an indicator compound. Peroxide values are expressed as milliequivalents (mEq) of oxygen per kg of oil with 1 mEq equal to 2 mmol of hydroperoxide. The most common titration method uses the hydroperoxide-promoted conversion of iodide to iodine. Iodine is then titrated with sodium thiosulfite to produce iodide that is measured with a starch indicator [82]. This method is relatively insensitive with a detection limit of 0.5 mEq $kg^{-1}$ oil and can require up to 5 g of lipid. Thus, it is only practical for isolated or bulk fats and oils. Lipid hydroperoxide-promoted oxidation of ferrous to ferric ions can also be used with ferric ions being detected with ferric ion-specific chromophores such as thiocyanate or xylenol orange [83]. These methods are much more sensitive than the iodine sodium thiosulfite titration methods. The chromophore formed from the thiocyanate–ferric complex has an extinction coefficient of $4.0 \times 10^4$ $M^{-1}$ $cm^{-1}$ allowing analysis to be performed with milligram quantities of lipids [83].

### 4.7.6.3 Secondary Lipid Oxidation Products

Secondary lipid oxidation products are compounds that arise from the decomposition of fatty acid hydroperoxides via reactions such as $\beta$-scission. As described above, these reactions can generate hundreds of different compounds, both volatile and nonvolatile, from the oxidation of food lipids. Since it is virtually impossible to measure all of these compounds simultaneously, these methods (see below) generally focus on the analysis of a single compound or class of compounds. A drawback of these methods is that the formation of secondary products derives from the decomposition of lipid hydroperoxides. Thus, in certain cases (e.g., presence of antioxidants), the concentrations of secondary products can be low while primary oxidation product concentrations are high. In addition, compounds in foods containing amine and sulfhydryl groups (e.g., proteins) can interact with secondary products that contain functional groups such as aldehydes, thus making them difficult to measure. An advantage of these measurements is that they measure many of the products from fatty acid decomposition, which are directly responsible for the off-flavors and off-aromas in rancid oils, and thus have higher correlation with sensory analysis.

*Analysis of volatile secondary products:* Volatile lipid oxidation products are typically measured by gas chromatography using direct injection, static or dynamic headspace, or solid-phase microextraction (SPME) [84]. Using these systems, lipid oxidation can be measured using specific products (e.g., hexanal for lipids high in $\omega$-6 fatty acids and propanal for lipids high in $\omega$-3 fatty acids), product classes (e.g., hydrocarbons or aldehydes), or by total volatiles as indicators. Each method can give different profiles of volatiles owing to differences in their ability to extract and collect the volatiles from the sample. The advantage of measuring volatile lipid oxidation products is the strong correlation with sensory analysis. The disadvantage is expense of instrumentation and the difficulty in analyzing large amounts of samples especially in lipids that are oxidizing rapidly (these techniques are often time consuming). In addition, these methods often use heating steps to increase the concentration of volatiles in the headspace above the samples. In some foods, such as meats, these heating steps may increase lipid oxidation rates by cooking the food. In general, lipids should be sampled at the lowest temperature possible. An additional problem is the loss of volatile compounds by processes such as steam distillation in frying oils.

*Carbonyls:* Carbonyls arising from lipid oxidation can be determined by reacting lipids with 2,4-dinitrophenylhydrazine to form corresponding hydrazones that absorb light at 430–460 nm.

This method is limited by the presence of other carbonyls in foods that can cause interference [82]. High performance liquid chromatography (HPLC) techniques have been developed to separate carbonyls arising from lipid oxidation from interfering compounds. However, these techniques are sophisticated and time consuming, and therefore not routinely used in food lipids.

Carbonyls can also be measured by conjugation with anisidine to form products that absorb at 350 nm [52]. This method is useful because it can measure nonvolatile, high molecular weight carbonyls. This makes it a useful method for frying oils where volatile oxidation products are lost by steam distillation. Anisidine is also used to measure oxidation in products such as fish oils since these oils commonly undergo extensive steam distillation during refining. Anisidine is therefore useful in fish oils because it can give an indication of the quality of the oil prior to steam distillation since nonvolatile, high molecular weight compounds are retained in the oil.

*Thiobarbituric acid (TBA):* The TBA assay is based on the reaction between TBA and carbonyls to form red, fluorescent adducts under acidic conditions [85]. The assay can be conducted on whole samples, sample extracts, or sample distillates, and adduct formation can be conducted under over a range of protocol temperatures (25–100°C) and times (15 min to 20 h). The compound often attributed to be the primary lipid oxidation product detected by TBA is malondialdehyde (MDA) whose TBA adduct absorbs strongly at 532 nm. MDA is a dialdehyde produced by a two-step oxidative degradation of fatty acids with three or more double bonds. This means that MDA yield during the oxidation of lipids is dependent on fatty acid composition with highly unsaturated fatty acids producing higher amounts of MDA. TBA can also react with aldehydic lipid oxidation products other than MDA, especially unsaturated aldehydes.

The TBA assay suffers from nonspecificity owing to its ability to react with nonlipid carbonyls such as ascorbic acid, sugars, and nonenzymatic browning products. These compounds can form TBA adducts that absorb over the range of 450–540 nm. Often it is more appropriate to refer to TBA reactive substances (TBARS to acknowledge that compounds in addition to MDA can generate pink chromophores). In order to decrease problems with interfering compounds, the TBA–MDA complex can be measured directly by fluorescence or HPLC techniques.

The TBA assay can be a useful method for analysis of lipid oxidation in foods since it is simple and inexpensive. However, the nonspecificity of the method requires an understanding of the test's limitations so that improper comparisons and conclusions are not made. To minimize potential misinterpretation of TBA analysis, it is suggested that analysis of fresh, nonoxidized samples be conducted to account for TBA reactive substances that do not arise from lipid oxidation. However, the TBA method should be avoided in foods where concentrations of interfering compounds are high. In addition, attempts to use TBA to compare oxidative changes in products with different fatty acid compositions are inappropriate since MDA yield varies with fatty acid composition.

## 4.8 FOOD LIPIDS AND HEALTH

### 4.8.1 Bioactivity of Fatty Acids

Dietary lipids have often been negatively associated with health. Since obesity is highly correlated with numerous diseases, such as heart disease and diabetes, the negative role of lipids in health is often attributed to their high caloric density of 9 kcal $g^{-1}$. Specific dietary lipids have also been associated with risk of heart disease owing to their ability to modulate LDL-cholesterol levels in the blood. This includes the saturated fatty acids that increase LDL-cholesterol levels and the unsaturated fatty acids that decrease LDL cholesterol. Since LDL-cholesterol levels are often associated with the development of heart disease, several dietary strategies have been proposed to decrease LDL cholesterol including reduction of dietary saturated fatty acids to $<7\%$ of calories, reduction of dietary cholesterol to $<200$ mg per day, and incorporation of dietary soluble fibers at 10–25 g per day [86].

#### 4.8.1.1 *Trans* Fatty Acids

*Trans* fatty acids have recently gained attention in their unique role in heart disease through their ability to both increase LDL-cholesterol and decrease high-density lipoprotein (HDL) cholesterol [87]. This behavior is partially due to the geometric configuration of *trans* fatty acids that is more similar to saturated fatty acids than unsaturated fatty acids. Originally, *trans* fatty acids are included in the unsaturated fatty acid category of the nutritional label in the United States even though their biological activities are very different. As of January 1, 2006, all foods are required to list *trans* fatty acid concentrations on their nutritional labels. Foods with less than 0.5 g of fat/serving do not have to label *trans* fatty acids as long as no claims are made about fat, fatty acids, or cholesterol content.

While a large amount of research has been devoted to the negative aspects of dietary lipids on health, evidence is growing that some dietary lipids can reduce the risk to several diseases. These bioactive lipids include $\omega$-3 fatty acids, phytosterols, carotenoids, and CLA.

#### 4.8.1.2 $\omega$-3 Fatty Acids

As agricultural practices have advanced, the profile of dietary lipids in Western societies has changed dramatically. Our ancestors are thought to have consumed diets with approximately equal amounts of $\omega$-6 and $\omega$-3 fatty acids. The development of modern agriculture increased the availability of refined fats, especially vegetable oils, changing our dietary $\omega$-6 to $\omega$-3 ratio to over 7:1. This is an extremely rapid change on an evolutionary timescale that is problematic since humans interconvert $\omega$-6 and $\omega$-3 fatty acids at slow rates. Levels of $\omega$-3 fatty acids in the diet are important because these bioactive lipids play a vital role in membrane fluidity, cellular signaling, gene expression, and eicosanoid metabolism. Therefore, consumption of dietary $\omega$-3 fatty acids is essential for promotion and maintenance of good health, especially for pregnant and lactating women, and individuals with coronary heart disease, diabetes, immune response disorders, and compromised mental health. There is strong evidence that the level of $\omega$-3 fatty acids currently consumed by the general population is inadequate [9]. Numerous food companies are attempting to increase the levels of these bioactive lipids in their products by direct incorporation of $\omega$-3 fatty acids into foods or by feeding $\omega$-3 fatty acids to livestock. These approaches are commonly impeded by the oxidative deterioration of the $\omega$-3 fatty acids during the processing and storage of fortified food products. A list of marine foods high in $\omega$-3 fatty acids is shown in Table 4.5. Seed oils rich in $\omega$-3 fatty acids, specifically linolenic acid, include soybean, canola, and flaxseed (linseed) (Table 4.2).

**TABLE 4.5**
**The $\omega$-3 Fatty Acid Content of Selected Fish**

| Fish | g $\omega$-3 Fatty Acids/100 g Fish |
|---|---|
| Tuna (white albacore) | 0.9 |
| Tuna (light) | 0.2 |
| Atlantic salmon (farmed) | 1.3–2.1 |
| Chinook salmon (wild) | 1.4 |
| Herring | 2.0 |
| Mackerel | 0.4–1.8 |
| Cod | 0.2 |
| Flounder | 0.5 |
| Catfish | 0.1 |

*Source:* Exler, J. (1987). *Composition of Foods: Finfish and Shellfish Products*. USDA Handbook 8-15, Washington, DC.

#### 4.8.1.3 Conjugated Linoleic Acid

The two double bonds of linoleic acid are normally in a methylene-interrupted system where two single bonds separate the double bonds. However, the double bond system is sometimes altered resulting in isomerization of the double bonds to a conjugated configuration. This isomerization can occur during processes such as hydrogenation and is common during the biohydrogenation process promoted by bacteria in ruminants. These isomers, known as CLA, have gained widespread interest because of their ability to inhibit cancer [89], lower blood cholesterol [90], inhibit the onset of diabetes, and influence weight gain [91]. The different isomers have different biological effects with 9-*cis*, 11-*trans* linoleic acid showing anticarcinogenic activity and 10-*trans*, 12-*cis* linoleic acid having the ability to influence body fat accumulation. The 9-*cis*, 11-*trans* isomer of CLA is the predominant isomer found in dairy and beef products. Molecular mechanisms of CLA bioactivity have been attributed to their ability to modulate eicosanoid formation and gene expression. Very few human clinical studies have been conducted to support the purported health benefits of CLA in humans.

#### 4.8.1.4 Phytosterols

The major phytosterols in foods are sitosterol, campesterol, and stigmasterol. Dietary phytosterols are practically nonabsorbed in the gastrointestinal tract. Their bioactivity lies in the fact that they can inhibit the absorption of both dietary and biliary (produced by intestinal cells) cholesterol [92]. The intake of 1.5–2 g per day of phytosterols can reduce LDL-cholesterol by 8–15%. Since phytosterols primarily inhibit cholesterol absorption, they are most effective when consumed with a cholesterol-containing meal. Phytosterols have very high melting points and thus exist as lipid crystals at the temperatures common to many foods. To minimize crystallization, the phytosterols are commonly esterified to unsaturated fatty acids to increase their lipid solubility.

#### 4.8.1.5 Carotenoids

Carotenoids are a diverse group ($>$600 different compounds) of yellow to red colored polyenes that are lipid soluble. Vitamin A is an essential nutrient obtained from carotenoids such as $\beta$-carotene. The bioactivity of other carotenoids has been a research area of great interest. This interest was initially focused on the antioxidant activity of carotenoids. However, when clinical trials were conducted to evaluate dietary $\beta$-carotene in subjects at risk to free radical stress (smokers), $\beta$-carotene was found to increase lung cancer rates [93]. It is unknown whether a similar effect would be observed with nonsmokers. Other carotenoids have been found to have health benefits. Lutein and zeaxanthin can enhance visual acuity and health [94]. Epidemiological studies have shown that the consumption of tomatoes is correlated with decreased risk for prostate cancer [95]. The health benefits of tomatoes have been attributed to the carotenoid, lycopene. Interestingly, cooked tomatoes have greater health benefits presumably owing to the thermally induced conversion of *trans*-lycopene to *cis*-lycopene isomers. The greater bioactivity of *cis*-lycopene isomers is thought to be because of their greater bioavailability.

### 4.8.2 Low Calorie Lipids

One of the other health concerns of dietary triacylglycerols is their high caloric density. Many attempts have been made to produce low fat foods that have the same sensory attributes as their full fat counterparts by using fat mimetics. Fat mimetics are nonlipid compounds such as proteins or carbohydrates that can produce fat-like properties at lower caloric values (e.g., 4 kcal $g^{-1}$ protein vs. 9 kcal $g^{-1}$ lipid). A similar approach has been attempted to produce lipid components with no calories or with lower caloric contents (fat substitutes). The first commercial noncaloric lipid was sucrose fatty acid esters (Proctor and Gamble's Olestra). This compound is noncaloric because the presence of $\geq$6 fatty acids esterified to sucrose sterically prevents lipase from hydrolyzing the ester

bond to release free fatty acids that can be absorbed into the blood. The nondigestiability of sucrose fatty acid esters means that they pass through the gastrointestinal tract and are excreted in the feces. This property can cause gastrointestinal problems such as diarrhea. Structured lipids with lower caloric density have also been used in the food industry (e.g., Nabisco's Salatrim). These products are based on the principle that only fatty acids at *sn*-1 and *sn*-3 of triacylglycerol are released as free fatty acids on hydrolysis by pancreatic lipase. If *sn*-1 and *sn*-3 have long-chain saturated fatty acids ($\geq$16 carbons), their release can lead to interactions with divalent cations to form insoluble soaps that are not readily bioavailable. Structured low calorie fats also use short-chain fatty acids ($\leq$6 carbons) at the *sn*-2 position. After hydrolysis by pancreatic lipase, the *sn*-2 monoacylglycerol is absorbed into the intestinal endothelial cells. The short-chain fatty acids at *sn*-2 eventually are metabolized in the liver where they yield fewer calories than long-chain fatty acids. The combination of both long-chain saturated fatty acids at *sn*-1 and *sn*-3 and short-chain fatty acids at *sn*-2 produce a triacylglycerol with 5–7 cal $g^{-1}$.

## 4.9 SUMMARY

Food lipids play an important role in food quality by contributing to attributes such as texture, flavor, nutrition, and caloric density. As knowledge of the nutritional importance of lipids continue to evolve, manufacturers will need to modify the physical and chemical properties of food lipids in order to produce healthy foods with high consumer acceptability. This means that foods will likely be produced to contain less of the nutritionally detrimental lipids (e.g., lower in total fat, saturated fat, and *trans* fatty acids). However, to accomplish these goals, food chemists will need to have a strong understanding of how lipids impact texture and flavor. Food will also be produced to contain nutritionally beneficial lipids such as $\omega$-3 fatty acids and phytosterols. A thorough understanding of the physical properties and chemical stability of lipids will be required to produce functional foods with bioactive lipids since these compounds can be chemically unstable ($\omega$-3 fatty acids) or difficult to physically incorporate into foods.

## REFERENCES


1. Faergemand, M. and Krog, N. (2003). Using emulsifiers to improve food texture. In *Texture in Foods, Volume 1: Semi-Solid Foods* (McKenna, B.M., ed.), CRC Press, Boca Raton, FL, chap. 10.
2. Bergenstahl, G. (1997). Physiochemical aspects of an emulsifier functionality. In *Food Emulsifiers and Their Applications* (Hasenhuettl, G.L. and Hartel, R.W., eds.), Chapman & Hall, New York, NY, chap. 6.
3. Bos, M., Nylander, T., Arnebrant, T., and Clark, D.C. (1997). Protein/emulsifier interactions. In *Food Emulsifiers and Their Applications* (Hasenhuettl, G.L. and Hartel, R.W., eds.), Chapman & Hall, New York, NY, chap. 5.
4. Deffenbaugh, L.B. (1997). Carbohydrate/emulsifier interactions. In *Food Emulsifiers and Their Applications* (Hasenhuettl, G.L. and Hartel, R.W., eds.), Chapman & Hall, New York, NY, chap. 4.
5. Krog, N.J. and Sparso, F.V. (2004). Food emulsifiers: their chemical and physical properties. In *Food Emulsions*, 4th edn. (Friberg, S., Larsson, K., and Sjoblom, J., eds.), Marcel Dekker, Inc., New York, NY, chap. 2.
6. Nielsen, S.S. (2003). *Food Analysis*, 3rd edn. Kluwer Academic, New York.
7. Wroltsad, R.E. (senior editor) (2005). *Current Protocols in Food Analytical Chemistry*. John Wiley & Sons, New York, NY.
8. O'Keefe, S.F. (2002). Nomenclature and classification of lipids. In *Food Lipids, Chemistry, Nutrition and Biotechnology* (Akoh, C.C. and Min, D.B., eds.), Marcel Dekker, Inc., New York, NY, pp. 1–40.
9. Simopoulos, A.P. (1999). Essential fatty acids in health and chronic disease. *Am. J. Clin. Nutr.* 70: 560S–569S.
10. O'Neil, M.J. (senior editor) (2001). *Merck Index*, 13th edn. Merck & Co., Inc., Whitehouse Station, NJ.
11. White, P.J. (2000). Fatty acids in oilseeds. In *Fatty Acids in Foods and Their Health Implications*, 2nd edn. (Chow, C.K., ed.), Marcel Dekker, Inc., New York, NY, pp. 209–238.

12. Ackman, R.G. (2000). Fatty acids in fish and shellfish. In *Fatty Acids in Foods and Their Health Implications*, 2nd edn. (Chow, C.K., ed.), Marcel Dekker, Inc., New York, NY, pp. 153–174.
13. Decker, E.A. (1996). The role of stereospecific saturated fatty acid position on lipid nutrition. *Nutr. Rev.* 54: 108–110.
14. Parish, E.J., Boos, T.L., and Li, S. (2002). The chemistry of waxes and sterols. In *Food Lipids, Chemistry, Nutrition and Biotechnology* (Akoh, C.C. and Min, D.B., eds.), Marcel Dekker, Inc., New York, NY, pp. 103–132.
15. Weiss, T.J. (1983). *Food Oils and Their Uses*, 2nd edn. AVI Publishing, Westport.
16. Gunstone, F.D. and Padley, F.B. (1997). *Lipid Technologies and Applications*. Marcel Dekker, Inc., New York, NY.
17. Akoh, C.C. and Min, D.B. (2002). *Food Lipids*, *Chemistry, Nutrition and Biotechnology*. Marcel Dekker, Inc., New York, NY.
18. Israelachvili, J.N. (1992). *Intermolecular and Surface Forces*. Academic Press, London.
19. Hartel, R.W. (2001). *Crystallization in Foods*. Aspen Publishers, Inc., Gaithersburg, MD.
20. Marangoni, A. and Narine, S. (2002). *Physical Properties of Lipids*. Marcel Dekker, Inc., New York, NY.
21. Larsson, K. (2004). Molecular organization in lipids and emulsions. In *Food Emulsions*. 4th edn. (Friberg, S., Larsson, K., and Sjoblom, J., eds.), Marcel Dekker, Inc., New York, NY, chap. 3.
22. Walstra, P. (1987). Fat crystallization. In *Food Structure and Behaviour* (Blanshard, J.M.V. and Lillford, P., eds.), Academic Press, London, chap. 5.
23. Walstra, P. (2003). *Physical Chemistry of Foods*. Marcel Dekker, Inc., New York, NY.
24. Formo, M.W. (1979). Physical properties of fats and fatty acids. In *Bailey's Industrial Oil and Fat Products*, Vol. 1, 5th edn. (Swern, D., ed.), John Wiley & Sons, New York, NY, chap. 3.
25. Gunstone, F.D. and Norris, F.A. (1983). *Lipids in Foods: Chemistry, Biochemistry and Technology*. Pergamon Press, Oxford.
26. Birker, P.J.M.W.L. and Padley, F.B. (1987). Physical properties of fats and oils. In *Recent Advances in Chemistry and Technology of Fats and Oils* (Hamilton, R.J. and Bhati, A., eds.), Elsevier Applied Science, London, chap. 1.
27. Timms, R.E. (1991). Crystallization of fats. *Chem. Ind.* May: 342–349.
28. Timms, R.E. (1995). Crystallization of fats. In *Developments in Oils and Fats* (Hamilton, R.J., ed.), Blackie Academic and Professional, London, chap. 8.
29. Coupland, J.N. and McClements, D.J. (1997). Physical properties of liquid edible oils. *J. Am. Oil Chem. Soc.* 74: 1559–1564.
30. Sherman, P. (1968). *Emulsion Science*. Academic Press, London.
31. Moran, D.P.J. (1994). Fats in spreadable products. In *Fats in Food Products*, Blackie Academic and Professional, London.
32. Pike, O.A. (2003). Fat characterization. In *Food Analysis*, 3rd edn. (Nielsen, S.S., ed.), Kluwer Academic, New York, NY, pp. 227–246.
33. McClements, D.J. (2004). *Food Emulsions: Principles, Practice and Techniques*, 2nd edn. CRC Press, Boca Raton, FL.
34. Hernqvist, L. (1984). On the structure of triglycerides in the liquid state and fat crystallinzation. *Fette Seifen Anstrichmittel* 86: 297.
35. Hutchings, J.B. (1999). *Food Color and Appearance*, 2nd edn. Aspen Publishers, Inc., Gaithersburg, MD.
36. Coupland, J. (2002). Determination of solid fat content by nuclear magnetic resonance. In *Current Protocols in Food Analytical Chemistry* (Wrolstad, R., ed.), John Wiley & Sons, New York, pp. D2.4.1–D2.4.18.
37. Lawler, P.J. and Dimick, P.S. (2002). Crystallization and polymorphism of fats. In *Food Lipids, Chemistry, Nutrition and Biotechnology* (Akoh, C.C. and Min, D.B., eds.), Marcel Dekker, Inc., New York, NY, chap. 9.
38. Bot, A., Floter, E., Lammers, J.G., and Pelan, E. (2003). Controlling the texture of spreads. In *Texture in Foods, Volume 1: Semi-Solid Foods* (McKenna, B.M., ed.), CRC Press, Boca Raton, FL, chap. 14.
39. Atkins, P.W. (1994). *Physical Chemistry*, 5th edn. Oxford University Press, Oxford.
40. Mullin, J.W. (2001). *Crystallization*, 4th edn. Butterworth-Heinmann, Oxford.
41. Dickinson, E., McClements, D.J., and Povey, M.J.W. (1991). Ultrasonic investigation of the particle size dependence of crystallization in n-hexadecane-in-water emulsions. *J. Coll. Interf. Sci.* 142: 103–110.

42. Boistelle, R. (1988). Fundamentals of nucleation and crystal growth. In *Crystallization and Polymorphism of Fats and Fatty Acids* (Garti, N. and Sato, K., eds.), Marcel Dekker, Inc., New York, NY, chap. 5.
43. Ghotra, B.S., Dyal, S.D., and Narine, S.S. (2002). Lipid shortenings: a review. *Food Res. Int.* 35: 1015–1048.
44. Garti, N. and Yano, J. (2001). The roles of emulsifiers in fat crystallization. In *Crystallization Processes in Fats and Lipid Systems* (Garti, N. and Sato, K., eds.), Marcel Dekker, Inc., New York, NY, p. 211.
45. Johnson, L.A. (2002). Recovery, refining, converting and stabilizing edible oils. In *Food Lipids, Chemistry, Nutrition and Biotechnology* (Akoh, C.C. and Min, D.B., eds.), Marcel Dekker, Inc., New York, NY, pp. 223–274.
46. Rousseau, D. and Marangoni, A.G. (2002). Chemical interesterification of food lipids: theory and practice. In *Food Lipids, Chemistry, Nutrition and Biotechnology* (Akoh, C.C. and Min, D.B., eds.), Marcel Dekker, Inc., New York, NY, pp. 301–334.
47. Willis, W.M. and Marangoni, A.G. (2002). Enzymatic interesterification. In *Food Lipids, Chemistry, Nutrition and Biotechnology* (Akoh, C.C. and Min, D.B., eds.), Marcel Dekker, Inc., New York, NY, pp. 1–40.
48. Flack, E. (1997). Butter, margarine, spreads and baking fats. In *Lipid Technologies and Applications* (Gunstone, F.D. and Padley, F.B., eds.), Marcel Dekker, Inc., New York, NY, pp. 265–304.
49. McClements, D.J. (2002). Theoretical prediction of emulsion color. *Adv. Coll. Interf. Sci.* 97: 63–89.
50. Lonchampt, P. and Hartel, R.W. (2004). Fat bloom in chocolate and compound coatings *Eur. J. Lipid Sci. Technol.* 106: 241–274.
51. Min, D.B. and Boff, J.M. (2002). Lipid oxidation in edible oil. In *Food Lipids, Chemistry, Nutrition and Biotechnology* (Akoh, C.C. and Min, D.B., eds.), Marcel Dekker, Inc., New York, NY, pp. 335–364.
52. Frankel, E.N. (1998). *Lipid Oxidation*. Oily Press, Scotland.
53. Huang, S.W., Frankel, E.N., and German, J.B. (1994). Antioxidant activity of alpha-tocopherols and gamma-tocopherols in bulk oils and in oil-in-water emulsions. *J. Agric. Food Chem.* 42: 2108–2114.
54. Abidi, S.L. and Rennick, K.A. (2003). Determination of nonvolatile components in polar fractions of rice bran oils. *J. Am. Oil Chem. Soc.* 80: 1057–1062.
55. Zhuang, H., Barth, M.M., and Hildebrand, D. (2002). Fatty acid oxidation in plant lipids. In *Food Lipids, Chemistry, Nutrition and Biotechnology* (Akoh, C.C. and Min, D.B., eds.), Marcel Dekker, Inc., New York, NY, pp. 413–464.
56. German, J.B. and Creveling, R.K. (1990). Identification and characterization of a 15-lipoxygenase from fish gills. *J. Agric. Food Chem.* 38: 2144–2147.
57. Decker, E.A. and McClements, D.J. (2001). Transition metal and hydroperoxide interactions: an important determinant in the oxidative stability of lipid dispersions. *Inform* 12: 251–255.
58. Girotti, A.W. (1998). Lipid hydroperoxide generation, turnover and effector action in biological systems. *J. Lipid Res.* 39: 1529–1542.
59. Halliwell, B. and Gutteridge, J.M. (1990). Role of free radicals and catalytic metal ions in human disease: an overview. *Meth. Enzymol.* 186: 1–88.
60. Faustman, C., Liebler, D.C., McClure, T.D., and Sun, Q. (1999). Alpha, beta-unsaturated aldehydes accelerate oxymyoglobin oxidation. *J. Agric. Food Chem.* 47: 3140–3144.
61. Smith, L.L. and Johnson, B.H. (1989). Biological activities of oxysterols. *Free Rad. Biol. Med.* 7: 285–332.
62. Liebler, D.C. (1993). The role of metabolism in the antioxidant function of vitamin E. *Crit. Rev. Toxicol.* 23: 147–169.
63. Buettner, G.R. (1993). The pecking order of free radicals and antioxidants: lipid peroxidation, $\alpha$-tocopherol, and ascorbate. *Arch. Biochem. Biophys.* 300: 535–543.
64. Shahidi, F. and Wanasundara, J.P.K. (1992). Phenolic antioxidants. *Crit. Rev. Food Sci. Nutr.* 32: 67–103.
65. Food and Nutrition Board, Institute of Medicine. (2001). Vitamin E. In *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids*. National Academy Press, Washington, DC, pp. 186–283.
66. Frankel, E.N., Huang, S.-W., Aeschbach, R., and Prior, E. (1996). Antioxidant activity of a rosemary extract and its constituents, carnosic acid, carnosol, and rosmarinic acid, in bulk oil and oil-in-water emulsion. *J. Agric. Food Chem.* 44: 131–135.

67. Aruoma, O.I., Halliwell, B., Aeschbach, R., and Löligers, J. (1992). Antioxidant and pro-oxidant properties of active rosemary constituents: carnosol and carnosol and carnosic acid. *Xenobiotica* 22: 257–268.
68. Mielche, M.M. and Bertelsen, G. (1994). Approaches to the prevention of warmedoven flavour. *Trend. Food Sci. Technol.* 5: 322–327.
69. Howard, L.R., Pandjaitan, N., Morelock, T., and Gil, M.I. (2002). Antioxidant capacity and phenolic content of spinach as affected by genetics and growing season. *J. Agric. Food Chem.* 50: 5891–5896.
70. Britz, S.J. and Kremer, D.F. (2002). Warm temperatures or drought during seed maturation increase free $\alpha$-tocopherol in seeds of soybean (glycine max {L.} Merr.). *J. Agric. Food Chem.* 50: 6058–6063.
71. Talcott, S.T., Howard, L.R., and Brenes, C.H. (2000). Antioxidant changes and sensory properties of carrot puree processed with and without periderm tissue. *J. Agric. Food Chem.* 48: 1315–1321.
72. Tong, L.M., Sasaki, S., McCelements, D.J., and Decker, E.A. (2000). Mechanisms of antioxidant activity of a high molecular weight fraction of whey. *J. Agric. Food Chem.* 48: 1473–1478.
73. Decker, E.A. (2002). Antioxidant mechanisms. In *Food Lipids, Chemistry, Nutrition and Biotechnology* (Akoh, C.C. and Min, D.B., eds.), Marcel Dekker, Inc., New York, NY, pp. 517–542.
74. Mahoney, J.R. and Graf, E. (1986). Role of $\alpha$-tocoperol, ascorbic acid, citric acid and EDTA as oxidants in a model system. *J. Food Sci.* 51: 1293–1296.
75. Sofos, J.N. (1986). Use of phosphates in low-sodium meat products. *Food Technol.* 40: 52–57.
76. Liebler, D.C. (1992). Antioxidant reactions of carotenoids. *Ann. NY Acad. Sci.* 691: 20–31.
77. Palozza, P. and Krinksky, N.I. (1992). Antioxidant effect of carotenoids *in vivo* and *in vitro*—an overview. *Meth. Enzymol.* 213: 403–420.
78. Kanner, J., German, J.B., and Kinsella, J.E. (1987). Initiation of lipid peroxidation in biological systems. *Crit. Rev. Food Sci. Nutr.* 25: 317–364.
79. Porter, W.L. (1993). Paradoxical behavior of antioxidants in food and biological systems. *Tox. Indus. Health* 9: 93–122.
80. Chen, H., Lee, D.J., and Schanus, E.G. (1992). The inhibitory effect of water on the $Co^{2+}$ and $Cu^{2+}$ catalyzed decomposition of methyl linoleate hydroperoxides. *Lipids* 27: 234–239.
81. Beuge, J.A. and Aust, S.D. (1978). Microsomal lipid peroxidation. *Meth. Enzymol.* 52: 302–310.
82. Pegg, R.B. (2002). Spectrophotometric measurement of secondary lipid oxidation products. In *Current Protocols in Food Analytical Chemistry* (Wrolstad, R., ed.), John Wiley & Sons, New York, NY, pp. D2.4.1–D2.4.18.
83. Shantha, N.C. and Decker, E.A. (1994). Rapid sensitive iron-based spectrophotometric methods for the determination of peroxide values in food lipids. *J. Assoc. Offic. Anal. Chem. Int.* 77: 421–424.
84. Larick, D.K. and Parker, J.D. (2002). Chromatographic analysis of secondary lipid oxidation products. In *Current Protocols in Food Analytical Chemistry* (Wrolstad, R., ed.), John Wiley & Sons, New York, pp. D2.2.1–D2.4.9.
85. Yu, T.C. and Sinnhuber, R.O. (1967). An improved 2-thiobarbituric acid (TBA) procedure for measurement of autooxidation in fish oils. *J. Am. Oil Chem. Soc.* 44: 256–261.
86. Grundy, S.M. (2001). United States cholesterol guidelines 2001: expanded scope of intensive low-density lipoprotein-lowering therapy. *Am. J. Cardiol.* 88: 23J–27J.
87. Willet, W.C., Stampfer, M.J., Manson, J.E., et al. (1993). Intake of *trans* fatty acids and risk of coronary heart disease among women. *Lancet* 341: 581–585.
88. Exler, J. (1987). *Composition of Foods: Finfish and Shellfish Products*. USDA Handbook 8-15, Washington, DC.
89. Ha, Y.L., Grimm, N.K., and Pariza, M.W. (1987). Anticarcinogens from fried ground beef: heat-altered derivatives of linoleic acid. *Carcinogenesis* 8: 1881–1887.
90. Lee, K.N., Kritchevsky, D., and Pariza, M.W. (1994). Conjugated linoleic acid and atherosclerosis in rabbits. *Atherosclerosis* 108: 19–25.
91. Park, Y., Storkson, J.M., Albright, K.J., Liu, W., and Pariza, M.W. (1999). Evidence that the *trans*-10, *cis*-12 isomer of conjugated linoleic acid induces body composition changes in mice. *Lipids* 34: 235–241.
92. Quilez, J., Garcia-Lorda, P., and Salas-Salvado, J. (2003). Potential uses and benefits of phytosterols in diet: present situation and future directions. *Clin. Nutr.* 22: 343–351.

93. Bendich, A. (2004). From 1989 to 2001: what have we learned about the "biological actions of beta-carotene"? *J. Nutr.* 134: 225S–230S.
94. Granado, F., Olmedilla, B., and Blanco, I. (2003). Nutritional and clinical relevance of lutein in human health. *Brit. J. Nutr.* 90: 487–502.
95. Nguyen, M.L. and Schwartz, S.J. (1999). Lycopene: chemical and biological properties. *Food Tech.* 53: 38–45.