23 chapter

Fat Characterization

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23.1 INTRODUCTION

Methods for characterizing edible **lipids**, fats, and oils can be separated into two categories: (1) those developed to analyze bulk oils and fats and (2) those focusing on analysis of foodstuffs and their lipid extracts. In evaluating foodstuffs, it is usually necessary to extract the lipids prior to analysis. In these cases, if sufficient quantities of lipids are available, methods developed for bulk fats and oils can be utilized.

The methods described in this chapter are divided into four sections. The first is traditional analytical methods for bulk fats and oils, many involving "wet chemistry." Then, two sections discuss methods of measuring lipid oxidation. Some of these methods utilize intact foodstuffs, but most require the lipids to be extracted from foodstuffs. Last addressed are methods for the analysis of lipid fractions, including fatty acids, triacylglycerols, and cholesterol.

Numerous methods exist for the characterization of lipids, fats, and oils [1–13]. This chapter includes methods required for the nutritional labeling of food and others appropriate for an undergraduate food analysis course. Many traditional "wet chemistry" methods have been supplemented or superseded by instrumental methods such as gas chromatography (GC), high-performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR), and Fourier transform infrared (FTIR) spectroscopy. Nonetheless, an understanding of basic concepts derived from traditional methods is valuable in learning more sophisticated instrumental methods.

Many of the methods cited are official methods of the AOAC International [1], American Oil Chemists' Society [2], or International Union of Pure and Applied Chemists [3]. The principles, general procedures, and applications are described for the methods. Refer to the specific methods cited in Table 23.1 for detailed information on procedures.

23.1.1 Definitions and Classifications

As explained in Chap. 17, the term **lipids** refers to a wide range of compounds soluble in organic solvents but only sparingly soluble in water. Chapter 17 also outlines the general classification scheme for lipids. The majority of lipids present in foodstuffs are of the following types: (1) fatty acids and their glycerides, including mono-, di-, and triacylglycerols, (2) phospholipids, (3) sterols (including cholesterol), (4) waxes, and (5) lipid-soluble pigments and vitamins. The commonly used terms monoglyceride, diglyceride, and triglyceride are synonymous with the proper nomenclature terms monoacylglycerol, diacylglycerol, and triacylglycerol, respectively.

In contrast to lipids, the terms fats and oils often refer to bulk products of commerce, crude or refined, that have already been extracted from animal products or oilseeds and other plants grown for their lipid content. The term **fat** signifies extracted lipids that are solid at room temperature, and **oil** refers to those that are liquid. However, the three terms, lipid, fat, and oil, often are used interchangeably.

The FDA has defined **fat content** for nutritional labeling purposes as the total lipid fatty acids expressed as triglyceride, rather than the extraction and gravimetric procedures used in the past (see also Chap. 17, Sect. 17.3.6.1).

> *"Fat, total" or "Total fat": A statement of the number of grams of total fat in a serving defined as total lipid fatty acids and expressed as triglycerides where fatty acids are aliphatic carboxylic acids consisting of a chain of alkyl groups and characterized by a terminal carboxyl group. Amounts shall be expressed to the nearest 0.5 (1/2) gram increment below 5 grams and to the nearest gram increment above 5 grams. If the serving contains less than 0.5 gram, the content shall be expressed as zero.* [21 CFR 101.9(c)(2)]

Fatty acids included in this definition may be derived from triacylglycerols, partial glycerides, phospholipids, glycolipids, sterol esters, or free fatty acids, but the concentration will be expressed as grams of triacylglycerols. This definition for nutritional labeling purpose requires that, rather than determining total fat via extraction and gravimetry (e.g., Soxhlet), total fat be based on GC of **fatty acid methyl esters** (FAMEs). Although this requires more complex and expensive analytical equipment, the analysis provides a better estimation of the fat content of foods.

Saturated fat is defined for nutritional labeling purposes as the sum (in grams) of all fatty acids without double bonds. Saturated fat is expressed to the closest 0.5 g below 5 g/serving and to the closest gram above 5 g. A food with less than 0.5 g of saturated fat per serving has the content expressed as zero. The optional category of **polyunsaturated fat** (PUFA) is defined as *cis, cis*-methylene-interrupted polyunsaturated fatty acids and has the same gram reporting requirements as saturated fats. Another optional category (unless certain label claims are made), **monounsaturated fat,** is defined as *cis*-monounsaturated fatty acids. The requirement that the fatty acids be *cis* prevents including fatty acids that contain *trans* isomers. *Trans* **fatty acids** must now be included on the nutritional label in the United States. The definition for nutritional labeling requires that the *trans* acids are not conjugated. Most *trans* fatty acids found in foods are monounsaturated, and methods for monounsaturated fatty acid and *trans* fatty acid analysis must distinguish between *cis* and *trans* monoenes, as well as the conjugated *cis-trans* fatty acids such as **conjugated linoleic acid** (CLA).

23.1 table Correlation of selected AOCS [2], AOAC [1], and IUPAC [3] methods

Method	*AOCS*	*AOAC*	*IUPAC*
Bulk fats and oils			
Refractive index	Cc 7-25	921.08	2.102
Melting			
Capillary tube melting point	Cc 1-25	920.157	
Slip melting point	Cc 3-25		
	Cc 3b-92		
DSC melting properties	Cj 1-94		
Dropping point	Cc 18-80		
Wiley melting point	Cc 2-38[a]	920.156	
Smoke, flash, and fire points	Cc 9a-48		
	Cc 9b-55		
Cold test	Cc 11-53	929.08	
Cloud point	Cc 6-25		
Color			
Lovibond	Cc 13e-92		
	Cc 13j-97		
Spectrophotometric	Cc 13c-50		2.103
Iodine value	Cd 1-25[a]	920.159	2.205
	Cd 1c-85		
	Cd 1d-87		
	Cd 1d-92	993.20	
	Cd 1e-01		
Saponification number	Cd 3-25	920.160	2.202
	Cd 3c-91		
	Cd 3a-94		
Free fatty acids (FFAs)	Ca 5a-40	940.28	
	Ca 5d-01		
Acid value	Cd 3d-63	969.17	2.201
Solid fat index (SFI)	Cd 10-57[a]		2.141
Solid fat content (SFC)	Cd 16b-93		2.150
Consistency, penetrometer method	Cc 16-60		
Spreadability	Cj 4-00		
Polar components in frying fats	Cd 20-91	982.27	2.507
Lipid oxidation – present status			
Peroxide value	Cd 8-53[a]	965.33	2.501
	Cd 8b-90		
p-Anisidine value	Cd 18-90		2.504
Hexanal (volatile organic compounds)	Cg 4-94		
Thiobarbituric acid (TBA) test	Cd 19-90		2.531
Conjugated dienes and trienes	Ti 1a-64	957.13	2.206
	Ch 5-91		
Lipid oxidation – oxidative stability			
Oven storage test	Cg 5-97		
Oil stability index (OSI)	Cd 12b-92		
Active oxygen method (AOM)	Cd 12-57[a]		2.506
Oxygen bomb			
Lipid fractions			
Fatty acid composition (including saturated/unsaturated, cis/trans)	Ce 1-62	963.22	2.302
	Ce 1a-13		
	Ce 1h-05	996.06	
	Ce 1i-07		
	Ce 1j-07		
	Ce 1e-91[a]		
	Ce 1f-96		

(continued)

23.1 table (continued)

Method	AOCS	AOAC	IUPAC
Fatty acid methyl esters (FAMEs)	Ce 2-66 Ce 2b-11 Ce 2c-11	969.33	2.301
trans Isomer fatty acids using IR spectroscopy	Cd 14-95 Cd 14d-99 Cd 14e-09 Cd 14f-14		2.207
Mono- and diacylglycerols	Cd 11-57 Cd 11b-91 Cd 11d-96	966.18	
Triacylglycerols	 Ce 5b-89 Ce 5c-93	986.19	
Cholesterol (and other sterols)		976.26	2.403

AOCS American Oil Chemists' Society, *AOAC* AOAC International, *IUPAC* International Union of Pure and Applied Chemists
[a]Though no longer current, these methods are included for reference because of their previous common use

There are several CLA isomers identified in ruminant lipids, although the 9 *cis* 11 *trans* isomer appears to be found in greatest abundance [4]. CLA isomers are thought to reduce the risk of cancer and other diseases.

> *"Trans fat" or "Trans": A statement of the number of grams of trans fat in a serving, defined as the sum of all unsaturated fatty acids that contain one or more isolated (i.e., nonconjugated) double bonds in a trans configuration, except that label declaration of trans fat content information is not required for products that contain less than 0.5 gram of total fat in a serving if no claims are made about fat, fatty acid or cholesterol content.* [21 CFR 101.9(c)(2)(ii)].

The presence of *trans* fat in foods has greatly declined due to this label requirement and also because the FDA has tentatively determined that the major source of trans fat in the diet, **partially hydrogenated oils** (PHO), is no longer generally recognized as safe (GRAS). However, some foods will still contain small amounts of *trans* fat because it occurs naturally in various animal products and edible oils.

23.1.2 Importance of Analyses

Such issues as the effect of dietary fat on health and food labeling requirements necessitate that food scientists be able not only to measure the total lipid content of a foodstuff but also to characterize it [4–10]. Health concerns require the measurement of such parameters as cholesterol and phytosterol contents, and amounts of *trans*, n-3/ω3, saturated, and mono- and polyunsaturated fatty acids. Lipid stability impacts not only the shelf life of food products but also their safety, since some oxidation products (e.g., malonaldehyde, cholesterol oxides) have toxic properties. Another area of interest is the analysis of oils and fats used in deep-fat frying operations [11]. Total polar materials and acid value are used as quality standards in deep-fat frying oil. Finally, the development of food ingredients composed of lipids that are not bioavailable (e.g., sucrose polyesters such as Olestra®) or lipids not contributing the normal 9 Cal/g to the diet (e.g., short- and medium-chain triglycerides such as Salatrim® and Caprenin®) accentuates the need to characterize the lipids present in food.

23.1.3 Lipid Content in Foods and Typical Values

Commodities containing significant amounts of fats and oils include butter; cheese; imitation dairy products such as margarine, spreads, shortening, frying fats, and cooking and salad oils; emulsified dressings such as mayonnaise, peanut butter, and confections; and muscle foods such as meat, poultry, and fish [12, 13]. Information is available summarizing the total fat content of foods (see Chap. 17, Table 17.2) as well as their constituent fatty acids [e.g., 14]. Ongoing studies are refining the quantities of saturated and unsaturated fat, *trans* isomers, cholesterol, cholesterol oxides, phytosterols, and other specific parameters in foods.

Because of their usefulness as food ingredients, it sometimes is important to know the physical and chemical characteristics of bulk fats and oils. Definitions and specifications for bulk fats and oils (e.g., soybean oil, corn oil, coconut oil), including values for many of the tests described in this chapter, can be found in Firestone [14], in the *Merck Index* [15], and in *Fats and Oils* [16]. Table 23.2 gives typical values for several of the tests for some of the common commercial fats and oils. It must be remembered that bulk fats

23.2 table Typical values of selected parameters for fats and oils

Fat/oil source	*Refractive index (40 °C)*	*Melting point (°C)*	*Iodine value*	*Saponification value*
Butterfat (bovine)	1.453–1.457		25–42	210–254
Cocoa butter	1.456–1.458	30–35	32–42	190–200
Coconut	1.448–1.450	21–26	5–13	242–265
Corn	1.465–1.468	−18 to−10	107–135	156–196
Cottonseed	1.458–1.466	−2	96–121	189–199
Lard			45–168	192–203
Menhaden			150–200	192–199
Olive		−3 to 0	75–94	184–196
Palm (*Elaeis guineensis*)	1.453–1.460	27–42.5	45–56	190–209
Palm kernel (*Elaeis guineensis*)	1.448–1.452	24–30	14–24	230–257
Peanut	1.460–1.465	−5 to −2	73–107	184–196
Rapeseed (canola)	1.465–1.467		110–126	182–193
Safflower seed	1.467–1.470	−5	136–151	186–203
Soybean	1.466–1.470		118–139	188–195
Sunflower seed	1.467–1.469		115–145	186–196
Tallow (beef)	1.450–1.458	45–48	33–50	190–202

Compiled from Firestone [14] with permission from AOCS Press, Copyright 2013

and oils can vary markedly in such parameters due to differences in source, composition, and susceptibility to deterioration. Foods containing even minor amounts of lipids (e.g., <1%) can have a shelf life limited by lipid oxidation and subsequent rancidity.

23.2 GENERAL CONSIDERATIONS

Various fat extraction solvents and methods are discussed in Chap. 17. For lipid characterization, extraction of fat or oil from foodstuffs can be accomplished by homogenizing with a solvent combination such as hexane-isopropanol (3:2, vol/vol) or chloroform-methanol (2:1, vol/vol). The solvent then can be removed using a rotary evaporator or by evaporation under a stream of nitrogen gas. Lipid oxidation during extraction and testing can be minimized by adding antioxidants [e.g., 10–100 mg of t-butylated hydroxytoluene (BHT)/L] to solvents and by taking other precautions such as flushing containers with nitrogen and avoiding exposure to heat and light [17].

Sample preparation is hastened through the use of **solid-phase extraction** (SPE), which consists of passing the lipid extract through a commercially available prepackaged absorbent (e.g., silica gel) that separates contaminants or various fractions based on polarity (see Chap. 14, Sect. 14.2.2.5, and Chap. 33, Sect. 33.2.2.3.2). Constituents present in lipid extractions that may present problems in lipid characterization include phosphatides, gossypol, carotenoids, chlorophyll, sterols, tocopherols, vitamin A, and metals.

Bulk oils such as soybean oil typically undergo the following purification processes: degumming, alkali or physical refining [removal of **free fatty acids** (FFAs)], bleaching, and deodorization after extraction from their parent source. Modifications such as fractionation, winterization, interesterification, and hydrogenation also may be a part of the processing, depending on the commodity being produced. Various methods discussed in this chapter can be used to monitor the refining process.

Changes that lipids undergo during processing and storage include hydrolysis (lipolysis), oxidation, and thermal degradation including polymerization (such as during deep-fat frying operations). These changes are discussed in the following sections on methods.

23.3 METHODS FOR BULK OILS AND FATS

Numerous methods exist to measure the characteristics of fats and oils. Some methods (e.g., titer test) have limited use for edible oils (in contrast to soaps and industrial oils). Other methods may require special apparatus not commonly available or may have been antiquated by modern instrumental procedures [e.g., volatile acid methods (Reichert-Meissl, Polenske, and Kirschner values) have been replaced largely by determination of fatty acid composition using GC]. Methods to determine impurities, including moisture, unsaponifiable material in refined vegetable oil, and insoluble impurities, also are not covered in this chapter. Defined methods exist for the sensory evaluation of fats and

oils (see AOCS Methods Cg 1-83 and Cg 2-83) but are outside the scope of this text.

23.3.1 Sample Preparation

Ensure that samples are visually clear and free of sediment. When required (e.g., iodine value), dry the samples prior to testing (AOAC Method 981.11). Because exposure to heat, light, or air promotes lipid oxidation, avoiding these conditions during sample storage will retard rancidity. If samples are solid or semisolid at room temperature, they should be melted and thoroughly mixed before sampling. Sampling procedures are available for bulk oils and fats (AOCS Method C 1-47).

23.3.2 Refractive Index

23.3.2.1 *Principle*

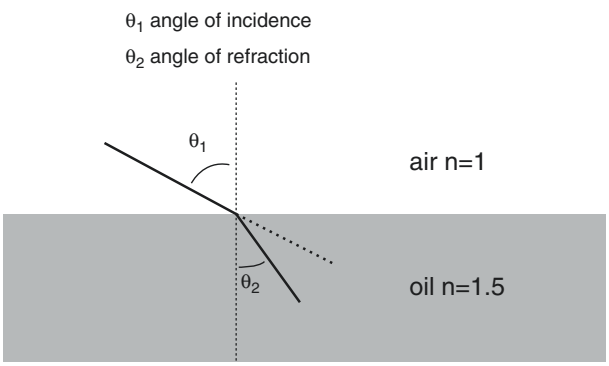
The **refractive index** (RI) of an oil is defined as the ratio of the speed of light in air (technically, a vacuum) to the speed of light in the oil. When a ray of light shines obliquely on an interface separating two materials, such as air and oil, the light ray is refracted in a manner defined by Snell's law, as shown in Eq. 23.1:

$$\theta_1 n_1 = \theta_2 n_2 \qquad (23.1)$$

where:

θ_1 = angle of the incident light
n_1 = refractive index of material 1
θ_2 = angle of the refracted light
n_2 = refractive index of material 2

As can be seen in Fig. 23.1 and from Eq. 23.1, if the angles of incidence and refraction and the refractive index (n) of one of the two materials are known, the refractive index of the other material can be determined. In practice, the θ_1 and n_1 are constant, so n_2 is determined by measuring θ_2.



23.1 figure Refraction of light in an air-oil interface

Because the frequency of light affects its refraction (violet light is refracted more than red light), white light can be dispersed or split after refraction through two materials of different refractive indexes (explaining the color separation of diamonds and rainbows). Refractometers often use monochromatic light (or nearly monochromatic light from the sodium doublet D line, that has 589.0 and 589.6 nm wavelengths or light-emitting diodes to provide 589.3 nm) to avoid errors from variable refraction of the different wavelengths of visible light.

23.3.2.2 *Procedure*

Samples are measured with a refractometer at 20 °C for oils and at specified higher temperatures for fats, depending on the temperature at which the fat is completely liquid.

23.3.2.3 *Applications*

RI is related to the amount of saturation in a lipid; the RI decreases linearly as iodine value (a measure of total unsaturation) decreases. RI also is used as a measure of purity and as a means of identification, since each substance has a characteristic RI. However, RI is influenced by such factors as FFA content, oxidation, and heating of the fat or oil. The RI of various lipids is shown in Table 23.2. A relatively saturated lipid such as coconut oil has a different RI (n = 1.448–1.450) compared to a relatively unsaturated lipid such as menhaden oil (n = 1.472).

23.3.3 Melting Point

23.3.3.1 *Principle*

The melting point may be defined in various ways, each corresponding to a different residual amount of solid fat. The **capillary tube melting point**, also known as the **complete melting point** or **clear point**, is the temperature at which fat heated at a given rate becomes completely clear and liquid in a one-end closed capillary. The **slip melting point** is performed similarly to the capillary tube method and measures the temperature at which a column of fat moves in an open capillary when heated. The **dropping point** (also called the **dropping melting point** or **Mettler dropping point)** is the temperature at which the sample flows through a 0.11-in. hole in a sample cup placed in a specialized furnace. The **Wiley melting point** measures the temperature at which a 1/8 × 3/8-in. disk of fat, suspended in an alcohol-water mixture of similar density, changes into a sphere.

23.3.3.2 *Applications*

It appears the predominant method in the United States for measuring melting point is the dropping melting point. The procedure has been automated and therefore is not labor-intensive. The capillary tube

method is less useful for oils and fats (in comparison to pure compounds) since they lack a sharp melting point due to their array of various components. The slip melting point often is used in Europe, whereas the Wiley melting point was preferred previously in the United States; the latter is no longer a current AOCS Method. A disadvantage of the Wiley melting point is the subjective determination as to when the disk is spherical. A disadvantage of the slip melting point is its 16-h stabilization time.

23.3.4 Smoke, Flash, and Fire Points

23.3.4.1 *Principle*

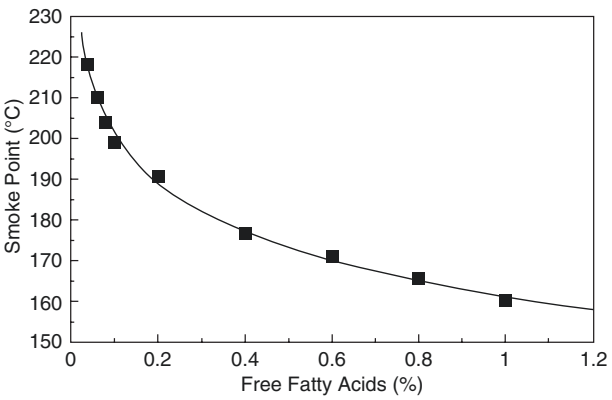
The **smoke point** is the temperature at which the sample begins to smoke when tested under specified conditions. The **flash point** is the temperature at which a flash appears at any point on the surface of the sample; volatile gaseous products of combustion are produced rapidly enough to permit ignition. The **fire point** is the temperature at which evolution of volatiles (by decomposition of sample) proceeds with enough speed to support continuous combustion.

23.3.4.2 *Procedure*

A cup is filled with oil or melted fat and heated in a well-lighted container. The smoke point is the temperature at which a thin, continuous stream of bluish smoke is given off. The flash point and fire point are obtained with continued heating, during which a test flame is passed over the sample at 5 °C intervals. For fats and oils that flash at temperatures below 149 °C, a closed cup is used.

23.3.4.3 *Applications*

These tests reflect the volatile organic material in oils and fats, especially FFAs (Fig. 23.2) and residual extraction solvents. Frying oils and refined oils should have smoke points above 200 °C and 300 °C, respectively.



23.2 figure Effect of free fatty acid content on smoke point of olive oil

23.3.5 Cold Test

23.3.5.1 *Principle*

The **cold test** is a measure of the resistance of an oil to crystallization. The absence of crystals or turbidity indicates proper winterizing.

23.3.5.2 *Procedure*

Oil is stored in an ice bath (0 °C) for 5.5 h and observed for crystallization.

23.3.5.3 *Applications*

The cold test is a measure of success of the winterizing process. It ensures that oils remain clear even when stored at refrigerated temperatures. Winterizing subjects an oil to cold temperatures and then separates the crystallized material from the bulk. This results in an oil that will not cloud at low temperature. This is useful for ensuring that oils remain clear in products, such as salad dressings, that will be stored at refrigeration temperatures after opening.

23.3.6 Cloud Point

23.3.6.1 *Principle*

The **cloud point** is the temperature at which a cloud is formed in a liquid fat due to the beginning of crystallization.

23.3.6.2 *Procedure*

The sample is heated to 130 °C and then cooled with agitation. The temperature of first crystallization is taken to be the point at which a thermometer in the fat is no longer visible.

23.3.7 Color

Two methods for measuring the color of fats and oils are the **Lovibond** method and the **spectrophotometric** method.

23.3.7.1 *Procedure*

In the Lovibond method, oil is placed in a standard sized glass cell and visually compared with red, yellow, blue, and neutral color standards. Results are expressed in terms of the numbers associated with the color standards. Automated colorimeters are available.

For the spectrophotometric method, the sample is heated to 25–30 °C, placed in a cuvette, and absorbance read at the following wavelengths: 460, 550, 620, and 670 nm. The photometric color index is calculated as shown in Eq. 23.2 AOCS Method Cc 13c-50:

$$\text{Photometric color index} = 1.29(A_{460}) + 69.7(A_{550}) + 41.2(A_{620}) - 56.4(A_{670}) \quad (23.2)$$

23.3.7.2 *Applications*

The color of fats and oils is most commonly evaluated using the Lovibond method. Oils and fats from different sources vary in color. But if refined oil is darker than expected, it is probably indicative of improper refinement or abuse [16]. Though specifically developed for testing the color of cottonseed, soybean, and peanut oils, the spectrophotometric method is probably applicable to other fats and oils as well.

23.3.8 Iodine Value

23.3.8.1 *Principle*

The iodine value (or iodine number) is a measure of degree of unsaturation, which is the number of carbon-carbon double bonds in relation to the amount of fat or oil. **Iodine value** is defined as the grams of iodine absorbed per 100 g of sample. The higher the amount of unsaturation, the more iodine is absorbed and the higher the iodine value.

A common practice is to determine **calculated iodine value** from the fatty acid composition (see Sect. 23.6.1) using AOCS Recommended Practice Cd 1c-85. The calculated iodine value is not meant to be a rapid method but instead gives two results (iodine value of triacylglycerols and FFAs) from one analysis (fatty acid composition).

23.3.8.2 *Procedure*

A quantity of fat or oil dissolved in solvent is reacted, avoiding light, with a measured amount of iodine or some other halogen such as ICl or IBr. Halogen addition to double bonds takes place (Eq. 23.3). A solution of potassium iodide is added to reduce excess ICl to free iodine (Eq. 23.4). The liberated iodine is then titrated with a sodium thiosulfate standard using a starch indicator (Eq. 23.5), and the iodine value is calculated (Eq. 23.6):

$$\underset{(\text{excess})}{\text{ICl}} + \text{R}-\text{CH}=\text{CH}-\text{R} \rightarrow \text{R}-\text{CHI}-\text{CHCl}-\text{R} + \underset{(\text{remaining})}{\text{ICl}} \qquad (23.3)$$

$$\text{ICl} + 2\text{KI} \rightarrow \text{KCl} + \text{KI} + \text{I}_2 \qquad (23.4)$$

$$\text{I}_2 + \underset{(\text{blue})}{\text{starch}} + 2\text{Na}_2\text{S}_2\text{O}_3 \rightarrow 2\text{NaI} + \underset{(\text{colorless})}{\text{starch}} + \text{Na}_2\text{S}_4\text{O}_6 \qquad (23.5)$$

$$\text{Iodine value} = \frac{(B-S)\times N\times 126.9}{W\times 1000}\times 100 \qquad (23.6)$$

where:

Iodine value = g iodine absorbed per 100 g of sample
B = volume of titrant (mL) for blank
S = volume of titrant (mL) for sample
N = normality of $Na_2S_2O_3$ (mol/L)
126.9 = MW of iodine (g/mol)
W = sample mass (g)
1,000 = conversion of units (mL/L)

Calculated iodine value is obtained from fatty acid composition using Eq. 23.7 for triacylglycerols. A similar equation allows calculation of the iodine value of FFAs:

$$\begin{aligned}\text{Iodine value (triglycerides)} = {} & (\%\ \text{hexadecenoic acid} \times 0.950) \\ & + (\%\ \text{octadecenoic acid} \times 0.860) \\ & + (\%\ \text{octadecadienoic acid} \times 1.732) \\ & + (\%\ \text{octadecatrienoic acid} \times 2.616) \\ & + (\%\ \text{eicosenoic acid} \times 0.785) \\ & + (\%\ \text{docosenoic acid} \times 0.723) \qquad (23.7)\end{aligned}$$

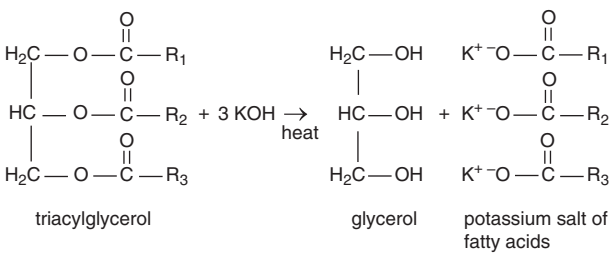
23.3.8.3 *Applications*

Iodine value is used to characterize oils, to follow the hydrogenation process in refining, and as an indication of lipid oxidation, since there is a decline in unsaturation during oxidation. The calculated value tends to be low for materials with a low iodine value and for oils with greater than 0.5% unsaponifiable material (e.g., fish oils). The Wijs iodine procedure uses ICl and the Hanus procedure uses IBr. The Wijs procedure may be preferable for highly unsaturated oils as it reacts faster with the double bonds.

23.3.9 Saponification Value

23.3.9.1 *Principle*

Saponification is the process of breaking down or degrading a neutral fat into glycerol and fatty acids by treatment of the fat with alkali (Eq. 23.8):



(23.8)

The **saponification value** (or saponification number) is defined as the amount of alkali necessary to saponify a given quantity of fat or oil. It is expressed as the milligrams of KOH required to saponify 1 g of the sample. The saponification value is an index of the mean molecular weight of the triacylglycerols in the sample. The mean molecular weight of the triacylglycerols may be divided by 3 to give an approximate mean molecular weight for the fatty acids present; the smaller the saponification value, the longer the average fatty acid chain length.

In common practice, the **calculated saponification value** is determined from the fatty acid composition (see Sect. 23.6.2) using AOCS Recommended Practice Cd 3a-94.

23.3.9.2 *Procedure*

Excess alcoholic potassium hydroxide is added to the sample, and the solution is heated to saponify the fat (Eq. 23.8). The unreacted potassium hydroxide is back-titrated with standardized HCl using phenolphthalein as the indicator and the saponification value is calculated (Eq. 23.9):

$$\text{Saponification value} = \frac{(B-S)\times N\times 56.1}{W} \quad (23.9)$$

where:

Saponification value = mg KOH per g of sample
B = volume of titrant (mL) for blank
S = volume of titrant (mL) for sample
N = normality of HCl (mmol/mL)
56.1 = MW of KOH (mg/mmol)
W = sample mass (g)

The **calculated saponification value** is obtained from fatty acid composition using Eq. 23.10. The **fractional molecular weight** of each fatty acid in the sample must be determined first by multiplying the fatty acid percentage (divided by 100) by its molecular weight. The **mean molecular weight** is the sum of the fractional weights of all the fatty acids in the sample:

$$\text{Calculated saponification value} = \frac{3\times 56.1\times 1000}{(\text{mean molecular weight}\times 3)+92.09-(3\times 18)} \quad (23.10)$$

where:

Calculated saponification value = mg KOH per g of sample
3 = number of fatty acids per triacylglycerol
56.1 = MW of KOH (g/mol)
1,000 = conversion of units (mg/g)
92.09 = MW of glycerol (g/mol)
18 = MW of water (g/mol)

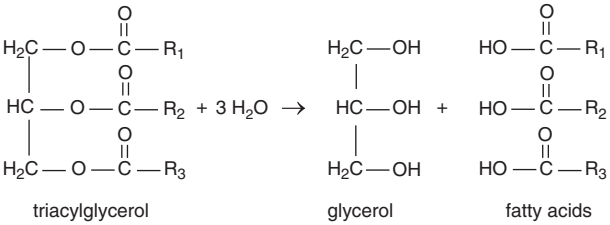
23.3.9.3 *Applications*

Saponification value is useful for determining the average fatty acid chain length of an oil of fat. The calculated saponification value is not applicable to fats and oils containing high amounts of unsaponifiable material, FFAs (>0.1 %), or mono- and diacylglycerols (>0.1 %).

23.3.10 Free Fatty Acids (FFAs) and Acid Value

23.3.10.1 *Principle*

Measures of fat acidity normally reflect the amount of fatty acids hydrolyzed from triacylglycerols (Eq. 23.11):



(23.11)

FFA is the percentage by weight of a specified fatty acid (e.g., percent oleic acid). **Acid value** (AV) is defined as the mg of KOH necessary to neutralize the free acids present in 1 g of fat or oil. The AV is often used as a quality indicator in frying oils, where a limit is 2 mg KOH/g oil is sometimes used. In addition to FFAs, acid phosphates and amino acids also can contribute to acidity. In samples containing no acids other than fatty acids, FFA and acid value may be converted from one to the other using a conversion factor (Eq. 23.12). Acid value conversion factors for lauric and palmitic are 2.81 and 2.19, respectively:

$$\%\,\text{FFA (as oleic)}\times 1.99 = \text{acid value} \quad (23.12)$$

Sometimes the acidity of edible oils and fats is expressed as milliliters of NaOH (of specified normality) required to neutralize the fatty acids in 100 g of fat or oil [8].

23.3.10.2 *Procedure*

To a liquid fat sample, neutralized 95 % ethanol and phenolphthalein indicator are added. The sample then is titrated with KOH and the percent FFA calculated (Eq. 23.13):

$$\%\,\text{FFA (as oleic)} = \frac{V\times N\times 282}{W\times 1000}\times 100 \quad (23.13)$$

where:

% FFA = percent free fatty acid (g/100 g), expressed as oleic acid
V = volume of KOH titrant (mL)
N = normality of KOH titrant (moL/L)
282 = MW of oleic acid (g/mol)
W = sample mass (g)
1,000 = conversion of units (mL/L)

23.3.10.3 *Applications*

In crude fat, FFA or acid value estimates the amount of oil that will be lost during refining steps designed to remove fatty acids. In refined fats, a high acidity level means a poorly refined fat or fat breakdown after storage or use. However, if a fat seems to have a high amount of FFAs, it may be attributable to acidic additives (e.g., citric acid added as a metal chelator) since any acid will participate in the reaction [16]. If the fatty acids liberated are volatile, FFA or acid value may be a measure of hydrolytic rancidity.

23.3.11 Solid Fat Content

23.3.11.1 *Principle*

The amount of solids in a fat, termed the **solid fat content** (SFC), is determined using either continuous wave or pulsed NMR. Chapter 10 explains NMR as it relates to measuring the solid content of fats and other foods. Comparison between samples must be made using SFC values taken at the same temperature. Note that the standard temperature used varies from country to country.

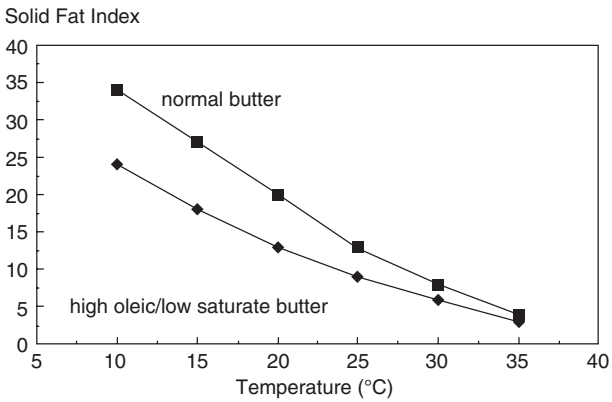
Originally, the amount of solids in a fat was estimated using the **solid fat index** (SFI). SFI is measured using dilatometry, which determines the change in volume with change in temperature. As solid fat melts, it increases in volume. Plotting volume against temperature gives a line at which the fat is solid, a line at which it is liquid, and a melting curve in between. The SFI is the volume of solid fat divided by the volume between the upper and lower lines, expressed as a percentage [16]. Though the equipment is expensive, SFC is preferred over SFI because it measures the actual fat content (it is not an estimate), is less subject to error, and takes less time.

23.3.11.2 *Applications*

The amount of solid fat phase present in a plastic fat (e.g., margarine, shortening) depends on the type of fat, its history, and the temperature of measurement. The proportion of solids to liquids in the fat and how quickly the solids melt have an impact on functional properties, such as the mouthfeel of a food. An example of SFI use is shown in Fig. 23.3; the butter with high oleic and low saturated fatty acid composition has lower solid fat and is softer as it melts over the temperature range 10–35 °C and is more easily spreadable at refrigeration temperatures.

23.3.12 Consistency and Spreadability

The textural properties of plasticized fats (e.g., shortenings, margarine, butter) can be measured using such tests as consistency and spreadability. The consistency method described has been used for several decades,



23.3 figure SFI curves of butter with normal and high oleic/low saturated fatty acid compositions

whereas the spreadability method is a more recently approved method that utilizes texture analysis instruments. The **penetrometer method** of determining consistency measures the distance a cone-shaped weight will penetrate a fat in a given time period. The **spreadability** test delineates the parameters for using a Texture Technologies TA-XT2 Texture Analyzer® (or similar instrument) to determine the force needed to compress a sample. See Chap. 29 for general approaches to characterizing the rheological properties of foods; many aspects can be applied to fats and oils.

The penetrometer method is useful for measuring the consistency of plastic fats and solid fat emulsions. Like SFC, consistency is dependent on the type of fat, its history, and the temperature during measurement. The spreadability method is applicable to lipid-containing solid suspensions, emulsion, and pastes that can maintain their shape at the temperature used for the analysis, including products such as peanut butter and mayonnaise.

23.3.13 Polar Components in Frying Fats

Methods used to monitor the quality of the oil or fat used in deep-fat frying operations are based on the physical and chemical changes that occur, which include an increase in each of the following parameters: viscosity, foaming, FFAs, degree of saturation, hydroxyl and carbonyl group formation, and saponification value. Standard tests used in the evaluation of frying fats include quantitating polar components, conjugated dienoic acids, polymers, and free fatty acids. In addition, there are several rapid tests useful in day-to-day quality assurance of deep-fat frying operations [11].

23.3.13.1 *Principle*

Deterioration of used frying oils and fats can be monitored by measuring the polar components, which

include monoacylglycerols, diacylglycerols, FFAs, and oxidation products formed during heating of foodstuffs. Nonpolar compounds are primarily unaltered triacylglycerols. The polar compounds in a sample can be separated from nonpolar compounds using chromatographic techniques.

23.3.13.2 *Procedure*

Polar components are measured by dissolving the fat sample in light petroleum ether-diethyl ether (87:13), then applying the solution to a silica gel column. Polar compounds are adsorbed onto the column. Nonpolar compounds are eluted, the solvent evaporated, the residue weighed, and the total polar components estimated by difference. Quality of the determination can be verified by eluting polar compounds and separating polar and nonpolar components using thin-layer chromatography (TLC).

23.3.13.3 *Applications*

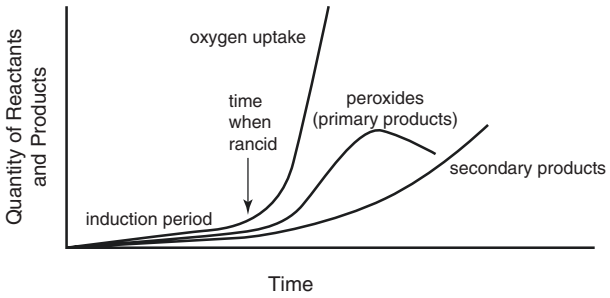
A suggested limit of 27% polar components in frying oil is a guide for when it should be discarded. A limitation of this method is the sample run time of 3.5 h [11]. The acid value is often determined as an alternate indicator of frying oil deterioration; however, rapid procedures based on dielectric constant are becoming more widely used because of their speed.

23.4 LIPID OXIDATION: MEASURING PRESENT STATUS

23.4.1 Overview

The term **rancidity** refers to the off odors and flavors resulting from lipolysis **(hydrolytic rancidity)** or lipid oxidation **(oxidative rancidity). Lipolysis** is the hydrolysis of fatty acids from the glyceride molecule. Because of their volatility, hydrolysis of short-chain fatty acids can result in off odors. Fatty acids shorter than C12 (lauric acid) can produce off odors in foods. Free C12 is often associated with a soapy taste but no aroma. FFAs longer than C12 do not cause significant impairment in taste or odor.

Lipid oxidation (also called autoxidation) as it occurs in bulk fats and oils proceeds via a self-sustaining free radical mechanism. Depending on the fatty acid composition, the prooxidants to which it is exposed, the type and amount of antioxidants present, and other factors, the decomposition of fats and oils can proceed via various mechanisms that produce many different compounds. The traditional explanation is that the initial or primary products are hydroperoxides that undergo scission to form various secondary products including aldehydes, ketones, organic acids, and hydrocarbons [18] (see Fig. 23.4). However, recent reviews [9, 10] suggest secondary products, including epoxides, dimers,



23.4 figure

Traditional portrayal of changes in quantities of lipid oxidation reactants and products over time. Recent reviews suggest greater complexity in possible reactions than shown here, where secondary products can be produced simultaneously with hydroperoxides (Adapted from Labuza [18], with permission, Copyright CRC Press, Boca Raton, FL, ©1971)

and polymers, can be produced simultaneously with hydroperoxides.

Many methods have been developed to measure the different compounds as they form or degrade during lipid oxidation. In the early stages of deterioration, effective measures of lipid oxidation include oxygen consumption, conjugated dienes, and hydroperoxide formation using peroxide value. Both conjugated dienes and hydroperoxides decompose, however, and are not valid measures in later stages or isolated samples. Other measures of lipid oxidation include the *p*-anisidine value, measurement of volatile organic compounds (e.g., aldehydes), and thiobarbituric acid reactive substances (TBARS). Some of these procedures have been modified (especially with respect to sample size) for use in biological tissue assays [19]. Other methods that monitor lipid oxidation (and that vary in usefulness) include the iodine value, acid value, Kreis test, and oxirane test, as well as the measurement of conjugated dienes and trienes, total and volatile carbonyl compounds, polar compounds, and hydrocarbon gases [6, 13]. Since the system is dynamic, it is recommended that two or more methods be used to obtain a more complete understanding of lipid oxidation. In addition, free radicals not only oxidize lipids but may co-oxidize other molecules such as proteins, nucleic acids, polysaccharides, vitamins, and pigments. Schaich et al. [10] suggest other measurements be made in addition to monitoring lipid oxidation, methods such as protein oxidation and pigment bleaching.

While quantitating lipid oxidation using methods such as those listed above is usually adequate, in some cases, it may be necessary to *visualize the location* of lipid molecules and lipid oxidation within a food or raw ingredient. **Fluorescence microscopy** with stains specific to lipids can be applied to such a problem (see Chap. 32, Sect. 32.2.2.3). For example, the dye Nile blue

(with the active ingredient Nile red) can be combined with a lipid-containing sample and the preparation viewed under a fluorescence microscope [20–22]. Lipids will appear an intense yellow fluorescence, with the intensity of the fluorescence changed by the nature of the lipids and by lipid oxidation. Example applications include localizing oxidized lipids in a cereal product, visualizing interactions between lipids and emulsifiers, and localizing lipids in cheeses, frosting, and chocolates.

23.4.2 Sample Preparation

Most methods require lipid extraction prior to analysis (see Sect. 23.2). However, variations of some methods (e.g., some TBARS tests) may begin with the original foodstuff.

23.4.3 Peroxide Value

23.4.3.1 *Principle*

Peroxide value is defined as the milliequivalents (mEq) of peroxide per kilogram of sample. It is a redox titrimetric determination. The assumption is made that the compounds reacting under the conditions of the test are peroxides or similar products of lipid oxidation.

23.4.3.2 *Procedure*

The fat or oil sample is dissolved in glacial acetic acid-isooctane (3:2). Upon addition of excess potassium iodide, which reacts with the peroxides, iodine is produced (Eq. 23.14). The solution then is titrated with standardized sodium thiosulfate using a starch indicator (Eq. 23.14). Peroxide value is calculated as shown in Eq. 23.15:

$$\mathrm{ROOH} + \mathrm{K^+I^-} \xrightarrow{\mathrm{H^+,heat}} \mathrm{ROH} + \underset{(\text{excess})}{\mathrm{K^+OH^-}} + \mathrm{I_2} \qquad (23.14)$$

$$\mathrm{I_2} + \underset{(\text{blue})}{\text{starch}} + 2\mathrm{Na_2S_2O_3} \rightarrow 2\mathrm{NaI} + \underset{(\text{colorless})}{\text{starch}} + \mathrm{Na_2S_4O_6} \qquad (23.15)$$

$$\text{Peroxide value} = \frac{(S-B)\times N}{W}\times 1000 \qquad (23.16)$$

where:

Peroxide value = mEq peroxide per kg of sample
S = volume of titrant (mL) for sample
B = volume of titrant (mL) for blank
N = normality of $Na_2S_2O_3$ solution (mEq/mL)
1,000 = conversion of units (g/kg)
W = sample mass (g)

23.4.3.3 *Applications*

Peroxide value measures a transient product of oxidation (i.e., after forming, peroxides and hydroperoxides break down to form other products). Though peroxide values measured on a given sample stored over time increase and then decrease (see Fig. 23.4), products are usually determined (using sensory analysis) to be rancid during the period of increasing values. For determination in foodstuffs, a disadvantage of this method is the 5 g fat or oil sample size required; it is difficult to obtain sufficient quantities from foods low in fat. This method is empirical and any modifications may change results. Despite its drawbacks, peroxide value is one of the most common tests of lipid oxidation.

High-quality, freshly deodorized fats and oils will have a peroxide value of zero. Peroxide values >20 correspond to very poor-quality fats and oils, which normally would have significant off flavors. For soybean oil, peroxide values of 1–5, 5–10, and >10 correspond to low, medium, and high levels of oxidation, respectively (AOCS Method Cg 3-91).

23.4.4 *p*-Anisidine Value and Totox Value

23.4.4.1 *Principle*

The ***p*-anisidine value** estimates the amount of α- and β-unsaturated aldehydes (mainly 2-alkenals and 2,4-dienals), which are secondary oxidation products in fats and oils. The aldehydes react with *p*-anisidine to form a chromogen that is measured spectrophotometrically. The **totox value** tends to indicate the total oxidation of a sample using both the peroxide and *p*-anisidine values (Eq. 23.16):

$$\text{Totox value} = p\text{-anisidine value} + (2\times \text{peroxide value}) \qquad (23.17)$$

23.4.4.2 *Procedure*

The ***p*-anisidine value** by convention is defined as 100 times the absorbance at 350 nm of a solution containing precisely 1 g of test oil diluted to 100 ml using a mixture of solvent (isooctane) and *p*-anisidine.

23.4.4.3 *Applications*

Since peroxide value measures hydroperoxides (which increase then decrease) and *p*-anisidine value measures aldehydes (decay products of hydroperoxides which continually increase), the totox value usually rises continually during the course of lipid oxidation. Fresh soybean oil should have a *p*-anisidine value <2.0 and a totox value <4.0 (AOCS Method Cg 3-91). Though not common in the United States, *p*-anisidine and totox values are extensively used in Europe [16].

23.4.5 Volatile Organic Compounds

23.4.5.1 *Principle*

Volatile organic compounds (VOCs) present in fats, oils, and foodstuffs are related to flavor, quality, and oxidative stability. These compounds include secondary products of lipid oxidation that can be responsible for the off flavors and odors of oxidized fats and oils (AOCS Method Cg 3-91). The compounds formed will vary depending on the fatty acid composition of the

sample and environmental conditions. Commonly measured compounds include propanal, pentanal, hexanal, and 2,4-decadienal. It is commonly performed using **static (equilibrium) headspace analysis**, which entails the chromatographic analysis of a set volume of vapor obtained from the headspace above a sample held in a closed container (see Chap. 14, Sect. 14.2.2.2). The introduction of **solid-phase microextraction** (SPME) improved the sensitivity of static headspace analysis of VOC because the volatiles are concentrated on the SPME fiber (Chap. 14, Sect. 14.2.2.5).

23.4.5.2 *Procedure*

General guidelines regarding GC parameters are given in AOCS Method Cg 4-94, under the heading of static headspace; a review of research literature will indicate current practices used with various commodities. Typically, a small sample of the commodity is placed in a container having a septum cap. An internal standard may be added, for example, 4-heptanone [23]. The container is sealed and then heated for a given time. Heating increases the concentration of headspace volatiles [24]. Using a gastight syringe, an aliquot of the vapors in the container headspace then is removed and injected into a GC equipped with a flame ionization detector or mass selective detector. Automated headspace samplers are available that both heat the sample and ensure a constant volume is being analyzed. The quantity of aldehydes, including hexanal and pentanal, and other volatiles of interest then is calculated from peak areas (see Chaps. 12 and 14).

23.4.5.3 *Applications*

The formation of aldehydes, such as hexanal and pentanal, may correlate well with sensory determination of lipid oxidation since they are major contributors to off flavors in some food commodities. The quantity of other volatile compounds resulting from lipid oxidation can be obtained simultaneously with aldehyde measurement, and may enhance the characterization of lipid oxidation in various food commodities. An advantage of this method is that since volatiles are collected from the headspace of the sample vial, lipid extraction is not required (i.e., intact foodstuffs can be analyzed). However, various SPME fibers are available, and the specific fiber used may not absorb all the volatiles produced.

23.4.6 Thiobarbituric Acid Reactive Substances Test

23.4.6.1 *Principle*

The **thiobarbituric acid reactive substances** (TBARS) test, also known as the **thiobarbituric acid** (TBA) test, measures secondary products of lipid oxidation, primarily **malonaldehyde**. It involves reaction of malonaldehyde (or malonaldehyde-type products including unsaturated carbonyls) with TBA to yield a colored compound that is measured spectrophotometrically. The food sample may be reacted directly with TBA but is often distilled to eliminate interfering substances, and then the distillate is reacted with TBA. Many modifications of the test have been developed over the years.

23.4.6.2 *Procedure*

In contrast to the direct method for fats and oils (see Table 23.1), a commonly used procedure [25, 26] is outlined here that requires distillation of the food commodity prior to determining TBARS. A weighed sample is combined with distilled water and mixed. The pH is adjusted to 1.2 and the sample is transferred to a distillation flask. After addition of BHT (optional), antifoam reagent, and boiling beads, the sample is distilled rapidly and the first 50 ml collected. An aliquot of the distillate is combined with TBA reagent and heated in a boiling water bath for 35 min. Absorbance of the solution is determined at 530 nm, and, using a standard curve, absorbance readings are typically converted to milligrams of malonaldehyde (or TBARS) per kilograms of sample.

23.4.6.3 *Applications*

The TBA test correlates better with sensory evaluation of rancidity than does peroxide value but, like peroxide value, it is a measure of a transient product of oxidation (i.e., malonaldehyde and other carbonyls readily react with other compounds). An alternative to the spectrophotometric method described is to determine the actual content of malonaldehyde using HPLC analysis of the distillate.

Despite common use, tests for measuring malonaldehyde are only applicable to foods containing fatty acids with at least three double bonds and therefore are not useful in many food systems [10].

23.4.7 Conjugated Dienes and Trienes

23.4.7.1 *Principle*

Double bonds in lipids are changed from nonconjugated to conjugated bonds upon oxidation. Fatty acid **conjugated diene hydroperoxides** formed on lipid oxidation absorb UV light at about 232 nm and **conjugated trienes** at about 270 nm. Conjugated products of lipid oxidation should not be confused with conjugated linoleic acid isomers, which are produced by incomplete hydrogenation in ruminants.

23.4.7.2 *Procedure*

A homogeneous lipid sample is weighed into a graduated flask and brought to volume with a suitable solvent (e.g., isooctane, cyclohexane). A 1% solution (e.g., 0.25 g sample in 25 ml) is made, then diluted or concentrated if necessary to obtain an absorbance

between 0.1 and 0.8. The diluted solution must be completely clear. Absorbance is measured with an ultraviolet (UV) spectrophotometer, using the pure solvent as a blank.

A common practice is to calculate the amount of conjugated dienes from chromatographic analysis of fatty acids (see Chap. 14).

23.4.7.3 *Applications*

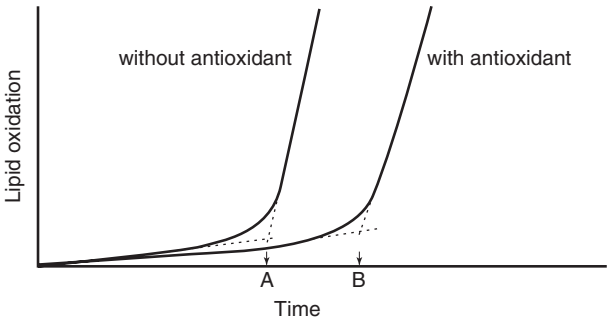
The conjugated diene and triene methods are useful for monitoring the early stages of oxidation. The magnitude of the changes in absorption is not easily related to the extent of oxidation in advanced stages. Olive oil grades are based on ultraviolet absorbance.

23.5 LIPID OXIDATION: EVALUATING OXIDATIVE STABILITY

23.5.1 Overview

Because of their inherent properties (e.g., the amount of unsaturation and the presence of natural antioxidants) as well as external factors (e.g., added antioxidants, processing, and storage conditions), lipids and lipid-containing foodstuffs vary in their susceptibility to rancidity. The resistance of lipids to oxidation is known as **oxidative stability**. Inasmuch as determining oxidative stability using actual shelf life determinations at ambient conditions of storage (usually room temperature) requires months or even years, accelerated tests have been developed to evaluate the oxidative stability of bulk oils and fats and various foodstuffs. **Accelerated tests** artificially hasten lipid oxidation by exposing samples to heat, oxygen, metal catalysts, light, or enzymes. A major problem with accelerated tests is assuming reactions that occur at elevated temperatures or under other artificial conditions are the same as normal reactions occurring at the actual storage temperature of the product. An additional difficulty is ensuring that the apparatus is clean and completely free of metal contaminants and oxidation products from previous runs. Therefore, assuming lipid oxidation is the factor that limits shelf life, shelf life determinations at ambient conditions should accompany and hopefully validate the results of accelerated tests of oxidative stability.

The **induction period** is defined as the length of time before detectable rancidity or time before rapid acceleration of lipid oxidation. Induction period can be determined by such methods as calculating the maximum of the second derivative with respect to time or manually drawing tangents to the lines (Fig. 23.5). Measurement of the induction period allows a comparison of the oxidative stability of samples that contain differing ingredients or of samples held at varying



23.5 figure

A plot of lipid oxidation over time, showing the effect of an antioxidant on induction period. Time A is induction period of sample without antioxidant and Time B is induction period of sample with antioxidant

storage conditions, and it provides an indication of the **effectiveness of** various **antioxidants** in delaying lipid oxidation and thus prolonging the shelf life of fats, oils, and foodstuffs. Guidelines for conducting such tests have been established (see AOCS Method Cg 7-05). In contrast to measuring the effectiveness of antioxidants in prolonging food shelf life, **antioxidant capacity** refers to the ability of foods and ingredients to counter oxidative reactions in the human body and is discussed in Chap. 25.

23.5.2 Oven Storage Test

As a means of accelerating the determination of oxidative stability, the **oven storage test** is often used. Previously this test was an ill-defined procedure known as the Schaal oven test. It is now a recommended practice of the AOCS (AOCS Method Cg 5-97). This protocol consists of placing a fat or oil of known volume in a forced-draft oven at a temperature above ambient but less than 80 °C, with 60 °C being recommended. Such temperatures are desirable accelerated storage temperatures since the mechanism of oxidation in this range is the same as oxidation at room temperature. Tests should be conducted in the dark, the initial quality of the sample oil should be high, and the surface to volume ratio of the oil must be kept constant for all samples.

To determine an induction period and thus oxidative stability, the oven storage test must be combined with other methods of detecting rancidity, for example, sensory evaluation and peroxide value. More than one method should be used to determine the extent of oxidation; it is recommended that one method measure primary products of lipid oxidation (e.g., peroxide value, conjugated dienes) and the other method measure secondary products (e.g., VOC, *p*-anisidine value, TBARS, or sensory evaluation). Results of oxidative stability determinations obtained at approximately 60 °C correlate well with actual shelf life determinations [8].

23.5.3 Oil Stability Index

23.5.3.1 *Principle*

The **oil stability index** (OSI) determines induction period by bubbling purified air through an oil or fat sample held at an elevated temperature (often 110 °C or 130 °C), then passing the acidic volatiles (primarily formic acid) into a deionized water trap. The conductivity of the water is measured continuously, resulting in data similar to those shown in Fig. 23.5. Results should specify the air flow rate and temperature used, as well as induction period time. Two instruments that automate this method are the Rancimat® (Metrohm USA, Inc.) and the Oxidative Stability Instrument® (Ultra Scientific, Inc.). The outdated and labor-intensive **active oxygen method** (AOM) is similar to the OSI except induction period is determined by discontinuous measurements of either peroxide value or sensory evaluation of rancid odor.

23.5.3.2 *Applications*

These methods were designed originally to measure the effectiveness of antioxidants. The OSI is determined much faster than tests performed at oven storage test temperatures, but results from the latter may correlate better with actual shelf life. Extrapolating OSI results to actual shelf life is difficult because the high temperatures used result in the formation of compounds not present in samples held at ambient or slightly elevated temperature conditions [8, 27, 28].

Specification sheets for fats and oils often report AOM values to accommodate individuals working in this area who are familiar with AOM values. OSI values can be converted to AOM values.

Applicable to all fats and oils, the OSI has also been researched for applicability to certain low-moisture snack foods (e.g., potato chips and corn chips). Because of the continuous exposure to circulating air, samples that contain more than negligible amounts of water tend to dehydrate during the determination and are not likely to give reliable results because water activity can affect oxidation rates.

23.5.4 Oxygen Bomb

23.5.4.1 *Principle*

Inasmuch as lipid oxidation results in the uptake of oxygen from the surrounding environment (see Fig. 23.4), measuring the time required for the onset of rapid disappearance of oxygen in a closed system provides a means of determining oxidative stability.

23.5.4.2 *Procedure*

The oxygen bomb consists of a heavy-walled container that has a pressure recorder attached. The sample is placed in the container, and oxygen is used to pressurize the container to 100 psi. The container then is placed in a boiling water bath. Induction period is determined by measuring the time until a sharp drop in pressure occurs, which corresponds with the rapid absorption of oxygen by the sample.

23.5.4.3 *Applications*

Oxygen bomb results may have a better correlation with rancidity shelf life tests than OSI values. Another advantage, compared to the OSI, is that the oxygen bomb method may be used with intact foodstuffs instead of extracted lipids [16].

23.6 METHODS FOR LIPID COMPONENTS

23.6.1 Overview

The lipid present in food commodities or bulk fats and oils can be characterized by measuring the amount of its various fractions, which include fatty acids, mono-, di-, and triacylglycerols, phospholipids, sterols (including cholesterol and phytosterols), and lipid-soluble pigments and vitamins. Another means of categorizing lipid fractions is inherent in nutrition labeling which involves the measurement of not only total fat but may also require quantification of saturated fat, monounsaturated fat, polyunsaturated fat, and *trans* isomer fatty acids. In addition, foods may contain lipids that do not contribute the same caloric content as normal lipids, for example, sucrose polyesters (e.g., Olestra®), medium-chain triglycerides, and triglycerides that contain short-chain fatty acids (e.g., Salatrim®, Caprenin®). Many of these fractions are determined readily by evaluating the component fatty acids. From the fatty acid composition, calculations are made to determine such parameters as total fat, saturated fat, calculated iodine value, and calculated saponification value.

In contrast, the measurement of total and saturated fat in foods containing Olestra® requires special consideration. AOAC peer-verified Method PVM 4:1995 outlines the use of lipase on the lipid extract, which yields fatty acids and unreacted Olestra®. The fatty acids are converted to calcium soaps and Olestra® is extracted and discarded. The precipitated soaps are converted back to fatty acids, which are subsequently analyzed via capillary GC [29]. Other procedures that have been studied include chromatographic separation using HPLC with an evaporative light-scattering detector (ELSD) [30]. Olestra®, like most lipids, will not absorb UV or VIS light appreciably, preventing analysis by using the common UV-VIS HPLC detectors. For this reason, detectors such as the refractive index (RID), transport flame ionization (TFID), or ELSD are required for analysis of most lipids.

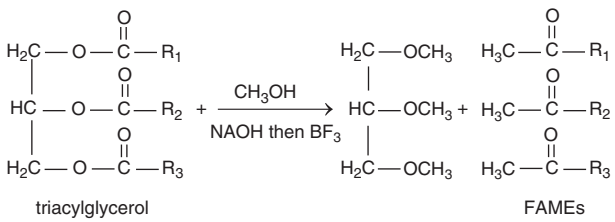
GC (see Chap. 14) is ideal for the analysis of many lipid components. GC can be used for determinations such as total fatty acid composition, distribution and position of fatty acids in lipid, sterols, studies of fat stability and oxidation, assaying heat or irradiation damage to lipids, and detection of adulterants and antioxidants [10]. Methods exist that detail the analysis of various lipid fractions using GC [5]. GC combined with **mass spectrometry** (MS) (see Chap. 11) is a powerful tool used in identification of compounds. **HPLC** (see Chap. 13) also is useful in lipid analyses, especially for components that are not readily volatilized, such as hydroperoxides and triacylglycerols [28]. **Thin-layer chromatography** (TLC) (see Chap. 12) has been used extensively in the past by lipid chemists. Partly due to low cost and ease, TLC is still useful, although many assays may be more quantitative or have better resolution using GC or HPLC.

23.6.2 Fatty Acid Composition and Fatty Acid Methyl Esters (FAMEs)

The **fatty acid composition,** or **fatty acid profile,** of a food product is determined by quantifying the kind and amount of fatty acids that are present, usually by extracting the lipids and analyzing them using **capillary GC** (also described in Chap. 17, Sect. 17.2.7).

23.6.2.1 *Principle*

To increase volatility before GC analysis, triacylglycerols are typically transesterified to form **fatty acid methyl esters** (FAMEs) (Eq. 23.18). Acyl lipids are readily transesterified using base such as sodium hydroxide and methanol. Sodium methoxide produced by this combination will create FAMEs from acyl lipids rapidly but will not react with free fatty acids. Acidic reagents such as methanolic HCL or boron trifluoride (BF_3) react rapidly with FFAs but more slowly with acyl lipids. Procedures such as the AOCS Method Ce 1b-89 (this is a joint method with AOAC 991.39) use a two-step methylation, first reacting the lipid with 0.5 *N* NaOH then with excess BF_3/methanol. This allows a rapid methylation of FFAs, acyl lipids, and phospholipids. The sodium hydroxide step is not a saponification procedure (i.e., hydrolysis of acyl groups); it is a direct transmethylation:



(23.18)

23.6.2.2 *Procedure*

The lipid is extracted from the food, for example, by homogenizing with a suitable solvent such as hexane-isopropanol (3:2, vol/vol) and then evaporating the solvent. The FAMEs are prepared by combining the extracted lipid with sodium hydroxide methanol and internal standard in isooctane and then heating at 100 °C for 5 min. The sample is cooled and then excess BF_3-methanol is added with further heating (100 °C for 30 min). After addition of saturated aqueous sodium chloride, additional isooctane, and mixing, the upper isooctane solution containing the FAMEs is removed and dried with anhydrous Na_2SO_4, and then diluted to a concentration of 5–10 % for injection onto the GC.

Several methods (see Table 23.1) describe procedures and conditions for using GC to determine fatty acid composition. AOCS Method Ce 1b-89 is specific for marine oils, and AOCS Method Ce 1f-96 is specifically suited for determining *trans* isomer fatty acids.

23.6.2.3 *Applications*

Determination of the fatty acid composition of a product permits the **calculation** of the following categories of fats that pertain to health issues and food labeling: percent saturated fatty acids, percent unsaturated fatty acids, percent monounsaturated fatty acids, percent polyunsaturated fatty acids, CLAs, and percent *trans* isomer fatty acids. Calculation of fatty acids as a percentage is referred to as **normalization**, i.e., the areas of all of the FAMEs are summed and the percent area of each fatty acid is calculated relative to the total area. This is a reasonable procedure because with flame ionization detectors (FID), the weight of fatty acids in a mixture closely parallels the area on the chromatogram. However, this is not absolutely correct. Theoretical correction factors are needed to correct for the FID response which is different depending on the level of unsaturation in FAMEs [31]. A chromatogram showing separation of FAMEs of varying length and unsaturation is shown in Chap. 17, Fig. 17.4. The separation of FAMEs on this SP2560 column is typical of what is seen when using a highly polar (biscyanopropyl polysiloxane) column [32].

The separation of FAMEs on GC columns depends on the polarity of the liquid phase. On nonpolar liquid phases [such as 100 % dimethyl polysiloxane (DB-1, HP-1, CPSil5CB) or 95 % dimethyl, 5 % diphenyl polysiloxane (DB5, HP5, CPSil8CB)], FAMEs are separated largely based on their boiling points. This results in the elution order 18:3n-3>18:3n-6>18:1n-9>18:0>20:0. On phases of medium polarity [such as 50 % cyanopropylphenyl polysiloxane (DB225, HP225, CPSil43CB)], the order of elution is changed because of the interaction of the pi electrons of the double bonds with the liquid

phase. The order of elution on these columns would be 18:0>18:1n-9>18:2n-6>18:3n-3>20:0 (first eluted to last). When the polarity of the liquid is increased further with 100% biscyanopropyl polysiloxane columns (SP2560, CPSil88), the greater interaction of the double bonds with the very polar liquid phase results in an elution pattern 18:0>18:1n-9>18:2n-6>20:0>18:3n-3. As the liquid phase polarity increases, the effect of double bonds on retention time increases. Additionally, *trans* fatty acids interact less effectively with the liquid phase than *cis* acids for steric reasons, so *trans* acids will elute before the corresponding *cis* acid; see Fig. 8.5 where 18:1Δ9 *trans* (elaidate) elutes before 18:1Δ9 (oleic acid), and 18:2Δ9 *trans* Δ12 *trans* (linoelaidate) elutes before linoleic acid (18:2n-6, 18:2 Δ9 *cis* Δ12 *cis*) on this highly polar 100% biscyanopropyl polysiloxane column. It also can be seen that gamma linolenic (18:3n-6) elutes before linolenic acid (18:3n-3). Because the double bonds are closer to the methyl side of the FAMEs in 18:3n-3, the double bonds can more effectively interact with the liquid phase, resulting in greater retention by the column.

The complexity of the fatty acids found in various foods will affect the details of the GC analysis that can be used. Analysis of FAMEs of a vegetable oil is quite simple and can easily be accomplished in less than 20 min using a column with a medium polarity liquid phase. The fatty acids present in most vegetable oils range from C14 to C24. Coconut and palm kernel oils also contain shorter-chain fatty acids such as C8–C12. Dairy fats contain butyric acid (C4) and other short-chain fatty acids, whereas peanut oil contains C26 at around 0.4–0.5% of the total FAMEs. Marine lipids contain a much wider range of fatty acids and require care in the separation and identification of FAMEs, many of which have no commercially available standards.

Trans fatty acids in foods originate from three main sources: biohydrogenation in ruminants, incomplete hydrogenation in the conversion of liquid oils to plastic fats, and high-temperature exposure during deodorization. The *trans* fatty acids formed from these three processes are quite different and require careful attention to achieve accurate analysis.

Separation of *trans* FAMEs is facilitated by selection of the most polar column phases available. Currently, Supelco SP2560 and Chrompak CPSil88 are most often used for analysis of *trans* fatty acids [32]. These columns have liquid phases based on 100% biscyanopropyl polysiloxane. Even with optimized temperature programming and column selection, resolution of *trans* isomers from partially hydrogenated vegetable oil mixtures is incomplete and facilitated by the use of a Fourier transform infrared (FTIR) detector (Chap. 8, Sect. 8.3.1.2) or mass spectrometry (Chap. 11).

23.6.3 *trans* Isomer Fatty Acids Using Infrared Spectroscopy

Most natural fats and oils extracted from plant sources contain only isolated (i.e., methylene interrupted, nonconjugated) *cis* double bonds. Fats and oils extracted from animal sources may contain small amounts of *trans* double bonds. Inasmuch as the *trans* isomer is more thermodynamically stable, additional amounts of *trans* double bonds can be formed in fats and oils that undergo oxidation, or during processing treatments such as extraction, heating, and hydrogenation. Ongoing studies are evaluating the health effects of dietary lipids that contain *trans* fatty acids.

Measurement of *trans* isomer fatty acids is commonly done using GC techniques, such as AOCS Method Ce 1f-96 (see Sect. 23.6.2). However, this section describes the use of infrared (IR) spectroscopy for determining *trans* isomer fatty acids.

23.6.3.1 *Principle*

The concentration of *trans* fatty acids is measurable in lipids from an absorption peak at 966 cm^{-1} in the IR spectrum.

23.6.3.2 *Procedure*

AOCS Method Cd 14-95 requires liquid samples be converted to methyl esters and dissolved in an appropriate solvent that does not absorb in the IR region strongly, due to planar (carbon disulfide) or tetrahedral (carbon tetrachloride) symmetry. The absorbance spectra between 1,050 and 900 cm^{-1} are obtained using an infrared spectrometer (see Chap. 8). Methyl elaidate is used as an external standard in calculating the content of *trans* double bonds. Alternately, AOCS Method Cd 14d-96 determines total *trans* fatty acids using attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy (see Chap. 8, Sects. 8.3.1.2 and 8.3.2).

23.6.3.3 *Applications*

The methods described will only detect isolated (i.e., nonconjugated) *trans* isomers. This is especially important when oxidized samples are of interest since oxidation results in a conversion from nonconjugated to conjugated double bonds. Also, AOCS Method Cd 14-95 is restricted to samples containing at least 5% *trans* isomers, and AOCS Method Cd 14d-96 is limited to samples containing at least 0.8% *trans* isomer fatty acids. For samples containing less than 0.8% *trans* double bonds, a capillary GC method (AOCS Method Ce 1f-96) is recommended.

23.6.4 Mono-, Di-, and Triacylglycerols

Mono-, di-, and triacylglycerols may be determined using various techniques (see Table 23.1). Older

methods use titrimetric approaches, whereas newer methods utilize chromatographic techniques, including HPLC and GC. Short nonpolar columns and very high temperatures are needed for analysis of intact triacylglycerols by GC. Section 23.6.6 describes the use of TLC to separate lipid classes, including mono-, di-, and triacylglycerols.

23.6.5 Cholesterol and Phytosterols

Many methods exist for the quantification of cholesterol and phytosterols in various matrices. Consulting research literature will give an indication of current practice and methods that may be less laborious or adapted for use with specific foodstuffs.

23.6.5.1 *Principle*

The lipid extracted from the food is saponified. The saponification process is a hydrolysis, with acyl lipids being converted to water-soluble FFA salts. Other components (called the unsaponifiable or nonsaponifiable matter) do not change in solubility after hydrolysis and thus remain soluble in organic solvents. Cholesterol (in the unsaponifiable fraction) is extracted and derivatized to form trimethylsilyl (TMS) ethers or acetate esters. This increases their volatility and reduces problems of peak tailing during chromatography. Quantitation is achieved using capillary GC.

23.6.5.2 *Procedure*

AOAC Method 976.26 outlined here is representative of the various procedures available for cholesterol determination. Lipids are extracted from the food and saponified, and the unsaponifiable fraction is extracted. This is accomplished by filtering an aliquot of the chloroform layer through anhydrous sodium sulfate and evaporating to dryness in a water bath using a stream of nitrogen gas. Concentrated potassium hydroxide and ethanol are added and the solution is refluxed. Aliquots of benzene and 1 *N* potassium hydroxide are added and then shaken. The aqueous layer is removed and the process is repeated with 0.5 *N* potassium hydroxide. After several washes with water, the benzene layer is dried with anhydrous sodium sulfate and an aliquot is evaporated to dryness on a rotary evaporator. The residue is taken up in dimethylformamide. An aliquot of this sample is derivatized by adding hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS). Water (to react with and inactivate excess reagent) and an internal standard in heptane are added, and then the solution is centrifuged. A portion of the heptane layer is injected into a GC equipped with a nonpolar column. The HMDS and TMCS reagents are rapidly inactivated by water, and thus the reaction conditions must remain anhydrous.

23.6.5.3 *Applications*

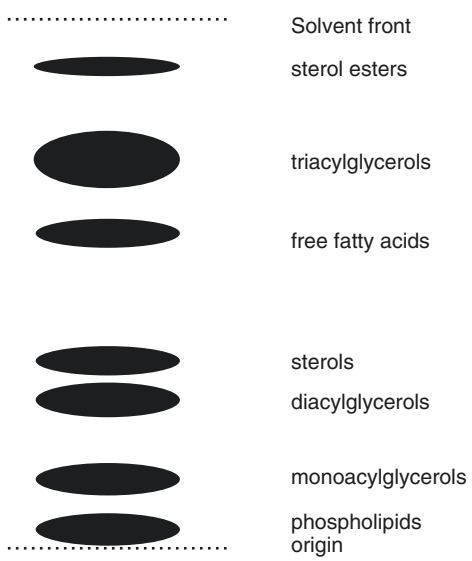
GC quantitation of cholesterol is recommended since many spectrophotometric methods are not specific for cholesterol. In the past, samples such as eggs and shrimp have had their cholesterol contents overestimated by relying on less specific colorimetric procedures. Other GC, HPLC, and enzymatic methods are available. For example, cholesterol methods developed for frozen foods [33] and meat products [34] eliminate the fat extraction step, directly saponifying the sample; compared to the AOAC method outlined previously, they are more rapid and avoid exposure to toxic solvents.

Cholesterol oxidation products as well as phytosterols can be quantified using the GC procedure outlined for cholesterol. A wide range of methods for analysis of sterols exist in the literature; most use TMS ether formation to increase volatility of the hydroxide-containing sterols and to improve chromatographic resolution (reduce peak tailing).

23.6.6 Separation of Lipid Fractions by Thin-Layer Chromatography

23.6.6.1 *Procedure*

Thin-layer chromatography (TLC) is performed using silica gel G as the adsorbent and hexane-diethyl ether-formic acid (80:20:2 vol/vol/vol) as the eluting solvent system (Fig. 23.6). Plates are sprayed with 2′,7′-dichlorofluorescein in methanol and placed under ultraviolet light to view yellow bands against a dark background [5].



23.6 figure Schematic thin-layer chromatography (TLC) separation of lipid fractions on silica gel G (Adapted with permission from Christie [5])

23.6.6.2 *Applications*

This procedure permits rapid analysis of the presence of the various lipid fractions in a food lipid extract. For small-scale preparative purposes, TLC plates can be scraped to remove various bands for further analysis using GC or other means. Many variations in TLC parameters are available that will separate various lipids. Thin-layer plates can be impregnated with silver nitrate to allow separation of FAMEs based on their number of double bonds. FAMEs with six double bonds are highly retained by the silver ions on the plate; FAMEs with no double bonds are only slightly retained. This allows a separation of FAMEs based on number of double bonds, which can be useful when identifying FAMEs in complex mixtures (bands are scraped off, eluted with solvent, then analyzed by GC).

23.7 SUMMARY

The importance of fat characterization is evident in many aspects of the food industry, including ingredient technology, product development, quality assurance, product shelf life, and regulatory aspects. Lipids are closely associated with health; the cholesterol or phytosterol compositions, and amounts of *trans*, saturated, and n-3/ω3 fatty acids are of great concern to consumers.

The methods described in this chapter help to characterize bulk oils and fats and the lipids in foodstuffs. A summary of some of the more common tests is given in Table 23.3. Methods described for bulk oils and fats can be used to determine characteristics such as melting point; smoke, flash, and fire points; color; degree of unsaturation; average fatty acid chain length; and amount of polar components. The peroxide value, TBA, and hexanal tests can be used to measure the present status of a lipid with regard to oxidation, while the OSI can be used to predict the susceptibility of a lipid to oxidation and the effectiveness of antioxidants. Lipid fractions, including fatty acids, triacylglycerols, phospholipids, and cholesterol, are commonly analyzed by chromatographic techniques such as GC and TLC.

The methods discussed in this chapter represent only a few of the many tests that have been developed

23.3 table Summary of some of the common tests used to characterize fats and oils in bulk products or foodstuffs

Test	*Indicated*	*Actually measured*
Iodine value	Degree of unsaturation	Iodine required for absorption by double bonds
Saponification value	Mean molecular weight of triacylglycerols	Alkali required to saponify fat/oil
Free fatty acids	Fatty acid hydrolysis from triglycerides (hydrolytic rancidity)	Potassium hydroxide necessary to neutralize free acids
Solid fat content	Proportion of solid vs. liquid fat	Percent solid fat, by continuous wave or pulsed NMR
Conjugated dienes and trienes	Oxidative rancidity (current status)	Change from nonconjugated to conjugated bonds, due to early stage of lipid oxidation
Peroxide value (PV)	Oxidative rancidity (current status)	Peroxides, due to early stages of lipid oxidation
Totox value	Oxidative rancidity (current status)	Peroxide and p-anisidine values, due to early and later stages of lipid oxidation
Volatile organic compounds	Oxidative rancidity (current status)	Volatile organic compounds (e.g., hexanal, pentanal, pentane), secondary products of lipid oxidation
Thiobarbituric acid reactive Substances (TBARS)	Oxidative rancidity (current status)	Malonaldehyde and similar compounds, as secondary products of lipid oxidation
Oven storage test	Oxidative stability (predicted)	Induction period after exposure to elevated temperature, measured using PV or sensory evaluation
Oil stability index	Oxidative stability (predicted)	Induction period after exposure to air and high temperature, based on acidic volatiles, measured by conductivity
Fatty acid methyl esters (FAMES)	Fatty acid composition of fats and oils; calculated total fat (nutritional labeling)	Chromatographic separation and quantification of individual fatty acids after fat extraction and hydrolysis of triacylglycerols
Trans fatty acids	Trans fat (nutritional labeling)	Chromatographic separation and quantification of fatty acids containing trans bonds after fat extraction and hydrolysis of triacylglycerols
Cholesterol	Cholesterol (nutritional labeling)	Chromatographic determination of cholesterol after fat extraction and saponification

to characterize lipid material. Consult the references cited for additional methods or more detailed explanations. Time, funding, availability of equipment and instruments, required accuracy, and purpose all will dictate the choice of method to characterize oils, fats, and foodstuffs containing lipids.

23.8 STUDY QUESTIONS

1. You want to compare several fat/oil samples for the chemical characteristics listed below. For each characteristic, name one test (give full name, not abbreviation) that could be used to obtain the information desired:
   (a) Degree of unsaturation
   (b) Predicted susceptibility to oxidative rancidity
   (c) Present status with regard to oxidative rancidity
   (d) Average fatty acid molecular weight
   (e) Amount of solid fat at various temperatures
   (f) Hydrolytic rancidity

2. Your analysis of an oil sample gives the following results. What does each of these results tell you about the characteristics of the sample? Briefly describe the principle for each method used:
   (a) Large saponification value
   (b) Low iodine value
   (c) High TBA number
   (d) High FFA content
   (e) High OSI

3. Define solid fat content and explain the usefulness of this measurement.
4. Peroxide value, TBA number, and hexanal content all can be used to help characterize a fat sample:
   (a) What do the results of these tests tell you about a fat sample?
   (b) Differentiate these three tests as to what chemical is being measured.

5. What methods would be useful in determining the effectiveness of various antioxidants added to an oil?
6. You are responsible for writing the specifications for vegetable oil purchased from your supplier for use in deep-fat frying several foods processed by your company. Itemize the tests you should require in your list of specifications (specific values for the tests are not needed). For each test, briefly state what useful information is obtained.
7. The Nutrition Education and Labeling Act of 1990, as amended in 2016 (see Chap. 3), requires that the nutrition label on food products contains information related to lipid constituents. In addition to the amount of total fat (see Chap. 17), the label must state saturated fat, *trans* fat, and cholesterol contents:
   (a) For a product such as traditional potato chips, explain an appropriate method for the analysis of each of these lipid constituents.
   (b) Compared with assays on traditional chips, how would the assays for total fat and saturated fat differ for potato chips made with Olestra®?

8. You have developed a new butter containing added fish oil, which is high in PUFA. Before placing the product on the market, you need to determine its shelf life. What method or methods would you use and why?
9. You work in quality control for a company that makes peanut butter. You received information that between July and August several lots of peanut butter may have been improperly stored and you are concerned about potential lipid oxidation in the product. A total of 50 lots of peanut butter are involved:
   (a) What test(s) would you use to measure lipid oxidation? Include your rationale for selecting the method.
   (b) How would you decide what to do with the peanut butter?

23.9 PRACTICE PROBLEMS

1. A 5.00-g sample of oil was saponified with excess KOH. The unreacted KOH was then titrated with 0.500 *N* HCl (standardized). The difference between the blank and the sample was 25.8 ml of titrant. Calculate the saponification value.
2. A sample (5.0 g) of food grade oil was reacted with excess KI to determine peroxide value. The free iodine was titrated with a standardized solution of 0.10 *N* $Na_2S_2O_3$. The amount of titrant required was 0.60 ml (blank corrected). Calculate the peroxide value of the oil.
3. You analyze the saponification value of an unknown. You use 4.0 g oil and 0.5 *N* HCl to titrate. The difference between the blank and the sample was 43 mL of titrant. What is the average fatty acid molecular weight of the oil?

4. You analyze an oil FAMEs by GC and find the following areas for your identified peaks:

16:0	2,853,369
18:0	1,182,738
18:1n-9	38,999,438
18:2n-6	14,344,172
18:3 n-3	2,148,207

Report the fatty acid composition as % and tentatively identify the oil.

Answers:

1. Saponification value =

$$\frac{25.8\text{mL} \times 0.500\ \text{mmoL/mL} \times 56.1\ \text{mg/mmol}}{5.00\text{g}} = 145$$

2. Peroxide value =

$$\frac{0.60\ \text{mL} \times 0.10\ \text{meq/mL} \times 1000}{5.0\text{g}} = 12\ \text{meq/kg}$$

3. Saponification value =

$$\frac{43\text{mL} \times 0.500\ \text{mol/mL} \times 56.1\text{mg/mmol}}{4.0\text{g}} = 302$$

$$302 = \left[3 \times 56.1 \times 1000\right] / \left\{\begin{array}{l}\left[\left(\text{Mean mol.wt} \times 3\right) + 92.09\right] \\ -\left(3 \times 18\right)\end{array}\right\}$$

$$\text{Mean mol.wt} = 172$$

4. Sum of all areas = 58,416,924

$$\text{Area \% } 16:0 = 100 \times 2,853,369 / 58,416,924 = 4.9\%$$

so,

16:0	4.9%
18:0	2.0%
18:1n-9	66.8%
18:2 n-6	22.7%
18:3n-3	3.7%

Based on the fatty acid composition, the oil is probably Canola oil.

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