
17 chapter

# Fat Analysis

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## 17.1 INTRODUCTION

### 17.1.1 Definitions

Lipids, proteins, and carbohydrates constitute the principal structural components of foods. Lipids are a group of substances that, in general, are soluble in ether, chloroform, or other organic solvents but are sparingly soluble in water. However, there exists no clear scientific definition of a lipid, primarily due to the water solubility of certain molecules that fall within one of the variable categories of food lipids [1]. Some lipids, such as triacylglycerols, are very hydrophobic. Other lipids, such as di- and monoacylglycerols, have both hydrophobic and hydrophilic moieties in their molecules and are soluble in relatively polar solvents [2]. Short-chain fatty acids such as C1–C4 are completely miscible in water and insoluble in nonpolar solvents [1].

As implied above, the most widely accepted definition is based on solubility as previously stated. While most macromolecules are characterized by common structural features, the designation of "lipid" being defined by solubility characteristics is unique to lipids [2]. Lipids comprise a broad group of substances that have some common properties and compositional similarities [3]. Triacylglycerols are fats and oils that represent the most prevalent category of the group of compounds known as lipids. The terms lipids, fats, and oils are often used interchangeably.

The term "lipid" commonly refers to the broad, total collection of food molecules that meet the definition previously stated. Fats generally refer to those lipids that are solid at room temperature, and oils generally refer to those lipids that are liquid at room temperature. While there may not be an exact scientific definition, the US Food and Drug Administration (FDA) has established a regulatory definition for nutrition labeling purposes. The FDA has defined total fat as the sum of fatty acids from C4 to C24, calculated as triglycerides. This definition provides a clear path for resolution of any nutrition labeling disputes.

### 17.1.2 General Classification

The general classification of lipids that follows is useful to differentiate lipids in foods [3]:

1. **Simple lipids:** ester of fatty acids with alcohol (i.e., fats, waxes)
2. **Compound lipids:** compounds containing groups in addition to an ester of a fatty acid with an alcohol (i.e., phospholipids, cerebrosides, and sphingolipids)
3. **Derived lipids:** substances derived from neutral lipids or compound lipids (e.g., fatty acids, long-chain alcohols, sterols, fat-soluble vitamins, and hydrocarbons)

### 17.1.3 Content of Lipids in Foods

Foods may contain any or all types of the lipid compounds previously mentioned. The lipid content in bovine milk (Table 17.1) illustrates the complexity and variability of lipids in a food system, having lipids that differ in polarity and concentrations.

Foods contain many types of lipids, but those that tend to be of greatest importance are the triacylglycerols and the phospholipids. **Liquid triacylglycerols** at room temperature are referred to as **oils**, such as soybean oil and olive oil, and are generally of plant origin. **Solid triacylglycerols** at room temperature are termed **fats**. Lard and tallow are examples of fats, which are generally from animals. The term *fat* is applicable to all triacylglycerols whether they are normally solid or liquid at ambient temperatures. Table 17.2 shows the wide range of lipid content in different foods.

17.1 table Lipids of bovine milk

| *Kinds of lipids* | *Percent of total lipids* |
|---|---|
| Triacylglycerols | 97–99 |
| Diacylglycerols | 0.28–0.59 |
| Monoacylglycerols | 0.016–0.038 |
| Phospholipids | 0.2–1.0 |
| Sterols | 0.25–0.40 |
| Squalene | Trace |
| Free fatty acids | 0.10–0.44 |
| Waxes | Trace |
| Vitamin A | (7–8.5 μg/g) |
| Carotenoids | (8–10 μg/g) |
| Vitamin D | Trace |
| Vitamin E | (2–5 μg/g) |
| Vitamin K | Trace |

Adapted from Patton and Jensen [4] with permission of Jenness and Patton [5] *Principles of Dairy Chemistry*. Jenness, R., and Patton, S. Copyright ©1959, John Wiley & Sons, Inc, with permission

17.2 table Fat content of selected foods

| *Food item* | *Percent fat (wet weight basis)* |
|---|---|
| Cereals, bread, and pasta | |
| Rice, white, long grain, regular, raw, enriched | 0.7 |
| *Sorghum* | 3.3 |
| Wheat, soft white | 2.0 |
| Rye | 2.5 |
| Wheat germ, crude | 9.7 |
| Rye bread | 3.3 |
| Cracked wheat bread | 3.9 |
| Macaroni, dry, enriched | 1.5 |
| Dairy products | |
| Milk, reduced fat, fluid, 2% | 2.0 |
| Skim milk, fluid | 0.2 |
| Cheddar cheese | 33.1 |
| Yogurt, plain, whole milk | 3.2 |
| Fats and oils | |
| Lard, shortening, oils | 100.0 |
| Butter, with salt | 81.1 |
| Margarine, regular, hard, soybean | 80.5 |
| Salad dressing | |
| Italian, commercial, regular | 28.3 |
| Thousand island, commercial, regular | 35.1 |
| French, commercial, regular | 44.8 |
| Mayonnaise, soybean oil, with salt | 79.4 |
| Fruits and vegetables | |
| Apples, raw, with skin | 0.2 |
| Oranges, raw, all commercial varieties | 0.1 |
| Blackberries, raw | 0.5 |
| Avocados, raw, all commercial varieties | 14.7 |
| Asparagus, raw | 0.1 |
| Lima beans, immature seeds, raw | 0.9 |
| Sweet corn, yellow, raw | 1.2 |
| Legumes | |
| Soybeans, mature seeds, raw | 19.9 |
| Black beans, mature seed, raw | 1.4 |
| Meat, poultry, and fish | |
| Beef, flank, separable lean and fat | 5.0 |
| Chicken, broilers or fryers, breast meat only | 1.2 |
| Bacon, pork, cured, raw | 45.0 |
| Pork, fresh, loin, whole, raw | 12.6 |
| Finfish, halibut, Atlantic and Pacific, raw | 2.3 |
| Finfish, cod, Atlantic, raw | 0.7 |
| Nuts | |
| Coconut meat, raw | 33.5 |
| Almonds, dried, unblanched, dry roasted | 52.8 |
| Walnuts, black, dried | 56.6 |
| Egg, whole, raw, fresh | 10.0 |

From US Department of Agriculture, Agricultural Research Service (2015) USDA National Nutrient Database for Standard Reference. Release 28 Nutrient Data Laboratory Home Page, http://ndb.nal.usda.gov

### 17.1.4 Importance of Analysis

An accurate and precise quantitative and qualitative analysis of lipids in foods is important for accurate nutritional labeling, to determine whether the food meets the standard of identity, and to ensure that the product meets manufacturing specifications. Inaccuracies in analysis may prove costly for manufacturers and could result in a product of undesirable quality and functionality.

### 17.1.5 General Considerations

By definition, lipids are soluble in organic solvents and insoluble in water. Therefore, water insolubility is the essential analytical property used as the basis for the separation of lipids from proteins, water, and carbohydrates in foods. Glycolipids are soluble in alcohols and have a low solubility in hexane. In contrast, triacylglycerols are soluble in hexane and petroleum ether, which are nonpolar solvents. The wide range of relative hydrophobicity of different lipids makes the selection of a single universal solvent impossible for lipid extraction of foods. Some lipids in foods are components of complex lipoproteins and lipopolysaccharides; therefore, successful extraction requires that bonds between lipids and proteins or carbohydrates be broken so that the lipids can be freed and solubilized in the extracting organic solvents.

## 17.2 SOLVENT EXTRACTION METHODS

### 17.2.1 Introduction

For routine quality control purposes, the total lipid content of a food is commonly determined by simple organic solvent extraction methods or by alkaline (Sect. 17.2.6.1) or acid (Sect. 17.2.6.2) hydrolysis followed by solvent extraction in a Mojonnier flask. For multicomponent food products, acid hydrolysis is often the method of choice. Solvent extraction methods may be used as a first step in the gas chromatographic determination of fatty acid content for nutrition labeling as required by FDA regulation in the United States for the determination of total fat (see Chap. 3).

The accuracy of direct solvent extraction methods (i.e., without prior acid or alkaline hydrolysis) greatly depends on the solubility of the lipids in the solvent used and the ability to separate the lipids from complexes with other macromolecules. The lipid content of a food determined by extraction with one solvent may be quite different from the content determined with another solvent of different polarity. In addition to solvent extraction methods, there are nonsolvent wet extraction methods and several instrumental methods that utilize the physical and chemical properties of lipids in foods for fat content determination.

Many of the methods cited in this chapter are official methods of AOAC International. Refer to these methods and other original references cited for detailed instructions of procedures. There are many methods available for the determination of lipid content. This chapter will focus on some of the primary methods in common use.

### 17.2.2 Sample Preparation

The validity of the fat analysis of a food depends on proper sampling and preservation of the sample before the analysis (see also Chap. 5). An ideal sample should be as close as possible in all of its intrinsic properties to the material from which it is taken. However, a sample is considered satisfactory if the properties under investigation correspond to those of the bulk material within the limits of the test [7].

The sample preparation for lipid analysis depends on the type of food and type and nature of lipids in the food [8]. The extraction method for lipids in liquid milk is generally different from that for lipids in solid soybeans. To analyze the lipids in foods effectively, knowledge of the structure, the chemistry, and the occurrence of the principal lipid classes and their constituents is necessary. Therefore, there is no single standard method for the extraction of all kinds of lipids in different foods. For the best results, sample preparation should be carried out under an inert atmosphere of nitrogen at low temperature to minimize chemical reactions such as lipid oxidation.

One or more preparatory steps are common in lipid analysis to aid in extraction: (1) removal of water, (2) reduction of particle size, (3) or separation of the lipid from bound proteins and/or carbohydrates through the use of techniques such as alkaline hydrolysis (Sect. 17.2.6.1) or acid hydrolysis (Sect. 17.2.6.2). The first two of these steps are described in sections immediately below.

#### 17.2.2.1 *Predrying Sample*

Lipids cannot be effectively extracted with ethyl ether from moist food because the solvent cannot easily penetrate the moist food tissues due to the hydrophobicity of the solvents used or the hydroscopic nature of the solvents. The ether, which is hygroscopic, becomes saturated with water and inefficient for lipid extraction. Drying the sample at elevated temperatures is undesirable because some lipids become bound to proteins and carbohydrates, and bound lipids are not easily extracted with organic solvents. Vacuum oven drying at low temperature or lyophilization increases the surface area of the sample for better lipid extraction. Predrying makes the sample easier to grind for better extraction, breaks fat-water emulsions to make fat dissolve easily in the organic solvent, and helps to free fat from the tissues of foods [7].

#### 17.2.2.2 *Particle Size Reduction*

The extraction efficiency of lipids from dried foods depends on particle size; therefore, adequate grinding is very important. The classical method of determining fat in oilseeds involves the extraction of the ground seeds with selected solvent after repeated grinding at low temperature to minimize lipid oxidation. For better extraction, the sample and solvent are mixed in a high-speed comminuting device such as a blender. It can be difficult to extract lipids from whole soybeans because of the limited porosity of the soybean hull and its sensitivity to dehydrating agents. The lipid extraction from soybeans is easily accomplished if the beans are broken mechanically by grinding. Extraction of fat from finished products can be a challenge, based on the ingredients (e.g., energy bars with nuts, caramel, protein, granola, soybean oil). Such products may best be ground after freezing with liquid nitrogen.

### 17.2.3 Solvent Selection

Ideal solvents for fat extraction should have a high solvent power for lipids and low or no solvent power for proteins, amino acids, and carbohydrates. They should evaporate readily and leave no residue, have a relatively low boiling point, and be nonflammable and nontoxic in both liquid and vapor states. The ideal solvent should penetrate sample particles readily, be in single component form to avoid fractionation, and be inexpensive and nonhygroscopic [6, 7]. It is difficult to find an ideal fat solvent to meet all of these requirements. Ethyl ether and petroleum ether are the most commonly used solvents, but pentane and hexane are used to extract oil from soybeans.

**Ethyl ether** has a boiling point of 34.6 °C and is a better solvent for fat than petroleum ether. It is generally expensive compared to other solvents, has a greater danger of explosion and fire hazards, is hygroscopic, and forms peroxides [6]. **Petroleum ether** is the low boiling point fraction of petroleum and is composed mainly of pentane and hexane. It has a boiling point of 35–38 °C and is more hydrophobic than ethyl ether. It is selective for more hydrophobic lipids, cheaper, less hygroscopic, and less flammable than ethyl ether. The detailed properties of petroleum ether for fat extraction are described in AOAC Method 945.16 [8].

A combination of two or three solvents is frequently used. The solvents should be purified and peroxide-free, and the proper solvent-solute ratio must be used to obtain the best extraction of lipids from foods [7].

### 17.2.4 Continuous Solvent Extraction Method: Goldfish Method

#### 17.2.4.1 *Principle and Characteristics*

For continuous solvent extraction, solvent from a boiling flask continuously flows over the sample held in a

17.1 figure Goldfish fat extractor (Courtesy of Labconco Corp., Kansas City, MO; www.labconco.com/_scripts/EditItem.asp?ItemID=487)

ceramic thimble. Fat content is measured by weight loss of the sample or by weight of fat removed.

The continuous methods give faster, more efficient extraction than semicontinuous extraction methods. However, they may cause channeling which results in incomplete extraction. The Goldfish test is an example of a continuous lipid extraction method [6, 7]. This method involves significant fire risk and has been discontinued in many laboratories, but is briefly described here for comparison to semicontinuous and discontinuous extraction methods (Sects. 17.2.5 and 17.2.6).

#### 17.2.4.2 *General Procedure*

The sample is weighed into a thimble. Fat is extracted from the sample with boiling ethyl ether in the Goldfish apparatus (Fig. 17.1). Fat content is calculated as given in Eq. 17.1:

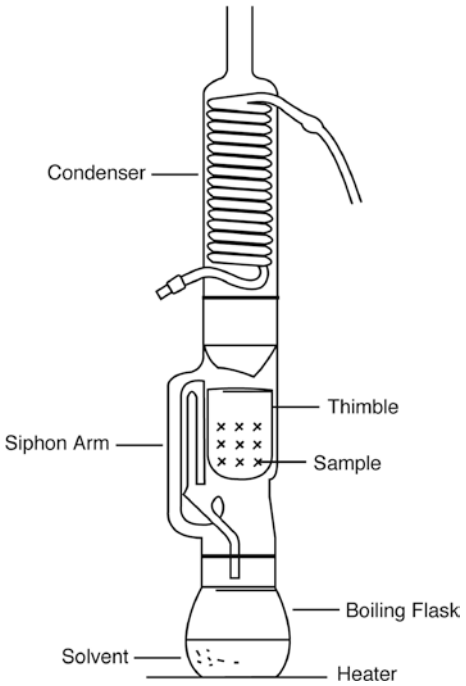
$$\%\ \text{Fat on dry weight basis} = (\text{g of fat in sample} / \text{g of dried sample}) \times 100 \quad (17.1)$$

### 17.2.5 Semicontinuous Solvent Extraction Method: Soxhlet Method

The Soxhlet method (AOAC Method 920.39C for Cereal Fat; AOAC Method 960.39 for Meat Fat) [8] is an example of the semicontinuous extraction method and is described below.

#### 17.2.5.1 *Principle and Characteristics*

For semicontinuous solvent extraction, the solvent builds up in the extraction chamber for 5–10 min and




17.2 figure Soxhlet extraction apparatus

completely surrounds the sample and then siphons back to the boiling flask. Fat content is measured by weight loss of the sample or by weight of fat removed.

This method provides a soaking effect of the sample and does not cause channeling. However, this method requires more time than the continuous method. Instrumentation for more rapid and automated versions of the Soxhlet method is available (e.g., Ankom XT15 Extractor, Ankom Technology, Macedon, NY; Soxtec™, FOSS in North America, Eden Prairie, MN).

#### 17.2.5.2 *General Procedure (See Fig. 17.2)*

For the Soxhlet or Goldfish methods, if the sample contains more than 10 % $H_2O$, dry the sample to constant weight at 95–100 °C under pressure ≤100 mmHg for about 5 h (AOAC Method 934.01). If analyzing a variety of food products, it may be more efficient to dry all samples, regardless of moisture content.

Samples are weighed into thimbles, placed in the Soxhlet apparatus (Fig. 17.2), and extracted with an appropriate solvent. As this procedure uses heat, it is more dangerous to use ethyl ether than other solvents. Many laboratories now use petroleum ether or hexane. Extraction time is 16 h in most cases. Certain products may lend themselves to shorter extraction time. The extract is then evaporated, and fat is determined gravimetrically. Fat content is calculated as in Eq. 17.2:

$$\%\ \text{Fat on dry weight basis} = (\text{g of fat in sample} / \text{g of dried sample}) \times 100 \quad (17.2)$$

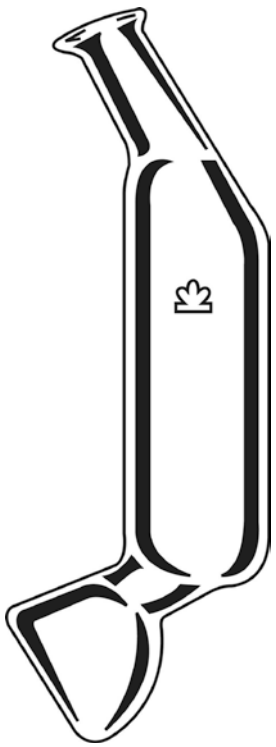
### 17.2.6 Discontinuous Solvent Extraction Methods

#### 17.2.6.1 *Alkaline Hydrolysis Method (Mojonnier Method)*

##### *17.2.6.1.1 Principle and Characteristics*

The terms Mojonnier fat, base hydrolysis, and alkaline hydrolysis are often used interchangeably to describe the following method. The term alkaline hydrolysis will be used in this chapter. After the use of ammonia to precipitate protein and free any bound fat, extraction is conducted with a mixture of ethyl ether and petroleum ether in a Mojonnier flask (Fig. 17.3), and the extracted fat is dried to a constant weight and expressed as percent fat by weight.

The alkaline hydrolysis test does not require removal of moisture from the sample, so it can be applied to both liquid and solid samples. The alkaline hydrolysis method was developed for and is applied primarily to dairy foods. If petroleum ether is used to purify the extracted fat, this method is very similar to the **Roese-Gottlieb method** (AOAC Method 905.02) in both principle and practice. The Mojonnier flasks (Fig. 17.3) are used not only for alkaline hydrolysis methods, but may also be used to conduct hydrolysis (acid, alkaline, or combination) prior to fat extraction and GC analysis for the determination of fat content via fatty acid profile analysis (Sect. 17.2.6), according to the US nutrition labeling regulations.



17.3 figure Mojonnier fat extraction flask (Courtesy of Kontes Glass Co., Vineland, NJ)

##### *17.2.6.1.2 General Procedure*

For AOAC Method 989.05 (Fat in Milk), the sample is prepared with appropriate warming and handling to ensure a homogenous sample, and then it is weighed into a Mojonnier flask. Ammonium hydroxide is added to precipitate the milk protein, and then ethanol is added to prevent gel formation. Following these treatments, fat is extracted first with ethyl ether to extract most lipids present, followed by petroleum ether to assist with removal of water from the ethyl ether and to assist with complete extraction of nonpolar lipids. Extractions are commonly repeated twice more to ensure complete extraction of lipids. The ether solution is decanted from the Mojonnier flask into the previously weighed Mojonnier fat dish. The solvent is evaporated, and fat content is determined gravimetrically, as in Eq. 17.3:

$$\%\ \text{Fat} = 100 \times \left\{ \left[ (\text{wt dish} + \text{fat}) - (\text{wt dish}) \right] - (\text{avg wt blank residue}) \right\} / \text{wt sample} \qquad (17.3)$$

A pair of reagent blanks must be prepared every day. For reagent blank determination, use 10 ml of distilled water instead of milk sample. The reagent blank should be <0.002 g.

#### 17.2.6.2 *Acid Hydrolysis Procedure*

##### *17.2.6.2.1 Principle and Characteristics*

A significant portion of the lipids in foods such as dairy, bread, flour, and animal products is bound to proteins and carbohydrates, and direct extraction with nonpolar solvents is inefficient. Such foods must be prepared for lipid extraction by acid hydrolysis. This includes a significant percentage of finished food products. Table 17.3 shows the inaccuracy that can occur if samples are not prepared by acid hydrolysis. Acid hydrolysis can break both covalently and ionically bound lipids into easily extractable lipid forms. Specifically, there are a variety of AOAC methods for

17.3 table Effects of acid digestion on fat extraction from foods

| | *Percent fat* | |
|---|---|---|
| | *Acid hydrolysis* | *No acid hydrolysis* |
| Dried egg | 42.39 | 36.74 |
| Yeast | 6.35 | 3.74 |
| Flour | 1.73 | 1.20 |
| Noodles | 3.77–4.84 | 2.1–3.91 |
| Semolina | 1.86–1.93 | 1.1–1.37 |

Adapted from Joslyn [6], p. 154, with permission

fat that involve an acid hydrolysis with HCl, followed by extraction with a combination of ethyl ether and petroleum ether [8]. Ethanol and solid hexametaphosphate may be added to facilitate separation of lipids from other components before food lipids are extracted with solvents [6, 7]. For example, the acid hydrolysis of two eggs requires 10 ml of HCl and heating in a water bath at 65 °C for 15–25 min or until the solution is clear [6].

#### *17.2.6.2.2 General Procedure*

For AOAC Method 922.06 (Fat in Flour), the sample is weighed into a Mojonnier flask. Hydrochloric acid digestion is carried out. Fat is extracted first with ethyl ether to extract most lipids present, followed by petroleum ether to assist with removal of water from the ethyl ether and to assist with complete extraction of nonpolar lipids. Extractions are commonly repeated twice more to ensure complete extraction of lipids. The ether solution is decanted from the Mojonnier flask into the previously weighed Mojonnier fat dish. The solvent is evaporated, and then fat is determined gravimetrically using Eq. 17.4:

$$\%\ \text{Fat} = 100 \times \left\{ \left[ \left( \text{wt dish} + \text{fat} \right) - \left( \text{wt dish} \right) \right] - \left( \text{avg wt blank residue} \right) \right\} / \text{wt sample} \tag{17.4}$$

### 17.2.6.3 *Chloroform-Methanol Procedure*

#### *17.2.6.3.1 Principle and Characteristics*

The combination of chloroform and methanol has been used commonly to extract lipids. The "Folch extraction" [9] applied to small samples, and the "Bligh and Dyer extraction" [10] applied to large samples of high moisture content, both utilize this combination of solvents to recover lipids from foods. These methods have been reviewed, and procedures were modified by Christie [11] and others. The Bligh and Dyer procedure [10] is a modification of the Folch extraction [9], designed for more efficient solvent usage for low-fat samples. The Christie modification [11] of these former methods replaced water with 0.88% potassium chloride aqueous solution to create two phases.

In both the modified Folch extraction and Bligh and Dyer procedure, food samples are mixed/homogenized in a chloroform-methanol solution, and the homogenized mixture is filtered into a collection tube. A 0.88% potassium chloride aqueous solution is added to the chloroform-methanol mixture containing the extracted fats. This causes the solution to break into two phases: the aqueous phase (top) and the chloroform phase containing the lipid (bottom). The phases are further separated in a separatory funnel or by centrifugation. After the evaporation of the chloroform, the fat can be quantitated by weight.

The various methanol-chloroform extraction procedures are rapid, well suited to low-fat samples, and can be used to generate lipid samples for subsequent fatty acid compositional analysis. The procedure has been more applied to basic commodities, rather than to finished product samples. For consistent results, the procedures must be followed carefully, including the ratio of chloroform and methanol. A cautionary note is that chloroform and methanol are highly toxic, so the extraction procedure must be done in well-ventilated areas.

#### *17.2.6.3.2 General Procedure*

For AOAC Method 983.23 (Fat in Foods), samples are extracted first with methanol, and then chloroform is added on top, and extraction is continued. Up to two additional such extractions may be performed. Potassium chloride is added to aid in separation of the layers. Solvent is evaporated, and fat is determined gravimetrically as in Eq. 17.5:

$$\%\ \text{Fat} = 100 \times \left\{ \left[ \left( \text{wt dish} + \text{fat} \right) - \left( \text{wt dish} \right) \right] - \left( \text{avg wt blank residue} \right) \right\} / \text{wt sample} \tag{17.5}$$

## 17.2.7 Total Fat by Gas Chromatography for Nutrition Labeling

### 17.2.7.1 *Principle*

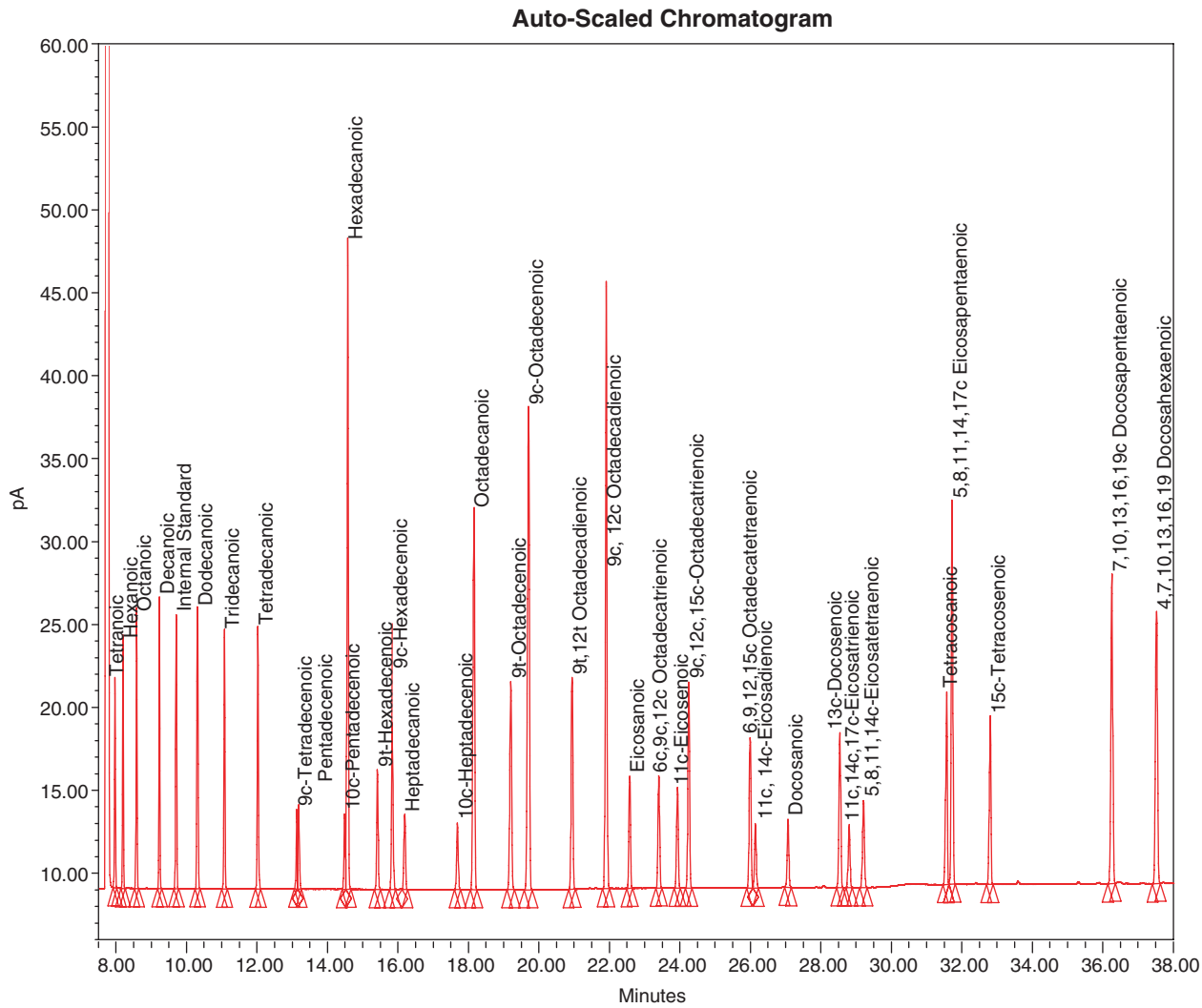
After adding an internal standard and an antioxidant, the sample is treated by acid and/or alkaline hydrolysis, and then fat is extracted with ether. Fatty acids are converted to fatty acid methyl esters (FAMEs), then separated by gas chromatography (GC), and quantitated against the internal standard, with the sum equal to total fat (AOAC Method 996.06).

The saturated and monounsaturated fats are calculated as the sum of the respective fatty acids. Monounsaturated fat includes only *cis* form. Trans fat can be quantified utilizing this method in conjunction with identification criteria established by the American Association of Oil Chemists (AOCS Method Ce 1h-05) [12] and Golay et al. [13].

### 17.2.7.2 *General Procedure*

The GC method for total fat (AOAC 996.06) is summarized as follows:

1. Add pyrogallic acid to sample.
2. Add internal standard (triundecanoin, $C_{11:0}$) to sample.
3. Subject sample to acid and/or alkaline hydrolysis.
4. Extract fat from sample with ethyl ether and/or petroleum ether.
5. Methylate fatty acids with boron trifluoride (in methanol) to form fatty acid methyl esters (FAMEs).




17.4 figure Fatty acid chromatogram

6. Separate FAMEs with GC capillary column and use detector to quantify peaks, compared to internal standard (Fig. 17.4).
7. Total fat is calculated as the sum of individual fatty acids expressed as triglyceride equivalents.

## 17.3 NONSOLVENT WET EXTRACTION METHODS

### 17.3.1 Babcock Method for Milk Fat

#### 17.3.1.1 *Principle*

In the Babcock method, $H_2SO_4$ is added to a known amount of milk in the Babcock bottle (Fig. 17.5). The sulfuric acid digests protein, generates heat, and releases the fat. Centrifugation and hot water addition isolate fat for quantification in the graduated portion of the test bottle. The fat is measured volumetrically, but the result is expressed as percent fat by weight.

#### 17.3.1.2 *Applications*

The Babcock method, which is a common official method for the determination of fat in milk (AOAC Method 989.04 and 989.10), takes about 45 min, and duplicate tests should agree within 0.1 %. The Babcock method does not determine the phospholipids in the milk products. It is not applicable to products containing chocolate or added sugar without modification because of charring of chocolate and sugars by sulfuric acid. A modified Babcock method is used to determine essential oil in flavor extracts (AOAC Method 932.11) and fat in seafood (AOAC Method 964.12).


17.5 figure

Babcock milk test bottles for milk (**a**), cream (**b**), and cheese (Paley bottle) (**c**) testing (Courtesy of Kimble Glass Co., Vineland, NJ)

### 17.3.2 Gerber Method for Milk Fat

#### 17.3.2.1 *Principle*

The principle of the Gerber method is similar to that of the Babcock method, but it uses sulfuric acid and amyl alcohol and a Gerber bottle (Fig. 17.6) (AOAC Method 2000.18). The sulfuric acid digests proteins and carbohydrates, releases fat, and maintains the fat in a liquid state by generating heat.

#### 17.3.2.2 *Applications*

The Gerber method is comparable to the Babcock method but is simpler and faster and has wider application to a variety of dairy products [14]. The isoamyl alcohol generally prevents the charring of sugar found with the regular Babcock method. This test is more popular in Europe than in America.

## 17.4 INSTRUMENTAL METHODS

Instrumental methods offer numerous attractive features compared to the previously described extraction methods. In general, they are rapid, some are nondestructive, and require minimal sample preparation and chemical consumption. These methods can provide significant labor savings when one must perform analysis of many samples on a daily basis.

17.6 figure

Gerber fat butyrometer

However, the equipment can be expensive, and some methods require the establishment of calibration curves specific to various compositions. Despite these drawbacks, several of the following instrumental methods are very widely used in quality control as well as research and product development applications. The following section describes several of these instrumental methods.

### 17.4.1 Infrared Method

The infrared (IR) method is based on absorption of IR energy by fat at a wavelength of 5.73 μm. The more the energy absorption at 5.73 μm, the higher the fat content of the sample [15]. (See Chap. 8 for a discussion of IR spectroscopy). Both near-infrared (NIR) or mid-infrared spectrophotometers are used in this method. For example, AOAC Method 2007.04 (Fat, Moisture, and Protein in Meat and Meat Products; specifies use of a FOSS Food Scan™ NIR spectrophotometer) is based on correlating NIR data with the values obtained from analysis by conventional methods, to then predict the concentration of fat, moisture, and protein in a sample being tested. Mid-IR spectroscopy is used in infrared milk analyzers to determine milk fat content (AOAC Method 972.16). NIR spectroscopy has been used to measure the fat content of commodities such as meats, cereals, and oilseeds in the laboratory and can be adapted for online measurement. NIR has been applied to near-line batch monitoring for food processing plants. NIR is an analytical technique that provides a prediction of chemical measurement. The NIR models are developed and validated to provide equivalent results to the wet chemical measurement. This method requires a comprehensive database of constituent values to create the model along with ongoing verification.

### 17.4.2 X-Ray Absorption Method

X-ray absorption is a rapid analysis method that has received interest in application to lipid analysis in meats [16]. This method can be adapted to in-line analysis of meats. Determination of the amount of fat in meat and meat products is based on the fact that the X-ray absorption of lean meat is higher than that of fat. This method has been used for the rapid determination of fat in meat and meat products using the standard curve of the relationship between X-ray absorption and fat content determined by a standard solvent extraction method [7]. For example, the MeatMaster™ II fat analysis instrument (Foss, Eden Prairie, MN), commonly used to rapidly determine the percent fat of meat products, is based on X-ray absorption.

### 17.4.3 Nuclear Magnetic Resonance

**Nuclear magnetic resonance** (NMR) can be used to measure lipids in food materials in a nondestructive way. It is one of the most popular methods for use in determining lipid melting curves to measure solid fat content (see Chap. 23), and more affordable instruments are becoming more popular for measuring total fat content. Total fat content can be measured using low-resolution pulsed NMR (AOAC Method 2008.06). (The principles and applications of NMR are described in Chap. 10). NMR analysis is a very rapid and accurate method, and while the principals of NMR are relatively complex, the use of NMR can be quite simple, especially due to the high degree of automation and computer control. Systems such as CEM Smart Trac II rapidly measure total fat and moisture.

### 17.4.4 Accelerated Solvent Extraction

**Accelerated solvent extraction** (ASE) was developed to replace Soxhlet and other extraction techniques for many samples. The automation and rapid extraction time of accelerated solvent extraction are its key advantages. Accelerated solvent extraction (Fig. 17.7) uses solvents at increased temperatures and pressures to accelerate the extraction process. Analytes are more soluble and thus dissolve more quickly in hot solvents. Pressurized solvent is heated well above its boiling point and introduced under high pressure to extract the fat from the sample. By using hot, pressurized liquid solvents, accelerated solvent extraction decreases the amount of solvent and the time needed to complete an extraction. Most extractions can be completed in less than 20 min, using less than 20 mL of solvent.

### 17.4.5 Supercritical Fluid Extraction

Fat extraction can be carried out using **supercritical fluid extraction** (SFE) instruments that use supercritical carbon dioxide as the solvent. Samples are weighed into extraction cells, the extraction chamber is heated and pressurized to a set value, and the supercritical fluid is then pumped through the extraction cell facilitating extraction of the target analytes from the sample matrix (Fig. 17.8). This technique is finding greater use because of the cost and environmental problems associated with the use and disposal of organic solvents. When pressurized $CO_2$ is heated above a certain critical temperature, it becomes a supercritical fluid which has some of the properties of a gas and some of a liquid. The fact that it behaves like a gas allows it to easily penetrate into a sample and extract the lipids, while the fact that it behaves like a fluid helps it dissolve lipids (especially at higher pressures). The pressure and temperature of the solvent are later reduced, which causes the $CO_2$ to turn to a gas, leaving the lipid fraction remaining. The lipid content of a food is determined by weighing the percentage of lipid extracted from the original sample [17].

17.7 figure Accelerated solvent extractor (Courtesy of Thermo Fisher Scientific, Waltham, MA)

## 17.5 COMPARISON OF METHODS

Various fat analysis methods are summarized and compared in Tables 17.4 and 17.5. Dried (or very low moisture content) samples are required for fat determination by the Soxhlet extraction method (traditional version or automated), which is applicable to many food commodities. If the samples are moist or liquid foods, the Mojonnier method is generally applicable to determination of the fat content. Acid hydro-

17.8 figure Supercritical fluid extractor (Courtesy of Parr Instrument Co., Moline, IL)

lysis or alkaline hydrolysis is widely used on many finished food products. The instrumental methods such as IR and NMR are very simple, reproducible, and fast and are gaining in popularity. The application of instrumental methods for fat determination generally requires a standard curve between the signal of the instrument analysis and the fat content obtained by a standard solvent extraction method. However, a rapid instrumental method can be used as a quality control method for fat determination of a specific food.

Major uses of the Soxhlet and Mojonnier methods include the following: (1) extract fat prior to GC analysis, (2) quality control of formulated products, (3) determine fat content during product development, (4) verify when fat content is <0.5 g/serving (so nutrient content claim can be made), and (5) defat samples prior to fiber analysis. Compared to GC analysis of fat content by AOAC Method 996.06, these three methods are faster and cheaper, but may give a higher fat content (which must be recognized when using these methods for product development).

## 17.6 SUMMARY

Lipids have often been defined by their solubility characteristics rather than by some common structural feature. Lipids in foods can be classified as simple, compound, or derived lipids. The lipid content of foods varies widely, but quantitation is important because of regulatory requirements, nutritive value, and functional properties. To analyze food for the fat content accurately and precisely, it is essential to have a comprehensive knowledge of the general compositions of the lipids in the foods, the physical and chemical properties of the lipids as well as the foods, and the principles of fat determination. There is no single standard method for the determination of fats in different foods. The validity of any fat analysis depends on proper sampling and preservation of the sample prior to analysis. Predrying of the sample, particle size reduction, and acid hydrolysis prior to analysis also may be necessary. The total lipid content of foods is commonly determined by organic solvent extraction methods, such as semicontinuous (e.g., Soxhlet) and discontinuous (e.g., Mojonnier), or by GC analysis for nutrition labeling. Nonsolvent wet extraction methods, such as the Babcock or Gerber, are commonly used for certain types of food products. Instrumental methods, such as NMR, infrared, ASE, SFE, and x-ray absorption, are also available for fat determination. These methods are rapid and may be useful for quality control but may require additional refinement in order to develop consistent correlation to a standard solvent extraction method.

## 17.7 STUDY QUESTIONS

1. What are some important considerations when selecting solvents to be used in continuous and noncontinuous solvent extraction methods?
2. To extract the fat from a food sample, you have the choice of using ethyl ether or petroleum ether as the solvent, and you can use either a Soxhlet or a Goldfish apparatus. What combination of solvent and extraction would you choose? Give all the reasons for your choice.
3. Itemize the procedures that may be required to prepare a food sample for accurate fat determination by a solvent extraction method (e.g., Soxhlet method). Explain why each of these procedures may be necessary.
4. You performed fat analysis on a new super energy shake (high carbohydrate and protein) using standard Soxhlet extraction. The value obtained for fat content was much lower than that expected. What could have caused the measured fat content to be low, and how would you modify the standard procedure to correct the problem?

17.4 table

Summary of fat analysis methods

| *Method* | *Principle* | *Advantages* | *Disadvantages* | *Applications* |
|---|---|---|---|---|
| Soxhlet | Fat is extracted, semicontinuously, with organic solvent. Fat content is measured by weight loss of sample or weight of fat removed | Provides for soaking effect. Does not cause channeling. Sample remains cool | Time to result is long. Errors low on many products with bound fat. Manual test | Grains, raw meat |
| Soxhlet (automated) | These methods take the traditional Soxhlet methodology described above and automate the process, removing some of the manual labor necessary to obtain results | Requires less labor. Faster time to result | Higher equipment cost | Same as Soxhlet |
| Mojonnier | Fat is extracted (discontinuous) with mixture of organic solvents. Extracted fat is dried to constant weight | Faster than Soxhlet | Errors low on many products with bound fat. Manual test | Same as above and on some nonprotein beverages |
| Mojonnier-base hydrolysis | Protein is precipitated with ammonia. Fat is extracted with mixture of organic solvents. Extracted fat is dried to constant weight | Best traditional method for dairy products | Manual test. Narrow range of scope | Intended for use on milk and milk-derived products |
| Mojonnier-acid hydrolysis | Organic matter (nonfat) is digested with acid. Fat is extracted with mixture of organic solvents. Extracted fat is dried to constant weight | Wide range of applicability for many food item | Manual test. More labor required than some methods | Applicable to most foods. Not applicable to raw soybeans |
| Chloroform-methanol | Fat is extracted with a combination of chloroform and methanol. Addition of potassium chloride causes the solution to break into two phases, with the fat in the chloroform phase. Evaporation of the chloroform allows for fat to be quantitated by weight | Wide range of applicability for many food item | Chloroform and methanol are both highly toxic; measures all fat-soluble materials. Manual test, significant labor expenditure. Need to deal with emulsions formed during extraction | Most foods |
| Gas chromatography (GC) | After adding an internal standard and a reagent to prevent oxidation, sample is treated by acid and/or alkaline hydrolysis, then fat is extracted with ether. Fatty acids are converted to fatty acid methyl esters (FAMEs). FAMEs are separated by GC and quantitated. Sum of fatty acids equals fat content | Measures only true fat, as defined by FDA. (Not other fat-soluble compounds) | Time consuming. Expensive. Many compounds to separate | Official method for nutrition labeling |
| Babcock | Sulfuric acid added digests protein, generates heat, and releases fat. After centrifugation of sample, fat content is measured volumetrically | Takes only ~45 min | Manual test. Get charring with high sugar products, so difficult to read volume of fat | Commonly applied to milk and other dairy products |

(continued)

17.4 table (continued)

| *Method* | *Principle* | *Advantages* | *Disadvantages* | *Applications* |
|---|---|---|---|---|
| Gerber | Similar to Babcock method but uses sulfuric acid and amyl alcohol | Compared to Babcock, it is simpler and faster, and the isoamyl alcohol prevents charring of sugar | Manual test | Commonly applied to milk and other dairy products, especially high sugar products |
| Infrared spectroscopy | NIR provides a prediction of fat measurement. Based on absorption of IR energy by fat at 5.73 μm. NIR models are developed and validated to provide equivalent results to classical methods | Rapid | Provides only an estimate of fat content; instrument must be calibrated against official method | Mid-IR spectroscopy is commonly used in infrared milk analyzers. NIR commonly used to measure fat content of meats, cereals, and oilseeds |
| Specific gravity | Fat is extracted from sample with the solvent perchloroethylene. Specific gravity of sample solvent extract is related to fat content | Rapid | Values are estimates, based on correlation chart created using an official method | Meat |
| NMR | Certain nuclei will absorb and re-emit RF energy over a narrow band of frequencies when placed in a static magnetic field. The frequency at which the NMR effect occurs for a given nuclear isotope is dependent on the magnetic field strength of the magnet, and the phenomenon is caused by the interaction between the nuclear magnetic dipole of a nucleus and the magnetic field it experiences. Fat is determined by pulsed radio frequency (RF) energy while within a static 0.47 T magnetic field. The resulting NMR signal is recorded and analyzed for the total proton activity of fat present in the sample [8] | Rapid. Very low labor cost. Tremendous potential in high-throughput fat testing laboratories | Expensive instrument. Range of applicability has not yet been totally demonstrated | Potential application to many foods |
| Accelerated solvent extraction (ASE) | Pressurized solvent is heated to well above its boiling point and introduced under high pressure to extract the fat from the sample | Very rapid | Instrument cost. May require some work to match classical results | Use in place of Soxhlet. Use for other fat-related tests |
| Supercritical fluid extraction (SFE) | The sample is heated and pressurized to a set value, and the supercritical fluid is then pumped through the extraction cell facilitating extraction of the fat from the sample matrix | Very rapid | Instrument cost. May require some work to match classical results | Use in place of Soxhlet. Use for other fat-related tests |

17.5 table Comparison of classical methods for fat analysis

| *Methods* | *Similarities* | *Differences* | |
|---|---|---|---|
| Soxhlet vs. Goldfish | Use for same applications (see list in Table 17.4)<br>Use extraction with organic solvents<br>Measure fat by wt. loss or wt. of fat removed (gravimetric)<br>Both take hours to do; sample must be dry or low in moisture<br>Use same type of cellulose "thimble" to hold sample for extraction | Soxhlet<br>Semicontinuous<br>Soaking effect<br>Sample stays cool<br>No channeling<br>May be slower (takes 4–16 h, depending on drip rate) | Goldfish<br>Continuous method<br>No soaking effect<br>Sample gets heated<br>Channeling likely<br>Extract for 4 h (so may be faster) |
| Babcock vs. Mojonnier | Can use on liquid or solid sample<br>Both developed originally for dairy | Babcock<br>Use no solvents (uses sulfuric acid)<br>Dairy products only<br>Measure fat volume | Mojonnier<br>Use multiple solvents<br>Any food product<br>Measure fat weight |
| Mojonnier vs. Soxhlet | Use for same applications<br>Gravimetric methods | *Mojonnier*<br>Use multiple solvents<br>Solid or liquid sample<br>Discontinuous method | *Soxhlet*<br>Use single solvent<br>Solid sample only (dry or low in moisture)<br>Semicontinuous method |
| Babcock vs. Gerber | Same applications (dairy)<br>Volumetric measurement | *Babcock*<br>Use only sulfuric acid<br>Use sulfuric acid to release fat<br>Would get charring with high sugar dairy product<br>Slower; more complex | *Gerber*<br>Use sulfuric acid plus isoamyl alcohol<br>Can use on high sugar dairy product<br>simpler and faster |
| Mojonnier vs. GC method | GC method commonly uses Mojonnier for initial fat extraction | Mojonnier<br>Common component of GC method<br>Measure by wt. of fat<br>Used for applications in summary table | GC<br>Requires acid and/or alkaline hydrolysis, and extraction is commonly done with Mojonnier-type flasks but has post steps to measure fat as sum of FAs<br>Used for nutrition labeling |

5. What is the purpose of the following chemicals used in the Mojonnier method?

   (a) Ammonium hydroxide
   (b) Ethanol
   (c) Ethyl ether
   (d) Petroleum ether

6. What is a key application of the GC method and what does it specifically quantify?
7. What is the purpose of the following procedures used in Babcock method?

   (a) Sulfuric acid addition
   (b) Centrifugation and addition of hot water

8. Which of the following methods are volumetric and which are gravimetric determinations of lipid content: Babcock, Soxhlet, Mojonnier, Gerber?

## 17.8 PRACTICE PROBLEMS

1. To determine the fat content of a semimoist food by the Soxhlet method, the food was first vacuum oven dried. The moisture content of the product was 25%. The fat in the dried food was determined by the Soxhlet method. The fat content of the dried food was 13.5%. Calculate the fat content of the original semimoist product.
2. The fat content of 10 g of commercial ice cream was determined by the Mojonnier method. The weights of extracted fat after the second extraction and the third extraction were 1.21 g and 1.24 g, respectively. How much of fat, as a percentage of the total, was extracted during the third extraction?

**Answers**

1. If the sample weight of a semimoist food is 10 g and the moisture content is 25%, the dried weight of the original food is 7.5 g (10 g × 75% = 7.5 g). If the fat content of the dried food is 13.5%, the 7.5 g of dried sample has 1.0125 g fat (7.5 g dried food ×13.5% fat = 1.0125 g fat). The 10 g of semimoist food contains the same amount of fat, i.e., 1.0125 g. Therefore, the fat content of the semimoist food is 10.125% (1.0125 g fat/10 g semimoist food).
2. [(1.24–1.21 g)/10 g] × 100 = 0.3%

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