Extraction and Measurement of Total Lipids

Lipids are soluble in organic solvents, but sparingly soluble or insoluble in water. The existing procedures for the extraction of lipids from source material usually involve selective solvent extraction and the starting material may be subjected to drying prior to extraction. Solubility of lipids is an important criterion for their extraction from source material and depends heavily on the type of lipid present, and the proportion of nonpolar (principally triacylglycerols) and polar lipids (mainly phospholipids and glycolipids) in the sample; therefore, several solvent systems might be considered, depending on the type of sample and its components. Incomplete removal of lipids via cold pressing can be employed, such as is in practice for the extraction of oil from flaxseed or screw-pressing of seeds like canola, prior to solvent extraction. The solvents of choice are usually hexane, in the case of Soxhlet (AOAC, 1995; see Basic Protocol 1) and Goldfisch methods (see Alternate Protocol 1), chloroform/methanol or chloroform/methanol/water, in the case of the Folch Method (Folch et al., 1957; see Basic Protocol 2) or its modified Bligh and Dyer Procedure (Bligh and Dyer, 1959; see Alternate Protocol 2), and *n*-propanol/water for high-amylose containing starchy foods (see Basic Protocol 3).

In addition, to release lipids from source material, such as those in starch, fish meal, or milk, it might be necessary to treat the sample with an acid prior to lipid extraction (see Basic Protocol 4). In the case of milk, addition of ammonium hydroxide is necessary to dissolve casein prior to lipid extraction, which will release the lipids from its surrounding matrix (e.g., from the film surrounding the fat globules in milk). Furthermore, in certain cases, it is necessary to predry the sample in order to allow efficient and complete extraction of lipids. Particle size reduction is another factor that may improve lipid extraction efficacy.

Accurate determination of lipids in foods is required for nutritional labeling, certification, or for evaluation of standard of identity and uniformity, as well as examination of their effects on functional and nutritional properties of foods. Following lipid extraction and precise quantitative analysis, lipids so obtained may be used for analysis of other lipid characteristics and properties provided that nondestructive and mild extraction procedures are employed that retain the integrity of lipids. Thus, determination of lipid classes, fatty acid composition (*UNIT D1.3*), and oxidative state of lipids (Chapter D2), amongst others, may be pursued following the extraction process.

CAUTION: The solvents used in these protocols are flammable, treat accordingly. See *APPENDIX 2B* for more information.

IMPORTANT NOTE: Use only ACS grade reagents and deionized water.

NOTE: A balance accurate to 0.1 mg is recommended for all measurements.

SOLVENT EXTRACTION OF OILSEEDS, NUTMEG, AND OTHER FOODS USING THE SOXHLET METHOD

BASIC PROTOCOL 1

Extraction of lipids into hexane, low-boiling petroleum ether (a mixture of pentanes and hexanes with a boiling point of 35° to 40°C), as well as diethyl ether, is easily achievable, provided that the moisture content of the food sample does not exceed 10%. This method may be used for quantitation of lipids in both low-fat and high-fat source materials but it removes mainly nonpolar lipids from samples as polar lipids are generally scarcely soluble in nonpolar solvents. In the case of high moisture foods, predrying of the sample may be necessary. Since high temperature drying procedures may adversely affect the

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oxidative state of lipids, predrying may be achieved using low temperature drying of the sample under vacuum (<100 mm Hg) at 40° to 50°C overnight, or 95° to 100°C for 5 hr (AOAC, 1995). In some cases, such as determination of fat content in meat samples by Goldfisch method (see Alternate Protocol 1), mixing of the sample with sand is necessary to avoid crust formation, which may lead to incomplete lipid extraction. This facilitates penetration of the solvent into the food matrix, increases surface area, and may hasten size reduction, if necessary. The Soxhlet extraction is the method commonly used; however, an alternate Goldfisch extraction procedure may also be employed for this purpose (see Alternate Protocol 1).

The Soxhlet extraction procedure is a semicontinuous process, which allows the buildup of the solvent in the extraction chamber for 5 to 20 min. The solvent surrounds the sample and is then siphoned back into the boiling flask (Fig. D1.1.1). Multiextractor units are available for extraction of lipids from several different samples or replicate runs of the same material. The procedure provides a soaking effect and does not permit channeling. The fact that polar and bound lipids are not removed is a drawback to the procedure (see Background Information).

Materials

60 g canola, mustard, soybean, or nutmeg seeds
Hexane

Coffee grinder
Soxhlet apparatus:
  Cellulose extraction thimbles and glass wool
  Condenser
  500-ml flat-bottom flask, predried with boiling chips or glass beads
  Heating mantle
  Soxhlet extractor
Rotary evaporator

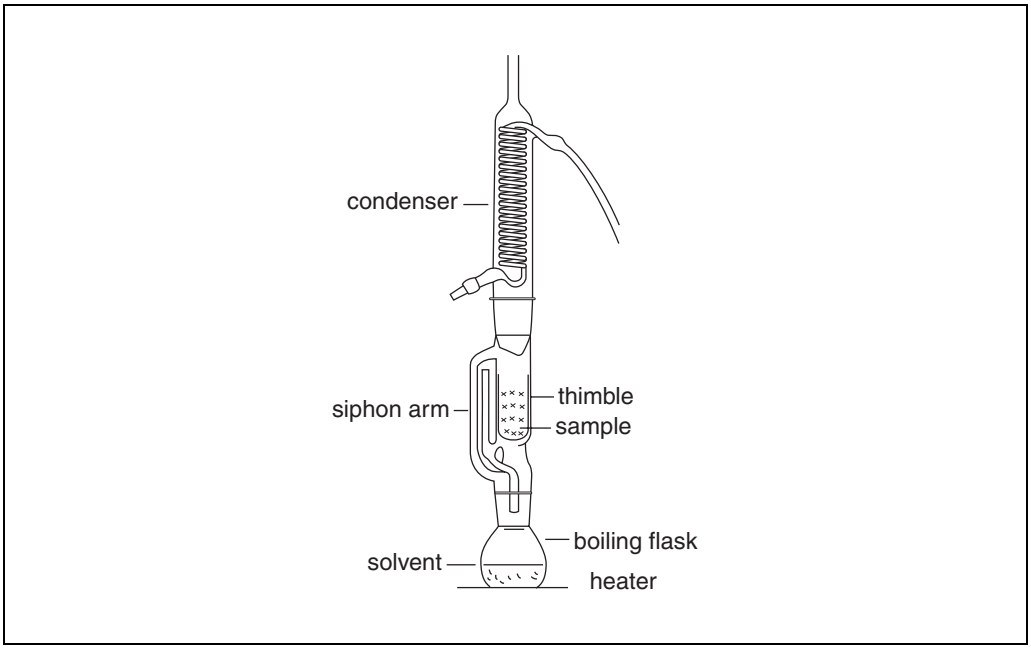

Figure D1.1.1 Soxhlet lipid extraction unit used for continuous extraction of analytes from a solid into an organic solvent. As the flask containing the solvent is heated, vapors rise in the larger outside tube, through the thimble containing the sample, enter the water-cooled condenser, and liquefy. When the liquid level in the extractor reaches the top of the siphon arm, the extract-enriched solvent returns to the flask.

1. Grind 60 g seeds or nutmeg in a coffee grinder with occasional shaking for 1 to 2 min.
2. Weigh exactly 20 æ 1 g of sample into cellulose extraction thimbles. Cover the top of each thimble with glass wool to prevent floating.
3. Weigh the predried flat-bottom extraction flask with a few boiling chips or glass beads.
4. Extract lipids with 150 to 200 ml of hexane at the boiling point for 7 to 12 h in a Soxhlet extractor (Fig. D1.1.1) using a heating mantle.

   The condensation rate for the solvent should be set at about 2 to 6 drops per second, depending on the extraction period envisaged. For longer extraction periods, a lower condensation rate is selected and vice versa. Usually an extraction period of 8 hr at a rate of 150 drops per min is considered adequate.

   The boiling point of hexane is ~69°C.
5. Let the sample cool.
6. Remove the solvent from the extract in a rotary evaporator at 40°C under reduced pressure.
7. Calculate the amount of lipid recovered and its percentage in the original sample as given below:

   Mass of lipid = (weight of the flask + boiling chips + extracted oil) − (weight of the flask + boiling chips)

   Lipid content (%) = mass of lipid extracted (g)/sample weight (g) × 100

GOLDFISCH METHOD FOR LIPID EXTRACTION

ALTERNATE PROTOCOL 1

The Goldfisch extraction procedure allows the solvent (usually hexane, petroleum ether, or diethyl ether) to continuously flow over the sample held in a ceramic thimble (Fig. D1.1.2). This procedure is again useful for determination of nonpolar lipids and is faster and more efficient than the Soxhlet extraction method (see Basic Protocol 1); however, it may cause channeling, which could render extraction of lipids incomplete. Although diethyl ether is a more efficient solvent, in general, danger of explosion, fire hazard, and a higher price are among the reasons for selection of low boiling petroleum ether (35° to 60°C) for lipid extraction.

Additional Materials *(also see Basic Protocol 1)*

10 g sample, predried
Petroleum ether

Goldfisch lipid extraction unit (e.g., Labconco Corp.):
- Extraction container, predried
- Glass holding tube
- Forced-air oven

Dessicator

CAUTION: The Goldfisch lipid extraction unit should be operated inside an explosion-proof fume hood to exhaust the vapors from flammable solvents used during normal operation. The fume hood should be equipped with a fire suppression device.

1. Weigh predried porous ceramic extraction thimbles and the predried extraction container. Add 10 g predried sample to the thimble and weigh again.
2. Place the ceramic extraction thimble into a glass holding tube.

3. Place ~40 ml of hexane or petroleum ether in the extraction container.
4. Set the equipment to extract for 4 to 7 hr.
5. Let the sample cool.
6. Remove the extraction container and then evaporate the solvent in the air overnight with subsequent heating at 95° to 100°C for 30 min using a forced-air oven.
7. Cool the beaker in a desiccator. Weigh the beaker and its contents. Calculate the weight of the lipid:

   Weight of lipid = (weight of container + extracted lipid) − (weight of container)

   Alternatively, the sample may be transferred before solvent removal to a tared round bottom-flask. Solvent may then be removed at 40°C under vacuum in a rotary evaporator.
8. Calculate the percentage of lipid in the sample as given below.

   Lipid content (%) = mass of lipid extracted (g)/sample weight (g) × 100

   Extracted lipids in this procedure may be used for certain further analysis, such as determination of fatty acid profile (UNIT D1.2) and oxidative state (UNITS D2.1 & D2.2) of the oil; however, for lipid classification, incomplete extraction of polar lipids into nonpolar solvents may not allow for accurate determination and quantitation.

BASIC PROTOCOL 2

CHLOROFORM/METHANOL/WATER EXTRACTION OF LIPIDS FROM MUSCLE FOODS

Mixtures of chloroform and methanol have had wide use as lipid extractants (e.g., Bligh and Dyer, 1959). This solvent system allows for extraction of both polar and nonpolar lipids, unlike extraction with hexane (see Basic Protocol 1 and Alternate Protocol 1). Optimum extraction may be achieved when water in the tissue, or that added to the medium, yields a monophasic solution. Subsequently, additional water and/or chloroform

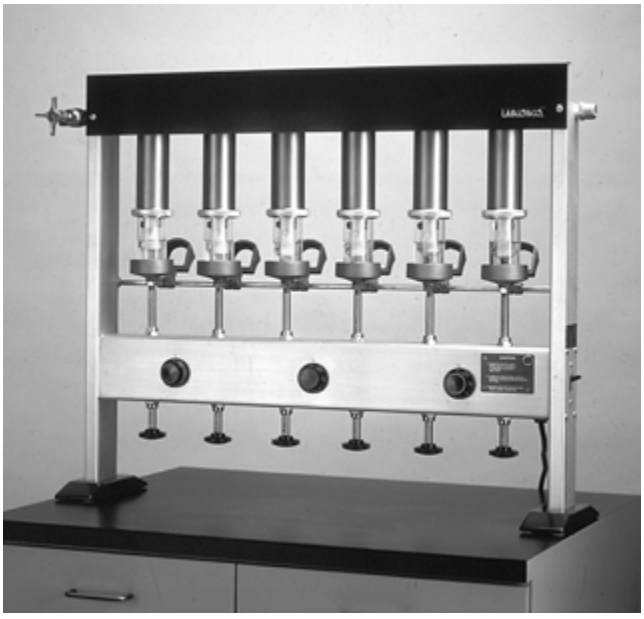

Figure D1.1.2 Goldfisch lipid extraction unit (Courtesy of Labconco Corporation, Kansas City, MO). A single beaker is used as the solvent chamber. Samples are placed between a boiling solvent and a cold surface. The solvent vaporizes, condenses on the cold surface, and washes down through the samples into the boiling solvent below.

may be introduced to produce a biphasic system. The lower chloroform layer includes the lipids and the top methanol-water layer generally contains the nonlipid components. The lipid in the chloroform layer is isolated using a separatory funnel.

CAUTION: Due to the hazardous nature of chloroform, procedures using chloroform must be conducted under a fume hood. See *APPENDIX 2B* for more information.

Materials

50 g small cut pieces of fish or meat
Methanol
Chloroform
1:1 (v/v) chloroform/methanol
Sodium sulfate, anhydrous

Waring blender or Polytron homogenizer
Tabletop centrifuge, rotor, and 50- to 500-ml tubes, or sintered glass funnel
Whatman No. 1 filter paper in a Buchner funnel
Separatory funnel
Rotary evaporator

1. Homogenize 50 g small cut pieces of fish or meat in a Waring blender, or with the aid of a Polytron homogenizer, in 100 ml of methanol. Add 50 ml chloroform. Homogenize for 2 min.

   If using a dry sample such as fish meal, use 10 to 15 g sample and add 35 to 40 ml water before homogenization.

2. Add 50 ml additional chloroform and homogenize 30 sec. Add 50 ml water and homogenize 30 sec.

3a. *For collecting liquid by centrifugation:* Transfer to 50 to 500 ml centrifuge tubes. Collect liquid in a tabletop centrifuge at 3300 × *g*, at 5° to 25°C. Decant and retain liquid.

3b. For collecting liquid by filtration: Filter through a sintered glass funnel or through Whatman No. 1 filter paper in a Buchner funnel with slight suction. Press the solids with the bottom of a beaker to ensure maximum solvent recovery. Retain liquids.

4. Repeat the procedure (step 3a or b) on the resulting solids, together with filter paper if applicable, by adding 20 ml 1:1 (v/v) chloroform/methanol. Retain liquids.

5. Combine the liquids and transfer into a separatory funnel.

   The bottom chloroform layer includes the lipids and should be retained for further work up.

6. Pass the chloroform layer through a 2.5-cm thick layer of anhydrous sodium sulfate using Whatman No. 1 filter paper in a funnel. Wash with 20 ml 1:1 (v/v) chloroform/methanol.

7. Remove the solvent using a rotary evaporator under vacuum, at 40°C. Calculate the weight of the lipid:

   Weight of lipid = (weight of container + extracted lipid) − (weight of container)

8. Determine the content of lipids in the sample by weight difference:

   Lipid content (%) = amount of lipid extracted (g)/weight of original sample (g) × 100

ALTERNATE PROTOCOL 2

CHLOROFORM/METHANOL/WATER EXTRACTION FROM SMALL SAMPLES

Extraction of lipid from small amounts (<1 g) of sample, such as that of rotifers, artemia, and larvae, may be achieved using an alternate procedure. In this approach, homogenization of sample is achieved using a vortex due to the delicate nature of tissues involved and the ease of their disintegration. The recovered lipids, similar to that in the original protocol (see Basic Protocol 2), may be used for further analysis.

Additional Materials *(also see Basic Protocol 2)*

300 to 400 mg sample to be analyzed (e.g., rotifers, artemia, larvae)
Methanol containing (optional) 500 ppm *tert*-butylhydroquinone (TBHQ)

15-ml test tube
Ultrasonic bath
Funnel and Whatman no. 1 filter paper

NOTE: In general, antioxidants (e.g., TBHQ), are required when extracting oils that contain highly unsaturated fatty acids with 3, 4, 5, or 6 double bonds.

1. Accurately weigh 300 to 400 mg of sample into a 15-ml test tube.
2. Add 4 ml methanol, containing 500 ppm *tert*-butylhydroquinone (TBHQ) as an antioxidant, if needed.
3. Add 2 ml chloroform and 0.4 ml water; homogenize (for larvae) and/or vortex for 30 sec. Sonicate in an ultrasonic bath for 15 min.
4. Add 2 ml chloroform and 2 ml water. Vortex for 30 sec.
5. Centrifuge the tubes at 3300 × *g* for 15 min, at 5° to 25°C.
6. Remove the upper layer (methanol/water) using a Pasteur pipette.

   There may be solids at the interface which should be allowed to remain in the tube.
7. Transfer the lower layer (methanol/chloroform) into a clear tube with a Pasteur pipette. Leave the solids behind, if present.
8. Reextract the solids left at the bottom of the test tube by repeating steps 4 to 7 using 2 ml of 1:1 (v/v) chloroform/methanol in step 4.
9. Pass the combined layers through a 2.5-cm thick layer of anhydrous sodium sulfate using Whatman no. 1 filter paper in a funnel, into a preweighed container suitable for a rotary evaporator.
10. Remove the solvents using a rotary evaporator under reduced pressure, at 40°C. Calculate the weight of the lipid:

    Weight of lipid = (weight of container + extracted lipid) − (weight of container)
11. Calculate the lipid content by weight difference, as given below:

    Lipid content (%) = amount of lipid extracts (gram)/weight of original sample (gram) × 100

n-PROPANOL/WATER EXTRACTION

BASIC PROTOCOL 3

Lipids in starchy foods may occur in the free as well as bound forms. The latter being either in the form of amylose inclusion complexes or linked via ionic or hydrogen bonding to the hydroxyl groups of the starch components. Free lipids are easily extractable at ambient temperatures, while use of nonalcoholic solvents for a prolonged period or disruption of the granular structure by acid hydrolysis (see Basic Protocol 4) may be required for the efficient extraction of bound lipids. While acid hydrolysis allows the release and quantitation of lipids, the procedure leads to destruction of the starch components; therefore, the alcohol extraction system involving propanol and water would be most desirable in these cases. This system removes both nonpolar and polar lipids from samples.

Materials

Cereal sample (e.g., wheat, barley) or potato starch
3:1 (v/v) *n*-propanol/water: mix well prior to use
Deionized H_2O

Coffee grinder (optional)
Soxhlet apparatus (see Basic Protocol 1)
Rotary evaporator

1. Grind a sample of cereal, such as wheat or barley, in a coffee grinder, or use potato starch.
2. Accurately weigh 5 to 10 g of sample into a cellulose extraction thimble. Cover the top with glass wool to prevent floating.
3. Weigh the thimble and its contents. Do the same with the flat-bottom flask containing a few boiling chips.
4. Add 150 to 200 ml 3:1 (v/v) *n*-propanol/water to the Soxhlet apparatus.
5. Heat the *n*-propanol/water to the boiling point (~85°C). Allow the continuous extraction to proceed for 8 to 12 h.
6. Remove the solvent using a rotary evaporator under vacuum. Calculate the weight of the lipid:

   Weight of lipid = (weight of container + extracted lipid) − (weight of container)

7. Calculate the lipid content by weight difference as given below:

   Lipid content (%) = mass of lipid extracted (g)/weight of the original sample (g) × 100

EXTRACTION OF LIPIDS REQUIRING ACID DIGESTION

BASIC PROTOCOL 4

To quantitate total lipids, defined as the sum of the free and bound lipids, both polar and nonpolar, acid hydrolysis may be necessary to release the bound lipids by dissociating lipid-starch and lipid-protein intermolecular forces. The resultant lipids may then be removed and measured; however, the nonlipid components so obtained are not usable for further analysis. Removal of some of the polar lipids may hinder the use of the extracted material for further analysis.

Materials

Starch or fish meal
6 N HCl
Sodium sulfate, anhydrous
n-hexane or 1:1 (v/v) chloroform/methanol

Coffee grinder
250-ml round-bottom flask
Whatman no. 1 filter paper and funnel
Rotary evaporator

1. Grind a sample of starch or fish meal into a powder in a coffee grinder.
2. Weigh 5 g of the ground sample into a 250-ml round-bottom flask.
3. Hydrolyze the sample of starch with 50 ml 6 N HCl at 70° to 80°C for 1 hr or the sample of fish meal at 110°C for 4 to 24 hr, until complete dissolution.

   The temperature can be controlled using a water or oil bath.

4. Extract the total lipids 3 times with 50 ml each of *n*-hexane (starch) or 1:1 (v/v) chloroform/methanol (fish), retaining the organic layers each time.
5. Combine the organic layers and pass through a 2.5-cm thick layer of anhydrous sodium sulfate using Whatman no. 1 filter paper in a funnel, into a preweighed container suitable for a rotary evaporator.
6. Remove the organic solvent at 40°C under reduced pressure using a rotary evaporator. Calculate the weight of the lipid:

   Weight of lipid = (weight of container + extracted lipid) − (weight of container)

7. Calculate the content of lipids by weight difference, as given below:

   Lipid content (%) = mass of lipid extracted (g)/weight of original sample (g) × 100

COMMENTARY

Background Information

Lipids are one of the major components of food and are present even in so-called "fat-free" foods at low levels often in the bound form. Lipids are defined as materials that are sparingly soluble or insoluble in water, but soluble in organic solvents such as hexane, diethyl ether, benzene, chloroform, methanol, or their selected mixtures (Nawar, 1996; deMan, 1999). There are several classes of lipids, all having similar and specific characteristics due to the presence of a major hydrocarbon portion in their molecules. Over 80% to 85% of lipids are triacylglycerols, which occur in many types according to the identity and positions of the three fatty acids involved. Those with a single kind of fatty acid in all three are called simple triacylglycerols. As an example, trimyristin, isolated from nutmeg, is composed of myristic acid (C14:0) and glycerol. Triacylglycerols belong to the nonpolar class of lipids, which also includes diacylglycerols, monoacylglycerols, free fatty acids, and fat soluble vitamins, as well as steroids, terpenes, hydrocarbons, carotenoids, and other matter which cannot be saponified (Christie, 1982). Nonpolar lipids, also referred to as simple lipids, afford one or two types of products upon hydrolysis. On the other hand, polar lipids generally consist of phospholipids and glycolipids, and their hydrolysis may afford three or more products, hence they are also known as complex lipids. Phospholipids are major constituents of cell wall materials. An example of a plant phospholipid is lecithin, which is found in many vegetable oil sources. Glycolipids consisting of lipids in conjugation with carbohydrates are also found abundantly in biological systems and foods.

To analyze lipids, it is necessary to first isolate them quantitatively from nonlipid components. Extraction of lipids from source materials, such as food, animal and plant tissues, or microorganism, should be carried out in a manner that avoids changes in the lipids or leads to the formation of artifacts. Thus, it might be necessary to deactivate enzymes that might hydrolyse lipids via heat treatment. Precaution must also be exercised to minimize oxidation of lipids, especially those containing polyunsaturated fatty acids. Use of an antioxidant (e.g., TBHQ)/antioxidant system (e.g., TBHQ/EDTA) might prove beneficial, particularly when dealing with the extraction of lipids with highly unsaturated fatty acids, such as

eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Lipids in foods exist in both the free and bound forms. While free lipids are easily extracted, bound lipids may require pretreatment with concentrated acids prior to their extraction (see Basic Protocol 4).

During the extraction process three types of interactions are usually disrupted, these are: van der Waals forces in lipid-lipid, lipid-protein, and liquid-carbohydrate complexes; electrostatic and hydrogen bonding interactions between lipids and proteins; and covalent bonding between lipids, carbohydrates, and proteins (Robyt and White, 1987). The solvent of choice depends on the type of lipid and the interactions to be disrupted. Thus, neutral lipids may be extracted with nonpolar solvents, while phospholipids and glycolipids are extracted with more polar solvent mixtures (Shahidi and Wanasundara, 1998).

The extraction and measurement of lipids may require several steps, these include: (1) Pretreatment, including drying, size reduction, and possibly acid hydrolysis to release lipids. (2) Homogenization of the tissue in the presence of a solvent/solvent system. (3) Separation of liquids from solids. (4) Removal of nonlipid contaminants. (5) Removal of solvent and drying. (6) Calculating the content of lipids by weight difference.

Procedures for isolation and measurement of lipids in foods include exhaustive Soxhlet extraction with hexane or petroleum ether (AOAC, 1995; see Basic Protocol 1), chloroform/methanol (Hanson and Olley, 1963; Ambrose, 1969), chloroform/methanol/water (Folch et al., 1957; Bligh and Dyer, 1959; see Basic Protocol 2 and Alternate Protocol 2), acid digestion followed by extraction (see Basic Protocol 4), or, for starchy material, extraction with *n*-propanol-water (e.g., Vasanthan and Hoover, 1992; see Basic Protocol 3). Each method has its own advantages and disadvantages and successful measurement of lipid content is often dictated by the type of sample and extraction medium employed. Commercial extraction and preparation of edible oils are explained in the literature (Williams, 1997).

In the Soxhlet extraction, usually dry or nearly dried material is subjected to semicontinuous extraction with hexane or petroleum ether. The drawbacks of this method are the length of time required for extraction and the fact that polar and bound lipids are not removed, offset by the fact that no channeling occurs. The Goldfisch method, on the other hand, is a continuous process and somewhat faster than that of the Soxhlet procedure. In the *n*-propanol-water extraction procedure, using Soxhlet, bound lipids in amylose and starchy food may be nearly completely released and polar lipids also quantitated. Nonetheless, total extraction and measurement of lipids may require acid digestion with strong sulfuric acid or usually 6 to 8 M HCl to release the bound lipids prior to their extraction into a desirable solvent. Meanwhile, the method of Folch et al. (1957) and that of Bligh and Dyer (1959) use chloroform/methanol/water to isolate total lipids. Use of chloroform is not ideal, but the method is of general use in wet foods, especially meat and fish. Recently, a rapid and simple method involving chloroform/methanol/water for determination of lipid content of seafoods has been reported by Lee et al. (1996). In addition, extraction of lipids from small amounts of material for determination and subsequent use for fatty acid analysis or directly for fatty acid profiling has been reported (Divakaran and Ostrowski, 1989; Garces and Mancha, 1993; Lugue de Castro and Garcia-Ayuso, 1998; Isik et al., 1999). Other methods have compared extraction with chloroform-based solvents with hexane/isopropanol (Gunnlaugsdottir and Ackman, 1993). Use of acetone/water has also been examined (Dambergs, 1956).

In addition to the above, other solvent systems and methodologies, both direct and indirect, have been explored for extraction and/or quantification of total lipids from different foods. As an example, Röse-Gottlieb extraction methodology (AOAC, 1995; James, 1995) is employed for extraction of lipids from milk and Schmid Bondzynski-Ratzlaff (Werner-Schmid) extraction of lipids from cheese is commonplace (AOAC, 1995; James, 1995). In the Röse-Gottlieb method, the sample is first treated with a solution of 25% (w/v) ammonia and then extracted repeatedly in the ethanol, diethyl ether, and hexanes (petroleum ether). The combined extracts are then dried and reextracted into petroleum ether followed by solvent removal and measurement. In the Mojonnier (AOAC, 1995; James, 1995) procedure, the combined extracts are then weighed without purification. The Schmidt-Bondzynski-Ratzlaff procedure uses successive extractions with diethyl ether after digestion with acid or base. Recently, use of analytical supercritical fluid extraction (SFE) for determination of fat content has been reported (Feller and King, 1996).

The indirect procedures are used primarily for measurement of lipids without isolation. The application of wide-line nuclear magnetic

resonance (NMR) for determination of oil content in oilseeds and use of infrared spectroscopy for determination of lipid in milk products has been reported (e.g., Shahidi and Wanasundara, 1998). Meanwhile, use of refractometry, density measurements and other techniques for lipid determination have been reported (See Pomeranz and Meloan, 1994).

Critical Parameters and Troubleshooting

In Soxhlet extractions (see Basic Protocols 1 and 3), the amount of solvent must be sufficient to cover the thimble and have at least 50 ml solvent in the flask and may require an additional amount of solvent. After completion of extraction, it is essential to let the system cool and let all the solvent transfer to the flask. In addition, accurate weighing of flask is essential. Use of gloves to avoid fingerprints on flasks which may affect the weights is critical.

There may be problems associated with phase separation due to the formation of emulsion (see Basic Protocol 2). Addition of a salt solution (KCl or NaCl) and further standing in a cold room is recommended.

Total removal of the solvent from extracted lipids is necessary (see Basic Protocols 1 to 4). Often keeping of the extract in a forced-air oven for 1 to 2 h is helpful.

If extracted lipids are to be used for further analysis, it is recommended that a certain volume of the extract be removed and used for this purpose prior to employing any harsh conditions for the removal of solvents. In this case, accurate measurement of values is essential.

Anticipated Results

Results for lipid content depends very much on the source material and the protocol used. Thus, oilseeds such as mustard, canola, and flax may contain 38% to 42% lipid, while starch may contain ≤1% total lipid. The content of lipids in meat and seafoods may vary from 1% to 30%. Nutmeg contains 25% to 30% lipids.

Time Considerations

For extraction of lipids, using all four protocols, approximately 35 to 40 hr is required. The time required for the Soxhlet method (see Basic Protocol 1) is up to 12 hr, the chloroform/methanol/water extraction (Basic Protocol 2), up to 12 hr, *n*-propanol/water extraction (see Basic Protocol 3), 5 to 6 hr, and extraction of lipids requiring acid digestion (see Basic Protocol 4), 5 to 8 hr.

Each of the alternate procedures requires the same time or less than that of the original.

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Provides general background and a cursory account of procedures available.

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