chapter 12

Fat Content Determination

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12.1 INTRODUCTION

12.1.1 Background

The term "lipid" refers to a group of compounds that are sparingly soluble in water but show variable solubility in a number of organic solvents (e.g., ethyl ether, petroleum ether, acetone, ethanol, methanol, benzene). The lipid content of a food determined by extraction with one solvent may be quite different from the lipid content as determined with another solvent of different polarity. Fat content is determined often by solvent extraction methods (e.g., Soxhlet, Goldfish, Mojonnier), but it also can be determined by nonsolvent wet extraction methods (e.g., Babcock, Gerber) and by instrumental methods that rely on the physical and chemical properties of lipids (e.g., infrared, density, X-ray absorption). The method of choice depends on a variety of factors, including the nature of the sample (e.g., dry versus moist), the purpose of the analysis (e.g., official nutrition labeling or rapid quality control), and instrumentation available (e.g., Babcock uses simple glassware and equipment; infrared requires an expensive instrument).

This experiment includes the Soxhlet, Goldfish, Mojonnier, and Babcock methods. If samples analyzed by these methods can be tested by an instrumental method for which equipment is available in your laboratory, data from the analyses can be compared. Low-moisture snack foods are suggested for analysis and comparison by the Soxhlet and Goldfish methods, and milk by the Mojonnier and Babcock methods. However, other appropriate foods could be substituted, and results compared between methods. Also, the experiment specifies the use of petroleum ether as the solvent for the Soxhlet and Goldfish methods, for reasons of cost and safety. Anhydrous ethyl ether could be used for both extraction methods, but appropriate precautions must be taken in handling the solvent, plus the snack foods would need to be dried before extraction. Using low-moisture content snack foods (versus dried samples) admittedly introduces a low bias with petroleum ether extraction, and requires that separate samples be analyzed for moisture content, to correct for moisture in the fat content calculations. In this laboratory exercise, the moisture content data are used to express the fat content on a wet and dry weight basis.

12.1.2 Reading Assignment

Ellefson, W.C. 2017. Fat analysis. Ch. 17, in *Food Analysis*, 5rd ed. S.S. Nielsen (Ed.), Springer, New York.

12.1.3 Objective

Determine the lipid contents of various snack foods by the Soxhlet and Goldfish methods, and determine the lipid content of milk by the Mojonnier and Babcock methods.

12.2 SOXHLET METHOD

12.2.1 Principle of Method

Fat is extracted, semicontinuously, with an organic solvent. Solvent is heated and volatilized, then is condensed above the sample. Solvent drips onto the sample and soaks it to extract the fat. At 15–20 min intervals, the solvent is siphoned to the heating flask, to start the process again. Fat content is measured by the weight loss of sample or weight of fat removed.

12.2.2 Chemicals

	CAS no.	*Hazards*
Petroleum ether	8032-32-4	Harmful, highly flammable, dangerous for environment
(or ethyl ether)	60-29-7	Harmful, extremely flammable

12.2.3 Hazards, Precautions, and Waste Disposal

Petroleum ether and ethyl ether are fire hazards; avoid open flames, breathing vapors, and contact with skin. Ether is extremely flammable, is hygroscopic, and may form explosive peroxides. Otherwise, adhere to normal laboratory safety procedures. Wear gloves and safety glasses at all times. Petroleum ether and ether liquid wastes must be disposed of in designated hazardous waste receptacles.

12.2.4 Supplies

- 3 Aluminum weighing pans, predried in 70 °C vacuum oven for 24 h
- Beaker, 250 mL
- Cellulose extraction thimbles, predried in 70 °C vacuum oven for 24 h
- Desiccator
- Glass boiling beads
- Glass wool, predried in 70 °C vacuum oven for 24 h
- Graduated cylinder, 500 mL
- Mortar and pestle
- Plastic gloves
- Snack foods (need to be fairly dry and able to be ground with a mortar and pestle)
- Spatula
- Tape (to label beaker)
- Tongs
- Weighing pan (to hold 30-g snack food)

12.2.5 Equipment

- Analytical balance
- Soxhlet extractor, with glassware
- Vacuum oven

12.2.6 Procedure

(Instructions are given for analysis in triplicate.)

1. Record the fat content of your snack food product as reported on the package label. Also record serving size so you can calculate g fat/100-g product.
2. Slightly grind ~30-g sample with mortar and pestle (excessive grinding will lead to greater loss of fat in mortar).
3. Wearing plastic gloves, remove three predried cellulose extraction thimbles from the desiccator. Label the thimbles on the outside with your initials and a number (use a lead pencil), then weigh accurately on an analytical balance.
4. Place ~2–3 g of sample in the thimble. Reweigh. Place a small plug of dried glass wool in each thimble. Reweigh.
5. Place the three samples in a Soxhlet extractor. Put ~350-mL petroleum ether in the flask, add several glass boiling beads, and extract for 6 h or longer. Place a 250-mL beaker labeled with your name below your samples on the Soxhlet extraction unit. Samples in thimbles will be placed in the beaker after extraction and before drying.
6. Remove thimbles from the Soxhlet extractor using tongs, air dry overnight in a hood, then dry in a vacuum oven at 70 °C, 25 in. mercury, for 24 h. Cool dried samples in a desiccator then reweigh.
7. Correct for moisture content of product as follows:
   (a) Using the remainder of the ground sample and three dried, labeled, and weighed aluminum sample pans, prepare triplicate 2–3-g samples for moisture analysis.
   (b) Dry sample at 70 °C, 25 in. mercury, for 24 h in a vacuum oven.
   (c) Reweigh after drying, and calculate moisture content of the sample.

12.2.7 Data and Calculations

Using the weights recorded in the tables below, calculate the percent fat (wt/wt) on a wet weight basis as determined by the Soxhlet extraction method. If the fat content of the food you analyzed was given on the label, report this theoretical value.

Name of snack food
Label g fat/serving
Label serving size (g)
Label g fat/100-g product

Data from Soxhlet extraction:

Rep	*Thimble (g)*	*Wet sample + thimble (g)*	*Wet sample (g)*	*Wet sample + thimble + glass wool (g)*	*Dry, extracted sample + thimble + glass wool (g)*	*Fat + moisture (g)*	*Fat + moisture (%)*	% Fat (wwb)	*% Fat (dwb)*
1									
2									
3									
									$\bar{X}$ =
									SD =

$$\%\left(\text{Fat + Moisture}\right)=\frac{\left[\left(\text{Wet sample + thimble + glass wool,g}\right)-\left(\text{Dry, extracted sample + thimble + glass wool,g}\right)\right]}{\left(\text{Wet sample,g}\right)}\times 100$$

Data from moisture analysis:

Rep	*Pan (g)*	*Pan + wet sample (g)*	*Pan + dried sample (g)*	*% Moisture*
1				
2				
3				
				$\bar{X}$ =
				SD =

Calculation of % moisture:

$$\frac{(\text{Wt of wet sample} + \text{Pan}) - (\text{Wt of dried sample} + \text{Pan})}{(\text{Wt of wet sample} + \text{Pan}) - (\text{Wt of pan})} \times 100$$

Calculation of % fat, wet weight basis:

$$\%\,\text{Fat}\,(\text{wt / wt, wet weight basis}) = (\%\,\text{Fat} + \%\,\text{Moisture}) - (\%\,\text{Moisture})$$

(*Note:* Use average % moisture in this calculation.)

Calculate % fat on a dry weight basis:

$$\%\ \text{Fat on dry basis} = (\%\ \text{Fat on wet basis} \times 100) / (100 - \%\ \text{Moisture content})$$

12.2.8 Questions

1. The Soxhlet extraction procedure utilized petroleum ether. What were the advantages of using it rather than ethyl ether?
2. What were the advantages of using the Soxhlet extraction method rather than the Goldfish extraction method?
3. If the fat content measured here differed from that reported on the nutrition label, how might this be explained?

12.3 GOLDFISH METHOD

12.3.1 Principle

Fat is extracted, continuously, with an organic solvent. Solvent is heated and volatilized, then is condensed above the sample. Solvent continuously drips through the sample to extract the fat. Fat content is measured by the weight loss of sample or weight of fat removed.

12.3.2 Chemicals

Same as for Sect. 12.2.2.

12.3.3 Hazards, Precautions, and Waste Disposal

Same as for Sect. 12.2.2.

12.3.4 Supplies

Same as for Sect. 12.2.2.

12.3.5 Equipment

- Analytical balance
- Goldfish extraction apparatus
- Hot plate
- Vacuum oven

12.3.6 Procedure

(Instructions are given for analysis in triplicate.)

Note: Analyze samples in triplicate.

1. Follow Steps 1–4 in Sect. 12.2.6.
2. Place the thimble in the Goldfish condenser bracket. Push the thimble up so that only about 1–2 cm is below the bracket. Fill the reclaiming beaker with petroleum ether (50 mL) and transfer to beaker. Seal beaker to apparatus using gasket and metal ring. Start the water flow through the condenser. Raise the hot plate up to the beaker, turn on, and start the ether boiling. Extract for 4 h at a condensation rate of 5–6 drops per second.
3. Follow Steps 6 and 7 in Sect. 12.2.6.

12.3.7 Data and Calculations

Record the data and do the calculations for the Goldfish method using the same tables and equations given for the Soxhlet method in Sect. 12.2.7. Calculate the percent fat (wt/wt) on a wet weight basis. If the fat content of the food you analyzed was given on the label, report this theoretical value:

Name of snack food
Label g fat/serving
Label serving size (g)
Label g fat/100-g product

12.3.8 Questions

1. What would be the advantages and disadvantages of using ethyl ether rather than petroleum ether in a solvent extraction method, such as the Goldfish method?
2. What were the advantages of using the Goldfish extraction method rather than the Soxhlet extraction method?
3. If the fat content measured here differed from that reported on the nutrition label, how might this be explained?

12.4 MOJONNIER METHOD

12.4.1 Principle

Fat is extracted with a mixture of ethyl ether and petroleum ether. The extract containing the fat is dried and expressed as percent fat by weight.

The assay uses not only ethyl ether and petroleum ether, but also ammonia and ethanol. Ammonia dissolves the casein and neutralizes the acidity of the product to reduce its viscosity. Ethanol prevents gelation of the milk and ether, and aids in the separation of the ether-water phase. Ethyl ether and petroleum ether

serve as lipid solvents, and petroleum ether decreases the solubility of water in the ether phase.

12.4.2 Chemicals

	CAS no.	*Hazards*
Ammonium hydroxide	1336-21-6	Corrosive, dangerous for the environment
Ethanol	64-17-5	Highly flammable
Petroleum ether	8032-32-4	Harmful, highly flammable, dangerous for environment
(Ethyl ether)	60-29-7	Harmful, extremely flammable

12.4.3 Hazards, Precautions, and Waste Disposal

Ethanol, ethyl ether, and petroleum ether are fire hazards; avoid open flames, breathing vapors, and contact with skin. Ether is extremely flammable, is hygroscopic, and may form explosive peroxides. Ammonia is a corrosive; avoid contact and breathing vapors. Otherwise, adhere to normal laboratory safety procedures. Wear gloves and safety glasses at all times. Petroleum ether and ether liquid wastes must be disposed of in designated hazardous waste receptacles. The aqueous waste can go down the drain with a water rinse.

12.4.4 Supplies

- Milk, whole and 2% fat
- Mojonnier extraction flasks, with stoppers
- Mojonnier fat dishes with lids
- Plastic gloves
- Tongs

12.4.5 Equipment

- Analytical balance
- Hot plate
- Mojonnier apparatus (with centrifuge, vacuum oven, and cooling desiccator)

12.4.6 Notes

Reagents must be added to the extraction flask in the following order: water, ammonia, alcohol, ethyl ether, and petroleum ether. The burets on the dispensing cans or tilting pipets are graduated for measuring the proper amount. Make triplicate determinations on both the sample and reagent blanks. The procedure given here is for fresh milk. Other samples may need to be diluted with distilled water in Step 2 and require different quantities of reagents in subsequent steps. Consult the instruction manual or AOAC International *Official Methods of Analysis* for samples other than fresh milk.

12.4.7 Procedure

(Instructions are given for analysis in triplicate.)

1. Turn on power unit and temperature controls for oven and hot plate on the fat side of the Mojonnier unit.
2. Warm milk samples to room temperature and mix well.
3. When oven is at 135 °C, heat cleaned fat dishes in oven under a vacuum of 20-in. mercury for 5 min. Handle dishes from this point on with tongs or gloves. Use three dishes for each type of milk samples, and two dishes for the reagent blank.
4. Cool dishes in cooling desiccator for 7 min.
5. Weigh dishes, record weight of each dish and its identity, and place dishes in desiccator until use.
6. Weigh samples accurately (ca. 10 g) into Mojonnier flasks. If weighing rack is used, fill curved pipettes and place in rack on the balance. Weigh each sample by difference.
7. Add chemicals for the first extraction in the order and amounts given below. After each addition of chemicals, stopper the flask and shake by inverting for 20 s.

	First extraction		*Second extraction*	
Chemicals	*Step*	*Amount (mL)*	*Step*	*Amount (mL)*
Ammonia	1	1.5	–	None
Ethanol	2	10	1	5
Ethyl ether	3	25	2	15
Petroleum ether	4	25	3	15

8. Place the extraction flasks in the holder of the centrifuge. Place both flask holders in the centrifuge. Operate the centrifuge to run at 30 turns in 30 s, to give a speed of 600 rpm (revolutions per minute). [In lieu of centrifuging, the flasks can be allowed to stand 30 min until a clear separation line forms, or three drops of phenolphthalein indicator (0.5% w/v ethanol) can be added during the first extraction to aid in determining the interface.]
9. Carefully pour off the ether solution of each sample into a previously dried, weighed, and cooled fat dish. Most or all of the ether layer should be poured into the dish, but none of the remaining liquid must be poured into the dish.
10. Place dishes with ether extract on hot plate under glass hood of Mojonnier unit, with power unit running. (If this hot plate is not available, use a hot plate placed in a hood, with the hot plate at 100 °C.)

11. Repeat the extraction procedure a second time for the samples in the Mojonnier flasks, following the sequence and amount given in the table above. Again, after each addition of chemicals, stopper the flask and shake by inverting for 20 s. Centrifuge the flasks again, as described above. Distilled water may be added now to the flask to bring the dividing line between ether and water layers to the center of the neck of flask. If this is done, repeat the centrifugation.
12. Pour ether extract into the respective fat dish (i.e., the ether for a specific sample should be poured into the same fat dish used for that sample from the first extraction), taking care to remove all the ether but none of the other liquid in the flask.
13. With lids ajar, complete the evaporation of ether, either very carefully on the hot plate (this can be problematic and a fire hazard) or open in a hood. In using a hot plate, the ether should boil slowly; not fast enough to cause splattering. If the plate appears to be too hot and boiling is too fast, only part of the dish should be placed on the hot plate. If instead using an operating hood, leave collection containers with lids ajar to have them evaporated by the next day.
14. When all the ether has been evaporated from the dishes, place the dishes in the vacuum oven 70–75 °C for 10 min with a vacuum of at least 20 in.
15. Cool the dishes in the desiccator for 7 min.
16. Accurately weigh each dish with fat. Record weight.

12.4.8 Data and Calculations

Calculate the fat content of each sample. Subtract the average weight of the reagent blank from the weight of each fat residue in the calculation.

12.4.9 Questions

1. List possible causes for high and low results in a Mojonnier fat test.
2. How would you expect the elimination of alcohol from the Mojonnier procedure to affect the results? Why?
3. How would you propose to modify the Mojonnier procedure to test a solid, nondairy product? Explain your answer.

12.5 BABCOCK METHOD

12.5.1 Principle

Sulfuric acid is added to a known amount of milk sample in a Babcock bottle. The acid digests the protein, generates heat, and releases the fat. Centrifugation and hot water addition isolate the fat into the graduated neck of the bottle. The Babcock fat test uses a volumetric measurement to express the percent of fat in milk or meat by weight.

12.5.2 Note

The fat column in the Babcock test should be at 57–60 °C when read. The specific gravity of liquid fat at that temperature is approximately 0.90 g per mL. The calibration on the graduated column of the test bottle reflects this fact and enables one to make a volumetric measurement, which expresses the fat content as percent by weight.

12.5.3 Chemicals

	CAS No.	*Hazards*
Glymol (red reader)	8042-47-5	Toxic, irritant
Sulfuric acid	7664-93-9	Corrosive

Rep	*Milk start (g)*	*Milk end (g)*	*Milk tested (g)*	*Dish (g)*	*Dish + fat (g)*	*Calculated % fat*
Reagent blanks:						
1						–
2						–
Samples:						$\bar{X}$ =
1						
2						
3						
						$\bar{X}$ =
						SD =

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

12.5.4 Hazards, Precautions, and Waste Disposal

Concentrated sulfuric acid is extremely corrosive; avoid contact with skin and clothes and breathing vapors. Wear gloves and safety glasses at all times. Otherwise, adhere to normal laboratory safety procedures. Sulfuric acid and glymol wastes must be disposed in a designated hazardous waste receptacle.

For safety and accuracy reasons, dispense the concentrated sulfuric acid from a bottle fitted with a repipettor (i.e., automatic bottle dispenser). Fit the dispenser with a thin, semirigid tube to dispense directly and deep into the Babcock bottle while mixing contents. Set the bottle with dispenser on a tray to collect spills. Wear corrosive- and heat-resistant gloves when mixing the sulfuric acid with samples.

12.5.5 Supplies

- 3 Babcock bottles
- Babcock caliber (or shrimp divider)
- Measuring pipette, 10 mL
- Pipette bulb or pump
- Plastic gloves
- Standard milk pipette (17.6 mL)
- Thermometer

12.5.6 Equipment

- Babcock centrifuge
- Water bath

12.5.7 Procedure

(Instructions are given for analysis in triplicate.)

1. Adjust milk sample to ca. 38 °C and mix until homogenous. Using a standard milk pipette, pipette 17.6 mL of milk into each of three Babcock bottles. After the pipette has emptied, blow out the last drops of milk from the pipette tip into the bottle. Allow milk samples to adjust to ca. 22 °C.
2. Dispense ca. 17.5 mL of sulfuric acid (specific gravity 1.82–1.83) and carefully add into the test bottle, with mixing during and between additions, taking care to wash all traces of milk into the bulb of the bottle. Time for complete acid addition should not exceed 20 s. Mix the milk and acid thoroughly. Be careful not to get any of the mixture into the column of the bottle while shaking. Heat generated behind any such lodged mixture may cause a violent expulsion from the bottle.
3. Place bottles in centrifuge heated to 60 °C. Be sure bottles are counterbalanced. Position bottles so that bottlenecks will not be broken in horizontal configuration. Be sure that the heater of the centrifuge is on.
4. Centrifuge the bottles for 5 min after reaching the proper speed (speed will vary depending upon the diameter of the centrifuge head).
5. Stop the centrifuge and add soft hot water (60 °C) until the liquid level is within 0.6 cm of the neck of the bottle. Carefully permit the water to flow down the side of the bottle. Again, centrifuge the bottles for 2 min.
6. Stop the centrifuge and add enough soft hot water (60 °C) to bring the liquid column near the top graduation of the scale. Again, centrifuge the bottles for 1 min.
7. Remove the bottles from the centrifuge and place in a heated (55–60 °C, preferably 57 °C) water bath deep enough to permit the fat column to be below the water level of the water bath. Allow bottles to remain at least 5 min before reading.
8. Remove the samples from the water bath one at a time, and quickly dry the outside of the bottle. Add glymol (red reader) to the top of fat layer. Immediately use a divider or caliper to measure the fat column to the nearest 0.05%, holding the bottle in a vertical position at eye level. Measure from the highest point of the upper meniscus to the bottom of the lower meniscus.
9. Reject all tests in which the fat column is milky or shows the presence of curd or charred matter, or in which the reading is indistinct or uncertain. The fat should be clear and sparkling, the upper and lower meniscus clearly defined, and the water below the fat column should be clear.
10. Record the readings of each test and determine the mean % fat and the standard deviation.

12.5.8 Data and Calculations

Rep	*Measured % fat*
1	
2	
3	
	$\bar{X}$ =
	SD =

12.5.9 Questions

1. What are the possible causes of charred particles in the fat column of the Babcock bottle?
2. What are the possible causes of undigested curd in the Babcock fat test?
3. Why is sulfuric acid preferred over other acids for use in the Babcock fat test?

RESOURCE MATERIALS

AOAC International (2016) Official methods of analysis, 20th edn, (On-line). AOAC International, Rockville, MD

Ellefson WC (2017) Fat analysis. Ch. 17. In: Nielsen SS (ed) Food analysis, 5th edn. Springer, New York

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