22

chapter

Fat Characterization

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22.1 Introduction
  22.1.1 Background
  22.1.2 Reading Assignment
  22.1.3 Overall Objective
22.2 Saponification Value
  22.2.1 Objective
  22.2.2 Principle of Method
  22.2.3 Chemicals

S.S. Nielsen, *Food Analysis Laboratory Manual*, Food Science Text Series,
DOI 10.1007/978-3-319-44127-6_22,

- 22.2.4 Reagents
- 22.2.5 Hazards, Precautions, and Waste Disposal
- 22.2.6 Supplies
- 22.2.7 Equipment
- 22.2.8 Procedure
- 22.2.9 Data and Calculations
- 22.2.10 Questions

22.3 Iodine Value
- 22.3.1 Objective
- 22.3.2 Principle of Method
- 22.3.3 Chemicals
- 22.3.4 Reagents
- 22.3.5 Hazards, Precautions, and Waste Disposal
- 22.3.6 Supplies
- 22.3.7 Equipment
- 22.3.8 Procedure
- 22.3.9 Data and Calculations
- 22.3.10 Questions

22.4 Free Fatty Acid Value
- 22.4.1 Objective
- 22.4.2 Principle of Method
- 22.4.3 Chemicals
- 22.4.4 Reagents
- 22.4.5 Hazards, Precautions, and Waste Disposal
- 22.4.6 Supplies
- 22.4.7 Equipment
- 22.4.8 Procedure
- 22.4.9 Data and Calculations
- 22.4.10 Questions

22.5 Peroxide Value
- 22.5.1 Objective
- 22.5.2 Principle of Method
- 22.5.3 Chemicals
- 22.5.4 Reagents
- 22.5.5 Hazards, Precautions, and Waste Disposal
- 22.5.6 Supplies
- 22.5.7 Equipment
- 22.5.8 Procedure
- 22.5.9 Data and Calculations
- 22.5.10 Questions

22.6 Thin-Layer Chromatography Separation of Simple Lipids
- 22.6.1 Objective
- 22.6.2 Principle of Method
- 22.6.3 Chemicals
- 22.6.4 Reagents
- 22.6.5 Hazards, Precautions, and Waste Disposal
- 22.6.6 Supplies
- 22.6.7 Equipment
- 22.6.8 Procedure
- 22.6.9 Data and Calculations
- 22.6.10 Questions

22.1 INTRODUCTION

22.1.1 Background

Lipids in food are subjected to many chemical reactions during processing and storage. While some of these reactions are desirable, others are undesirable; so, efforts are made to minimize the reactions and their effects. The laboratory deals with characterization of fats and oils with respect to composition, structure, and reactivity.

22.1.2 Reading Assignment

Pike, O.A., and O'Keefe, S.F. 2017. Fat characterization, Ch. 23 in *Food Analysis*, 5th ed. S.S. Nielsen (Ed.), Springer, New York.

22.1.3 Overall Objective

The overall objective of this laboratory is to determine aspects of the composition, structure, and reactivity of fats and oils by various methods.

22.2 SAPONIFICATION VALUE

22.2.1 Objective

Determine the saponification number of fats and oils.

22.2.2 Principle of Method

Saponification is the process of treating a neutral fat with alkali, breaking it down to glycerol and fatty acids. The saponification value (or number) is defined as the amount of alkali needed to saponify a given quantity of fat or oil, expressed as mg potassium hydroxide to saponify 1 g sample. Excess alcoholic potassium hydroxide is added to the sample, the solution is heated to saponify the fat, the unreacted potassium hydroxide is back-titrated with standardized hydrochloric acid using a phenolphthalein indicator, and the calculated amount of reacted potassium hydroxide is used to determine the saponification value.

22.2.3 Chemicals

	CAS No.	*Hazards*
Ethanol	64-17-5	Highly flammable
Hydrochloric acid (HCl)	7647-01-0	Corrosive
Phenolphthalein	77-09-8	Irritant
Potassium hydroxide (KOH)	1310-58-3	Corrosive

22.2.4 Reagents

(**It is recommended that these solutions be prepared by the laboratory assistant before class.)

- Alcoholic potassium hydroxide, ca. 0.7 *N* **
  Dissolve 40 g KOH, low in carbonate, in 1 L of distilled ethanol, keeping temperature below 15.5 °C while the alkali is being dissolved. The solution should be clear.
- Hydrochloric acid, ca. 0.5 *N*, accurately standardized **
  Prepare ca. 0.5 *N* HCl. Determine the exact normality using solution of standard base.
- Phenolphthalein indicator solution **
  1 %, in 95 % ethanol

22.2.5 Hazards, Precautions, and Waste Disposal

Use hydrochloric acid in a fume hood. Otherwise, adhere to normal laboratory safety procedures. Wear safety glasses at all times. Wastes likely may be put down the drain using a water rinse, but follow good laboratory practices outlined by environmental health and safety protocols at your institution.

22.2.6 Supplies

(Used by students)

- Air (reflux) condenser (650 mm long, minimum)
- Beaker, 250 mL (to melt fat)
- Buchner funnel (to fit side-arm flask)
- Boiling beads
- 2 Burets, 50 mL
- Fat and/or oil samples
- Filter paper (to fit Buchner funnel, to filter oil and melted fat)
- 4 Flasks, 250–300 mL, to fit condenser
- Mechanical pipettor, 1000 μL, with plastic tips (or 1 mL volumetric pipette)
- Side-arm flask

22.2.7 Equipment

- Analytical balance
- Hot plate or water bath (with variable heat control)

22.2.8 Procedure

(Instructions are given for analysis in duplicate.)

1. Melt any solid samples. Filter melted fat sample and oil sample through filter paper to remove impurities.
2. Weigh accurately ca. 5 g melted fat or oil into each of two 250–300 ml flasks that will connect to a condenser. Record weight of sample. Prepare sample in duplicate.
3. Add accurately (from a buret) 50 mL of alcoholic KOH into the flask.

4. Prepare duplicate blank samples with just 50 mL of alcoholic KOH in a 250–300 mL flask.
5. Add several boiling beads to the flasks with fat or oil sample.
6. Connect the flasks with the sample to a condenser. Boil gently but steadily on a hot plate (or water bath) until the sample is clear and homogenous, indicating complete saponification (requires ca. 30–60 min). (Note: The fumes should condense as low as possible in the condenser; otherwise, a fire hazard will be created.)
7. Allow samples to cool somewhat. Wash down the inside of the condenser with a little deionized distilled (dd) water. Disconnect flask from condenser. Allow the samples to cool to room temperature.
8. Add 1 mL phenolphthalein to samples and titrate with 0.5 *N* HCl (from a buret) until the pink color just disappears. Record the volume of titrant used.
9. Repeat Steps 5–8 with sample blanks. Reflux the blanks for the same time period as used for the sample.

22.2.9 Data and Calculations

Sample	*Weight (g)*	*Titrant volume (mL)*	*Saponification value*
1			
2			
			$\bar{X}$ =

Oil/fat sample type tested:

Blank titration (mL):

Sample 1 =
Sample 2 =
$\bar{X}$ =

Calculate the saponification number (or value) of each sample as follows:

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

where:

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

22.2.10 Questions

1. What is meant by unsaponifiable matter in lipid samples? Give an example of such a type of compound.
2. What does a high versus a low saponification value tell you about the nature of a sample?

22.3 IODINE VALUE

22.3.1 Objective

Determine the iodine value of fats and oils.

22.3.2 Principle of Method

The iodine value (or number) is a measure of the degree of unsaturation, defined as the grams of iodine absorbed per 100 g sample. In the assay, a measured quantity of fat or oil dissolved in solvent is reacted with a measured excess amount of iodine or some other halogen, which reacts with the carbon-carbon double bonds. After a solution of potassium iodide is added to reduce excess ICl to free iodine, the liberated iodine is titrated with a standardized solution of sodium thiosulfate using a starch indicator. The calculated amount of iodine reacted with the double bonds is used to calculate the iodine value.

22.3.3 Chemicals

	CAS no.	*Hazards*
Acetic acid (glacial)	64-19-7	Corrosive
Carbon tetrachloride (CCl_4)	56-23-5	Toxic, dangerous for the environment
Chloroform	67-66-3	Harmful
Hydrochloric acid (HCl)	7647-01-0	Corrosive
Iodine	7553-56-2	Harmful, dangerous for the environment
Potassium dichromate ($K_2Cr_2O_7$)	7789-00-6	Toxic, dangerous for the environment
Potassium iodide (KI)	7681-11-0	
Sodium thiosulfate	7772-98-7	
Soluble starch	9005-25-8	

22.3.4 Reagents

(**It is recommended that these solutions be prepared by the laboratory assistant before class.)

- Potassium iodide solution, 15 %
  Dissolve 150 g KI in dd water and dilute to 1 l.
- Sodium thiosulfate, 0.1 *N* standardized solution (AOAC Method 942.27) **
  Dissolve ca. 25 g sodium thiosulfate in 1 L dd water. Boil gently for 5 min. Transfer while hot to

a storage bottle (make sure bottle has been well cleaned and is heat resistant). Store solution in a dark, cool place. Use the following procedure to standardize the sodium thiosulfate solution: Accurately weigh 0.20–0.23 g potassium dichromate ($K_2Cr_2O_7$) (previously dried for 2 h at 100 °C) into a glass-stoppered flask. Dissolve 2 g potassium iodide (KI) in 80 mL chlorine-free water. Add this water to the potassium dichromate. To this solution, add, with swirling, 20 mL ca. 1 *M* HCl and immediately place in the dark for 10 min. Titrate a known volume of this solution with the sodium thiosulfate solution, adding starch solution after most of the iodine has been consumed.

- Starch indicator solution, 1 % (prepare fresh daily)**
  Mix ca. 1 g soluble starch with enough cold dd water to make a thin paste. Add 100 mL boiling dd water. Boil ca. 1 min while stirring.
- Wijs iodine solution **
  Dissolve 10 g ICl_3 in 300 mL CCl_4 and 700 mL glacial acetic acid. Standardize this solution against 0.1 *N* sodium thiosulfate (25 mL of Wijs solution should consume 3.4–3.7 mEq of thiosulfate). Then, add enough iodine to the solution such that 25 mL of the solution will require at least 1.5 times the milliequivalency of the original titration. Place the solution in an amber bottle. Store in the dark at less than 30 °C.

22.3.5 Hazards, Precautions, and Waste Disposal

Carbon tetrachloride and potassium chromate are toxic and must be handled with caution. Use acetic acid and hydrochloric acid in a fume hood. Otherwise, adhere to normal laboratory safety procedures. Wear safety glasses at all times. Carbon tetrachloride, chloroform, iodine, and potassium chromate must be handled as hazardous wastes. Other wastes likely may be put down the drain using a water rinse, but follow good laboratory practices outlined by environmental health and safety protocols at your institution.

22.3.6 Supplies

(Used by students)

- 2 Beakers, 250 mL (one to melt fat; one to boil water)
- Buchner funnel (to fit side-arm flask)
- Buret, 10 or 25 mL
- Fat and/or oil samples
- Filter paper (to fit Buchner funnel, to filter melted fat and oil)
- 4 Flasks, 500 mL, glass stoppered
- Graduated cylinder, 25 mL
- Graduated cylinder, 100 mL
- Mechanical pipettor, 1000 µL, with plastic tips (or 1 mL volumetric pipette)
- Side-arm flask
- Volumetric pipette, 10 mL
- Volumetric pipette, 20 mL

22.3.7 Equipment

- Analytical balance
- Hot plate

22.3.8 Procedure

(Instructions are given for analysis in duplicate.)

1. Melt any samples that are solid at room temperature by heating to a maximum of 15 °C above the melting point. Filter melted fat sample and oil sample through filter paper to remove impurities.
2. Weigh accurately 0.1–0.5 g sample (amount used depends on expected iodine number) into each of two dry 500 mL glass-stoppered flasks. Add 10 mL chloroform to dissolve the fat or oil.
3. Prepare two blanks by adding only 10 mL chloroform to 500 mL glass-stoppered flasks.
4. Pipette 25 mL Wijs iodine solution into the flasks. (The amount of iodine must be 50–60 % in excess of that absorbed by the fat.)
5. Let flasks stand for 30 min in the dark with occasional shaking.
6. After incubation in the dark, add 20 mL potassium iodide solution to each flask. Shake thoroughly. Add 100 mL freshly boiled and cooled water, washing down any free iodine on the stopper.
7. Titrate the iodine in the flasks with standard sodium thiosulfate, adding it gradually with constant and vigorous shaking until the yellow color almost disappears. Then add 1–2 mL of starch indicator and continue the titration until the blue color entirely disappears. Toward the end of the titration, stopper the flask and shake violently so that any iodine remaining in the chloroform can be taken up by the potassium iodide solution. Record the volume of titrant used.

22.3.9 Data and Calculations

Sample	*Weight (g)*	*Titrant volume (mL)*	*Iodine value*
1			
2			
			$\bar{X}$ =

Oil/fat sample type tested:

Blank titration (mL):

Sample 1 =
Sample 2 =
$\bar{X}$ =

Calculate the iodine value of each sample as follows:

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

where:

Iodine value = g iodine absorbed per 100 g of sample
B = volume of titrant (mL) for blank
S = volume of titrant (mL) for sample
N = normality of $Na_2S_2O_3$ (mol/1000 mL)
126.9 = MW of iodine (g/mol)
W = sample mass (g)

22.3.10 Questions

1. In the iodine value determination, why is the blank volume higher than that of the sample?
2. What does a high versus a low iodine value tell you about the nature of the sample?

22.4 FREE FATTY ACID VALUE

22.4.1 Objective

Determine the free fatty acid (FFA) value of fats and oils.

22.4.2 Principle of Method

Free fatty acid value, or acid value, reflects the amount of fatty acids hydrolyzed from triacylglycerols. Free fatty acid is the percentage by weight of a specific fatty acid. Acid value is defined as the milligrams of potassium hydroxide needed to neutralize the free acids present in 1 g of fat or oil. A liquid fat sample combined with neutralized 95% ethanol is titrated with standardized sodium hydroxide to a phenolphthalein endpoint. The volume and normality of the sodium hydroxide are used, along with the weight of the sample, to calculate the free fatty acid value.

22.4.3 Chemicals

	CAS no.	*Hazards*
Ethanol	64-17-5	Highly flammable
Phenolphthalein	77-09-8	Irritant
Sodium hydroxide (NaOH)	1310-73-2	Corrosive

22.4.4 Reagents

(**It is recommended that these solutions be prepared by the laboratory assistant before class.)

- Ethanol, neutralized
  Neutralize 95% ethanol to a permanent pink color with alkali and phenolphthalein.
- Phenolphthalein indicator**
  In a 100 mL volumetric flask, dissolve 1 g phenolphthalein in 50 mL 95% ethanol. Dilute to volume with dd water.
- Sodium hydroxide, 0.1 *N*, standardized **
  Use commercial product, or prepare as described in other laboratory manual chapters (Chaps. 2 and 21).

22.4.5 Hazards, Precautions, and Waste Disposal

Adhere to normal laboratory safety procedures. Wear safety glasses at all times. Wastes likely may be put down the drain using a water rinse, but follow good laboratory practices outlined by environmental health and safety protocols at your institution.

22.4.6 Supplies

(Used by students)

- Beaker, 250 mL (to melt fat)
- Buchner funnel (to fit side-arm flask)
- Buret, 10 mL
- 4 Erlenmeyer flasks, 250 mL
- Fat and/or oil samples
- Filter paper (to fit Buchner funnel, to filter melted fat and oil)
- Graduated cylinder, 100 mL
- Mechanical pipettor, 1000 μL, with plastic tips (or 1 mL volumetric pipette)
- Side-arm flask

22.4.7 Equipment

- Analytical balance
- Hot plate

22.4.8 Procedure

(Instructions are given for analysis in triplicate.)

1. Melt any samples that are solid at room temperature by heating to a maximum of 15 °C above the melting point. Filter melted fat sample and oil sample through filter paper to remove impurities.
2. As a preliminary test, accurately weigh ca. 5 g melted fat or oil into a 250 mL Erlenmeyer flask.
3. Add ca. 100 mL neutralized ethanol and 2 mL phenolphthalein indicator.

4. Shake to dissolve the mixture completely. Titrate with standard base (ca. 0.1 *N* NaOH), shaking vigorously until the endpoint is reached. This is indicated by a slight pink color that persists for 30 s. Record the volume of titrant used. Use the information below to determine if the sample weight you have used is correct for the range of acid values under which your sample falls. This will determine the sample weight to be used for Step 5.

The *Official Methods and Recommended Practices of the AOCS* (AOCS 2009) recommends the following sample weights for ranges of expected acid values:

FFA range (%)	*Sample (g)*	*Alcohol (mL)*	*Strength of alkali*
0.00–0.2	56.4 ± 0.2	50	0.1 *N*
0.2–1.0	28.2 ± 0.2	50	0.1 *N*
1.0–30.0	7.05 ± 0.05	75	0.25 *N*

5. Repeat Steps 1–3 more carefully in triplicate, recording each weight of the sample and the volume of titrant.

22.4.9 Data and Calculations

Sample	*Weight (g)*	*Titrant volume (mL)*	*FFA value*
1			
2			
3			
			$\bar{X}$ =
			SD =

Oil/fat sample type tested:

Calculate the FFA value of each sample as follows:

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

where:

% FFA = percent free fatty acid (g/100 g), expressed as oleic acid
V = volume of NaOH titrant (mL)
N = normality of NaOH titrant (mol/1000 mL)
282 = MW of oleic acid (g/mol)
W = sample mass (g)

22.4.10 Questions

1. What is a high FFA value indicative of relative to product history?
2. Why is the FFA content of frying oil important?
3. In a crude fat extract, FFA are naturally present, but they are removed during processing to enhance the stability of the fat. State and describe the processing step that removes the FFA naturally present.

22.5 PEROXIDE VALUE

22.5.1 Objective

Determine the peroxide value of fats and oils, as an indicator of oxidative rancidity.

22.5.2 Principle of Method

Peroxide value is defined as the milliequivalents of peroxide per kilogram of fat, as determined in a titration procedure to measure the amount of peroxide or hydroperoxide groups. To a known amount of fat or oil, excess potassium iodide is added, which reacts with the peroxides in the sample. The iodine liberated is titrated with standardized sodium thiosulfate using a starch indicator. The calculated amount of potassium iodide required to react with the peroxide present is used to determine the peroxide value.

22.5.3 Chemicals

	CAS no.	*Hazards*
Acetic acid (glacial)	64-19-7	Corrosive
Chloroform	67-66-3	Harmful
Hydrochloric acid (HCl)	7647-01-0	Corrosive
Potassium chromate ($K_2Cr_2O_7$)	7789-00-6	Toxic, dangerous for environment
Potassium iodide (KI)	7681-11-0	
Sodium thiosulfate	7772-98-7	
Soluble starch	9005-25-8	

22.5.4 Reagents

(**It is recommended that these solutions be prepared by the laboratory assistant before class.)

- Acetic acid-chloroform solution
  Mix three volumes of concentrated acetic acid with two volumes of chloroform.
- Potassium iodide solution, saturated **
  Dissolve excess KI in freshly boiled dd water. Excess solid must remain. Store in the dark. Test before use by adding 0.5 mL acetic acid-chloroform solution, and then add 2 drops 1% starch indicator solution. If solution turns blue, requiring >1 drop 0.1 *N* thiosulfate solution to discharge color, prepare a fresh potassium iodide solution.
- Sodium thiosulfate, 0.1 *N*, standard solution (AOAC Method 942.27) **
  Dissolve ca. 25 g sodium thiosulfate pentahydrate in 1 L dd water. Boil gently for 5 min.

Transfer while hot to a storage bottle (make sure bottle has been well cleaned and is heat resistant). Store solution in a dark, cool place. Use the following procedure to standardize the sodium thiosulfate solution: Accurately weigh 0.20–0.23 g potassium chromate ($K_2Cr_2O_7$) (previously dried for 2 h at 100 °C) into a glass-stoppered flask. Dissolve 2 g potassium iodide (KI) in 80 mL chlorine-free water. Add this water to the potassium chromate. To this solution, add, with swirling, 20 mL ca. 1 *M* HCl, and immediately place in the dark for 10 min. Titrate a known volume of this solution with the sodium thiosulfate solution, adding starch solution after most of the iodine has been consumed.

- Starch indicator solution, 1 % (prepare fresh daily)**
  Mix ca. 1 g soluble starch with enough cold dd water to make a thin paste. Add 100 mL boiling dd water. Boil ca. 1 min while stirring.

22.5.5 Hazards, Precautions, and Waste Disposal

Potassium chromate is toxic and must be handled with caution. Use hydrochloric acid in a fume hood. Otherwise, adhere to normal laboratory safety procedures. Wear gloves and safety glasses at all times. Chloroform and potassium chromate must be handled as hazardous wastes. Other wastes likely may be put down the drain using a water rinse, but follow good laboratory practices outlined by environmental health and safety protocols at your institution.

22.5.6 Supplies

(Used by students)

- Beaker, 250 mL (to melt fat)
- Buchner funnel (to fit side-arm flask)
- Buret, 25 mL or 50 mL
- 4 Erlenmeyer flasks, 250 mL, glass stoppered
- Fat and/or oil samples
- Filter paper (to fit Buchner funnel, to filter melted fat and oil)
- 2 Graduated cylinders, 50 mL
- Mechanical pipettor, 1000 μL, with plastic tips (or 1 mL volumetric pipette)
- Side-arm flask

22.5.7 Equipment

- Analytical balance
- Hot plate

22.5.8 Procedure

(Instructions are given for analysis in duplicate.)

1. Melt any samples that are solid at room temperature by heating to a maximum of 15 °C above the melting point. Filter melted fat sample and oil sample through filter paper to remove impurities.
2. Accurately weigh ca. 5 g fat or oil (to the nearest 0.001 g) into each of two 250 mL glass-stoppered Erlenmeyer flasks.
3. Add 30 mL acetic acid-chloroform solution and swirl to dissolve.
4. Add 0.5 mL saturated KI solution. Let stand with occasional shaking for 1 min. Add 30 mL dd water.
5. Slowly titrate samples with 0.1 *N* sodium thiosulfate solution, with vigorous shaking until yellow color is almost gone.
6. Add ca. 0.5 mL 1 % starch solution, and continue titration, shaking vigorously to release all iodine from chloroform layer, until blue color just disappears. Record the volume of titrant used. (If <0.5 mL of the sodium thiosulfate solution is used, repeat determination.)
7. Prepare (omitting only the oil) and titrate a blank sample. Record the volume of titrant used.

22.5.9 Data and Calculations

Sample	*Weight (g)*	*Titrant volume (mL)*	*Peroxide value*
1			
2			
			$\bar{X}$ =

Oil/fat sample type tested:

Blank titration (mL):

Sample 1 =
Sample 2 =
$\bar{X}$ =

Calculate the peroxide value of each sample as follows:

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

where:

Peroxide value = mEq peroxide per kg of sample
S = volume of titrant (mL) for sample
B = volume of titrant (mL) for blank
N = normality of $Na_2S_2O_3$ solution (mEq/mL)
1000 = conversion of units (g/kg)
W = sample mass (g)

22.5.10 Questions

1. What are some cautions in using peroxide value to estimate the amount of autoxidation in foods?

2. The peroxide value method was developed for fat or oil samples. What must be done to a food sample before measuring its peroxide value using this method?

22.6 THIN-LAYER CHROMATOGRAPHY SEPARATION OF SIMPLE LIPIDS

22.6.1 Objective

Separate and identify the lipids in some common foods using thin-layer chromatography (TLC).

22.6.2 Principle of Method

Like all types of chromatography, TLC is a separation technique that allows for the distribution of compounds between a mobile phase and a stationary phase. Most classes of lipids can be separated from each other by adsorption chromatography on thin layers. In TLC, a thin layer of stationary phase is bound to an inert support (i.e., glass plate, plastic, or aluminum sheet). The sample and standards are applied as spots near one end of the plate. For ascending chromatography, the plate is placed in a developing chamber, with the end of the plate nearest the spots being placed in the mobile phase at the bottom of the chamber. The mobile phase migrates up the plate by capillary action, carrying and separating the sample components. The separated bands can be visualized or detected and compared to the separation of standard compounds.

22.6.3 Chemicals

	CAS no.	*Hazards*
Acetic acid	64-19-7	Corrosive
Diethyl ether	60-29-7	Harmful, extremely flammable
Hexane	110-54-3	Harmful, highly flammable, dangerous for the environment
Sulfuric acid	7664-93-9	Corrosive

22.6.4 Reagents

- Chloroform/methanol, 2:1, v/v
- Mobile phase
  Hexane/diethyl ether/acetic acid, 78:20:2
- Standards
  Triacylglycerol, fatty acid, cholesteryl ester, and cholesterol
- Sulfuric acid solution
  Concentrated H_2SO_4, in 50 % aqueous solution

22.6.5 Hazards, Precautions, and Waste Disposal

Use acetic acid and sulfuric acid in a fume hood. Diethyl ether is extremely flammable, is hygroscopic, and may form explosive peroxides. Otherwise, adhere to normal laboratory safety procedures. Wear safety glasses at all times. Diethyl ether and hexane must be handled as hazardous wastes. Other wastes likely may be put down the drain using a water rinse, but follow good laboratory practices outlined by environmental health and safety protocols at your institution.

22.6.6 Supplies

- Capillary tubes (or syringes) (to apply samples to plates)
- Developing tank, with lid
- Filter paper, Whatman No. 1 (to line developing tank)
- Oil/fat food samples (e.g., hamburger, safflower oil) (prepare at a concentration of 20 μg/mL in 2:1 v/v chloroform-methanol solution)
- Pencil
- Thin-layer chromatography plates: Silica Gel 60, 0.25 mm thick coating on glass backing, 20 × 20 cm (EM Science)

22.6.7 Equipment

- Air blower (e.g., blow hair dryer)
- Oven

22.6.8 Procedure

Preparation of Silica Gel Plates

1. Place plates in oven at 110 °C for 15 min, then cool to ambient temperature (5 min).
2. With a pencil, draw a line to mark the origin, 2.5 cm from the bottom of the plate.
3. Make marks with a pencil to divide the plate into 10 "lanes" of equal width.
4. Use capillary tubes or syringes to apply approximately 10 μl of each standard and sample to a separate lane (use the middle eight lanes). The application should be done as a streak across the center of the lane origin. This is best accomplished with four spots of 2.5 μl each.
5. Below the origin line, write the identity of the sample/standard in each lane.
6. Allow spots to dry. You may accelerate drying by using a low-temperature air blower.
7. Write your name in the top right corner of the plate.

Development of Plates

1. Line the developing tank with Whatman no. 1 or similar filter paper.
2. Pour the mobile phase gently over the filter paper until the depth of solvent in the tank is approximately 0.5 cm. About 200 mL is required.
3. Place the lid on the tank and allow 15 min for the atmosphere in the tank to become saturated with solvent vapor.
4. Place the spotted TLC plate in the developing tank and allow it to develop until the solvent front reaches a point about 2 cm from the top of the plate.
5. Remove the plate from the tank and *immediately* mark the position of the solvent front. Evaporate the solvent in the fume hood.

Visualization of Lipids

1. In a well-ventilated fume hood, spray lightly with 50 % aqueous H_2SO_4. Allow to dry.
2. Heat plate for 5–10 min at 100–120 °C. Remove from oven, cool, and inspect. Handle the plate with caution as the surface still contains sulfuric acid.
3. Mark all visible spots at their center, and note the color of the spots.

22.6.9 Data and Calculations

For the spots of each of the standards and the samples, report the distance from the origin for the spot. Also for each spot, calculate the R_f value, as the distance from the origin to the spot divided by the distance from the origin to the solvent front. Using the R_f value of the standards, identify as many of the spots (bands) in the samples as possible.

Standard	*Distance from origin*	*R_f value*
Triacylglycerol		
Fatty acid		
Cholesteryl ester		
Cholesterol		

Sample spot number	*Distance from origin*	*R_f value*	*Identity*

Oil/fat sample type tested:

22.6.10 Questions

1. Explain the chemical structure of an ester of cholesterol.
2. Besides the four fat constituents used as standards, what other fat constituents might be found using a TLC method such as this?

Acknowledgments This laboratory exercise was developed with input from Dr Arun Kilara with Arun Kilara Worldwide, Northbrook, IL.

RESOURCE MATERIALS

AOCS (2009) Official methods and recommended practices of the AOCS, 6th edn. American Oil Chemists' Society, Champaign, IL

Pike OA, O'Keefe SF (2017) Fat characterization. Ch. 23. In: Nielsen SS (ed) Food analysis, 5th edn. Springer, New York