PRACTICAL WORK OF FOOD BIOCHEMISTRY

3rd year student of Food Biotechnology

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Contents

General presentation and objectives

This booklet is intended for third-year undergraduate students majoring in Food Biotechnology, within the Department of Biotechnology, Faculty of Natural and Life Sciences, at Ferhat Abbas University Sétif 1. It is structured into ten main practical work. By the end of this series of practical works, students will be able to:

- ✓ Apply the fundamental rules of good laboratory practice, including safety, hygiene, and accurate scientific reporting.
- ✓ Determine the moisture content and dry matter of food products using gravimetric analysis.
- ✓ Quantify food proteins using spectrophotometric and colorimetric methods.
- ✓ Isolate and characterize proteins from food matrices through precipitation techniques.
- ✓ Extract and measure total lipid content using standardized solvent extraction methods.
- ✓ Determine total and reducing sugar content in food products using colorimetric and titrimetric approaches.
- ✓ Identify and separate individual sugars by thin-layer chromatography.
- ✓ Assess the oxidative and hydrolytic quality of lipids through the determination of peroxide value and fat acidity.
- ✓ Critically interpret analytical results in relation to food quality standards and nutritional composition.
- ✓ Develop technical proficiency, precision, and autonomy in a food analysis laboratory environment.

Practical work 0: Introduction to Good Laboratory Practice

1. Objectives

At the end of this practical session, students should be able to:

- Understand the principles of Good Laboratory Practice (GLP)
- Identify laboratory hazards and apply safety rules
- Use basic laboratory equipment correctly
- Adopt proper scientific behavior (organization, traceability, hygiene)
- Record experimental data accurately in a lab notebook

2. Introduction

Working in a biotechnology or biochemistry laboratory requires strict rules to ensure:

- **Safety of people** (students, teachers, technicians).
- **Reliability of experimental results**.
- **Preservation of equipment and facilities**.

The aim of this first practical work is to introduce students to basic safety rules and the proper use of laboratory glassware.

3. General Safety Rules

- ✓ Wear a **lab coat** (cotton, buttoned, and clean).
- ✓ Wear **gloves** and **safety goggles** when handling chemicals or biological samples.
- ✓ Tie back **long hair** and avoid dangerous accessories (scarves, dangling jewelry).
- ✓ Never **eat, drink, or smoke** in the laboratory.
- ✓ Correctly label all **chemical reagents**.
- ✓ Handle **flammable solvents** away from flames or heat sources.
- ✓ Report immediately any **accident** or **glass breakage** to the instructor.
- ✓ Keep the working area **clean and organized**.

4. Rules for Good Experimental Conduct

- ❖ Read the **protocol** carefully before starting the experiment.
- ❖ Use the **appropriate glassware** for each manipulation.
- ❖ Avoid contamination by using **clean and dry equipment**.
- ❖ Record all observations in a **laboratory notebook**.

- ❖ Dispose of chemical and biological waste according to the **established procedures**.

5. Basic Glassware

Laboratory glassware comprises various types, each characterized by a specific function and a defined level of precision (Table 1, Figure 1).

Table 1: Laboratory glassware.

Glassware (French)	**Glassware (English)**	**Main Use**
Bécher	Beaker	Containing, heating, or mixing liquids
Éprouvette graduée	Graduated cylinder	Measuring liquid volumes
Fiole jaugée	Volumetric flask	Preparing solutions with precise concentration
Pipette graduée	Graduated pipette	Delivering a measured volume of liquid
Pipette jaugée	Volumetric pipette	Delivering one unique, precise volume
Propipette	Pipette filler	Assisting in liquid aspiration with a pipette
Burette	Burette	Performing accurate titrations
Erlenmeyer	Conical flask	Mixing liquids, carrying out titrations
Entonnoir	Funnel	Pouring liquids, filtering with paper
Tube à essai	Test tube	Small reactions, heating small volumes
Support élévateur	Laboratory jack	Supporting and adjusting the height of containers
Ballon rond	Round-bottom flask	Even heating, distillations
Ballon jaugé	Volumetric flask	Preparing standard solutions
Verrerie de montre	Watch glass	Evaporation, covering beakers

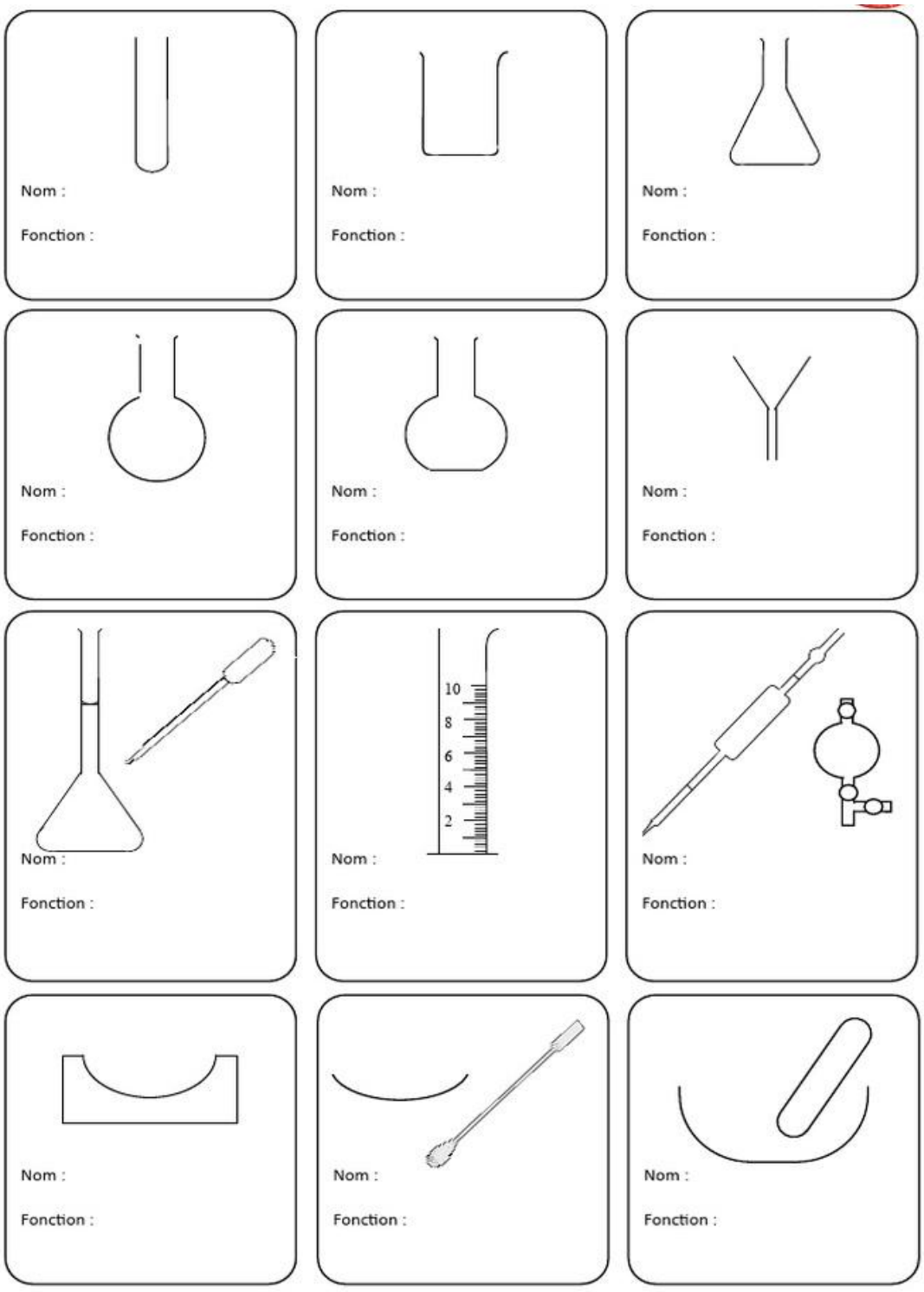

Figure 1: Laboratory glassware illustration.

Practical Work 1: Determination of moisture content and dry matter of flour

Objective

The objective of this session is to determine the moisture content (%MC) and the dry matter content (%DM) of a flour sample by desiccation, according to the protocol described below.

Materials and Reagents

- Flour
- Petri dish with lid
- Analytical balance (precision 0.001 g)
- Ventilated oven adjustable to 103±2°C
- Desiccator
- Tongs
- Timer

Experimental Protocol

1. **Preparation of the Petri Dish:**
    - Tare the Petri dish with its lid. Place the dish in the oven, with the lid removed, and dry at 103±2°C for 15 minutes.
    - Allow it to cool in the desiccator for 5 minutes.
    - Weigh the cooled dish with its lid and record this mass as m1 (mass of the empty dish and lid).
2. **Sample Weighing**
    - Place 5 g of flour into the Petri dish, spreading it evenly over the bottom.
    - Close the dish with its lid, then weigh the whole assembly (dish + lid + sample). Record this mass as m2.
3. **Drying in the Oven**
    - Place the dish containing the sample, with the lid removed, in the oven set at 103±2°C for 1 hour.
    - After 1 hour, quickly replace the lid and allow it to cool in the desiccator to room temperature.
    - Weigh the cooled assembly (dish + dry matter) and record the mass as m3.

4. **Repeated weighing sessions**
    - If the difference between two successive weighings is greater than 5 mg, return the dish to the oven (lid removed) for an additional half-hour.
    - Repeat this step until the difference between two successive weighings is less than or equal to 5 mg.

Calculations

1. **Moisture Content (% MC)**

$$\%MC = \frac{extracted\ water\ weight}{initial\ mass\ of\ sample} \times 100$$

2. **Dry Matter Content (% DM)**

$$\%DM = 100 - \%MC$$

Replicates

The experiment is repeated three times to ensure the reproducibility of the results.

Expected Results

- Initial mass : ... g
- Mass after drying: ... g
- extracted water weight: ... g
- % Moisture Content (MC): ... %
- % Dry Matter (DM): ... %

Conclusion

The results obtained will allow for the determination of the moisture and dry matter content of the flour sample studied, which are essential parameters for assessing the quality of food products.

Safety Instructions

- Handle hot objects (dishes, samples) with tongs to avoid burns.
- Ensure the oven is well-ventilated for the entire duration of the experiment.

Practical Work 2: Determination of milk proteins by the Bradford method

Objective

The objective of this practical work is to quantify the proteins present in a milk sample using the Bradford method, which is based on the binding of proteins with a specific dye.

1. Introduction

Protein determination by the Bradford method is based on the interaction between the Coomassie dye Brilliant Blue G-250 and proteins. This dye changes color when it binds to the aromatic amino acids in proteins, turning from reddish-brown to blue. The intensity of the blue color is proportional to the protein concentration in the sample and can be measured at a wavelength of 595 nm.

2. Materials and reagents

- Bradford reagent solution
- Milk samples (skim or whole)
- Standard protein bovine serum albumin 0.1% (BSA)
- Distilled water
- Test tubes
- Pipettes
- Spectrophotometer set at 595 nm
- Micropipettes and cones
- Sodium phosphate buffer (0.2M, pH 5.2)

3. Experimental protocol

3.1 Protocol for the preparation of Bradford reagent

1. Coomassie Brilliant Blue G-250: 100 mg
2. Ethanol (95%): 50 mL
3. Phosphoric acid or orthophosphoric acid : 100 mL
4. Distilled water: top up to 1 L

3.1.1 Preparation steps:

1. **Dissolving the dye**

Dissolve 100 mg of Coomassie Brilliant Blue G-250 in 50 mL of ethanol. Shake well until completely dissolved.

2. **Adding phosphoric acid**

Add 100 mL of concentrated phosphoric acid (85%) to the previous solution. Mix thoroughly.

3. **Dilution to 1 L**

Complete the volume of the solution with distilled water to reach 1 L. Mix the solution well.

4. **Filtration**

Filter the solution through a paper filter to remove undissolved particles. This will result in a clear reagent.

5. **Storage**

Store Bradford's reagent solution at room temperature in an amber bottle to protect it from light. The solution is stable for several weeks at room temperature and several months in the refrigerator.

3.2 Preparation of protein standards (standard curve)

1. Prepare a standard range of BSA at known concentrations (table 2):

Table 2: Standard curve of BSA and milk sample preparation

Tube number	1	2	3	4	5	6	X
BSA (µL)	0	20	40	60	80	100	100 µL of the
Buffer (µL)	100	80	60	40	20	0	diluted milk
Bradford reagent (mL)	5	5	5	5	5	5	5
Amount of protein (µg/ mL)	……..	……..	……..	……..	……..	……..	**……..**
Abs 595 nm	……..	……..	……..	……..	……..	……..	**……..**

2. In test tubes, add 100 µL of each BSA standard solution.
3. Add 5 mL of Bradford reagent to each tube containing BSA.
4. Mix gently by vortexing .
5. Incubate at room temperature for 5 to 10 minutes.
6. Measure the absorbance at 595 nm using the spectrophotometer.

7. Plot the calibration curve by plotting the absorbance as a function of the protein concentration (in µg/ mL).

3.2 Preparation of milk samples

1. Dilute the milk 1:100 in a volumetric flask (dilute 1 mL of milk to 100 mL with water).
2. Collect 100 µL of the diluted milk sample into a test tube.
3. Add 5 mL of Bradford reagent.
4. Mix gently by vortexing .
5. Incubate at room temperature for 5 to 10 minutes.
6. Measure the absorbance at 595 nm.

4. Calculation of protein concentration

1. Plot the absorbance of the milk sample on the calibration curve to determine the protein concentration.
2. Multiply the obtained concentration by the dilution factor (100) to calculate the actual protein concentration in the milk sample.

5. Expected results

Results should show a linear increase in absorbance with protein concentration for BSA standards. Diluted milk samples should give an absorbance value that allows the protein concentration in the milk to be deduced.

6. Discussion

- Compare the results obtained with the theoretical protein concentration values for milk (approximately 3.2 g/100 mL for whole milk).
- Discuss possible sources of error (dilutions, interference with other compounds, etc.).

Practical Work 3: Isolation of casein from milk by precipitation with acetic acid

Objectives

1. Learn how to isolate casein from milk.
2. Understand the role of pH modification and the use of organic solvents such as acetone.
3. Explore the precipitation properties of proteins under specific conditions.

Material and reagents

Material

- Beakers (100 mL and 250 mL)
- Glass stirring rod
- Funnel
- Filter paper or Büchner filter
- pH meter
- Magnetic stirrer
- Analytical balance
- Pipettes

Reagents

- Whole milk (200 mL)
- Acetic acid 10% (v/v)
- Acetone (pure)
- Distilled water

Experimental protocol

1. **Milk preparation**

Gently heat 50 mL of milk to 40°C in a beaker, stirring to prevent the formation of a surface film.

2. **Acidification**

Slowly add 10% acetic acid to the warm milk while stirring continuously.

Monitor the pH with the pH meter until reaching **pH 4.6** (isoelectric point of casein).

Once the pH is reached, observe the formation of a precipitate (casein).

3. **Filtration**

Let stand for 5 to 10 minutes to facilitate separation of the precipitate.

Vacuum filter the mixture using filter paper or a Büchner filter to collect the solid precipitate.

Keep the precipitate and discard the filtrate (whey).

4. **Washing**

Transfer the precipitate to a clean beaker.

Add 50 mL of distilled water and shake gently to remove impurities.

Filter again.

5. **Acetone treatment**

Add 10 mL of acetone to the precipitate to dehydrate and purify the casein.

Mix well, then filter immediately to recover the casein.

6. **Drying**

Leave the resulting precipitate to dry in the open air or in an oven at low temperature (30-40°C).

Observations

- Note the physical changes during the experiment (precipitation, color, texture).
- Describe the appearance of isolated casein after drying.

Calculations

Casein yield

$$Yeild\ (\%) = \frac{Masse\ of\ isolated\ casein\ (g)}{Initial\ mass\ of\ milk\ (g)} \times 100$$

Questions

1. Why does casein precipitate at pH 4.6?
2. What is the role of acetone in this procedure?
3. How could the method be modified to isolate other dairy proteins?

Safety and precautions

- Handle acetone and acetic acid with care.
- Work under a fume hood to avoid inhaling acetone fumes.
- Wear gloves, safety glasses and a lab coat.

Expected conclusion

Students will have isolated casein in solid form, understood the role of pH in protein precipitation, and learned the importance of organic solvents in protein purification.

Practical Work 4: Extraction of lipids from milk powder using the Soxhlet technic

Objectives

1. Soxhlet extraction technique.
2. Soxhlet apparatus for lipid extraction.
3. Quantify the lipid content of milk powder.
4. Analyze the results to interpret the effectiveness of the method.

Materials and reagents

Material

- Soxhlet extractor
- Round bottom flask (250 mL)
- Condenser
- Water heater
- Filter paper or extraction cartridge
- Accurate scale (to within 0.001 g)
- Mortar and pestle (to homogenize the sample if necessary)
- Pipette and graduated cylinder

Reagents

- Solvent: Hexane or petroleum ether (according to laboratory safety instructions)
- Milk powder (taken in sufficient quantity for analysis)

Protocol

Sample preparation

Weigh exactly 5 g of previously homogenized milk powder.

Insert the sample into an extraction cartridge or wrap it in compatible filter paper.

Assembling the Soxhlet apparatus

Fill the round-bottomed flask with approximately 150 mL of solvent.

Connect the flask, the Soxhlet extractor and the condenser, ensuring that the joints are airtight.

Install the assembly on the water heater (figure 2).

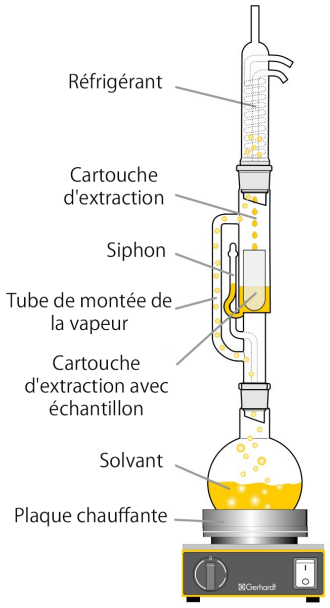

Figure 2: Soxhlet assembly

Extraction

Heat the system to gently boil the solvent.

Make sure the solvent condenses and falls back onto the sample.

Allow the extraction to continue for 6 to 8 cycles (approximately 3 hours.

Lipid recovery

Once the extraction is complete, collect the solvent containing the lipids in the flask.

Evaporate the solvent using a rotary evaporator or by moderate heating under a fume hood.

Lipid weighing

Weigh the flask containing the lipids after complete evaporation of the solvent.

Calculate the amount of lipids extracted by mass difference before and after extraction.

Calculations

$$\text{lipid yeild (\%)} = \frac{Mass\ of\ the\ extacted\ oil}{Mass\ of\ intial\ sample} \times 100$$

Expected results

Students should obtain a measurable amount of lipids, typically between 20% and 30% of the dry mass for milk powder.

Discussion Questions

How does the nature of the solvent influence the extraction yield?

Practical Work 5: Determination of total sugars in honey by the phenol–sulfuric acid method

1. Objective

To determine the total sugar content in honey using the Phenol–Sulfuric Acid Method.

2. Principle

In the Phenol–Sulfuric Acid Method:

- Carbohydrates are dehydrated by concentrated Sulfuric acid to form furfural derivatives
- These react with Phenol to produce a yellow-orange complex
- The absorbance is measured at 490 nm
- The intensity is proportional to total sugar concentration

3. Materials and Reagents

Equipment

- Spectrophotometer (490 nm)
- Test tubes
- Micropipettes
- Vortex mixer

Reagents

- 5% Phenol solution
- Concentrated Sulfuric acid
- Standard sugar solution (preferably Glucose)
- Distilled water

4. Sample Preparation (Honey)

Honey is rich in sugars (mainly glucose and fructose), so dilution is essential:

1. Weigh 1 g of honey
2. Dissolve in 100 mL distilled water (stock solution)
3. Further dilute (e.g., 1:10) to fit calibration range

5. Preparation of Calibration Curve

Prepare standard solutions of Glucose (0–100 µg/mL) (table 3):

Table 3: standard solutions of Glucose

Tube	Glucose (mL)	Distilled Water (mL)	Glucose concentration	Absorbance à 490 nm
0	0	1		
1	0.2	0.8		
2	0.4	0.6		
3	0.6	0.4		
4	0.8	0.2		
5	1.0	0		

6. Experimental Procedure

For **standards and samples**

1. Pipette 1 mL of solution into a test tube
2. Add 1 mL of 5% phenol
3. Rapidly add 5 mL concentrated Sulfuric acid
4. Mix immediately
5. Allow to stand for 10 minutes at room temperature
6. Measure absorbance at 490 nm

7. Results and Calculations

1. Plot calibration curve (Absorbance vs concentration)
2. Determine linear equation:

$$ABS = aC + b$$

3. Calculate sample concentration:

$$C = \frac{ABS - b}{a}$$

4. Apply dilution factor and express results:

$$\text{Total sugars (g/100 g honey)} = \frac{C \times V \times DF}{m}$$

Where:

- C = concentration from curve (mg/mL)
- V = total volume (mL)
- DF = dilution factor
- m = mass of honey (g)

8. Expected Results

- Honey contains **~70–80% sugars**
- Major sugars:
    - Glucose (~30–35%)
    - Fructose (~35–40%)

9. Discussion Questions

1. Why is dilution necessary for honey analysis?
2. Why is Phenol used in this method?
3. What types of sugars are detected by this method?
4. What are the limitations of this assay?

10. Conclusion

The Phenol–Sulfuric Acid Method is a **rapid and sensitive method** for determining total sugars in honey, suitable for routine food analysis.

Practical Work 6: Determination of reducing sugars by the luff–schoorl method

1. Introduction and objectives

Reducing sugars such as glucose, fructose, lactose, and maltose are characterized by the presence of a free carbonyl group capable of acting as a reducing agent. The Luff–Schoorl method is a classical analytical technique based on redox reactions, widely used in food analysis for the quantification of reducing sugars in products such as juices, milk, honey, and cereals.

This method remains important in teaching laboratories as it introduces students to indirect titration (iodometry) and fundamental analytical chemistry concepts.

2. Principle

- Reducing sugars react with copper (II) ions (Cu^{2+}) in alkaline medium, reducing them to copper(I) (Cu^{+}).
- The excess (unreacted) Cu^{2+} is treated with potassium iodide (KI), leading to the release of iodine (I_2).
- The liberated iodine is titrated with sodium thiosulfate ($Na_2S_2O_3$).

* The volume of thiosulfate used is inversely proportional to the concentration of reducing sugars.

3. Reagents

Luff–Schoorl reagent

Potassium iodide solution (KI, 30%)

Sulfuric acid (H_2SO_4)

Sodium thiosulfate solution ($Na_2S_2O_3$, 0.1 N)

Starch solution (indicator)

Standard glucose solution

Distilled water

4. Apparatus

Burette

Volumetric pipettes

Erlenmeyer flasks

Reflux flask or boiling setup

Hot plate or heating mantle

Analytical balance

5. Experimental Procedure

5.1 Sample Preparation

Dilute the food sample if necessary

Filter to remove suspended particles

5.2 Reaction with Luff–Schoorl Reagent

Pipette 25 mL of Luff reagent into an Erlenmeyer flask

Add 10 mL of the sample

Heat to boiling and maintain for 10 minutes

Cool rapidly under running water

5.3 Iodometric Titration

Add 10 mL of KI (30%)

Add 25 mL of H_2SO_4

Titrate immediately with 0.1 N $Na_2S_2O_3$

Near the endpoint, add a few drops of starch indicator

Continue titration until the blue color disappears

Record the volume of thiosulfate used

5.4 Blank Determination

Perform the same procedure using distilled water instead of the sample.

6. Calculation of Results

Let:

V_0 = volume of $Na_2S_2O_3$ for the blank

V_1 = volume for the sample

The difference ($V_0 - V_1$) is used to determine the amount of reducing sugars using Luff–Schoorl tables or calibration data.

General expression:

$$Reducing\ sugars\ (g/L) = f(V0 - V1)$$

7. Results and Interpretation

Students should:

Record titration volumes accurately

Calculate reducing sugar content

Compare results with a standard solution

Analyze possible sources of error (heating time, endpoint detection, reagent accuracy)

8. Conclusion

The Luff–Schoorl method provides a reliable indirect determination of reducing sugars and introduces key analytical concepts such as:

- Redox reactions
- Back titration (iodometry)
- Analytical precision and error sources.

Practical Work 7: Determination of honey sugar composition by Thin-Layer Chromatography (TLC)

1. **Introduction and objective**

Thin-Layer Chromatography (TLC) is a qualitative analytical technique. Its purpose is to separate the components of a mixture and to identify a compound, verify its purity, or monitor the progress of a reaction by analyzing successive samples from the reaction medium in order to demonstrate the appearance of products and/or the disappearance of reactants. The aims of this practical work is the determination of honey sugar composition by Thin-Layer Chromatography (TLC).

2. Principle of the Technique

Consider a mixture of compounds that needs to be separated. In TLC, the mixture is deposited on a porous adsorbent solid called the stationary phase, which coats an inert rigid plate. The lower part of the plate is placed in contact with a solvent called the mobile phase, which rises by capillary action (figure 3). This process is known as elution, and the mobile phase is called the eluent. During elution, the different compounds in the mixture migrate to varying heights on the plate due to competition among three phenomena:

- Adsorption of the compounds onto the stationary phase;
- Solubilization of the compounds in the eluent;
- Adsorption of the eluent onto the stationary phase, replacing the adsorbed compounds and thereby pushing them upward.

These three phenomena are governed by weak interactions such as Van der Waals forces and hydrogen bonds. To optimize separation, interactions among the three components (compound, adsorbent, and eluent) must be considered.

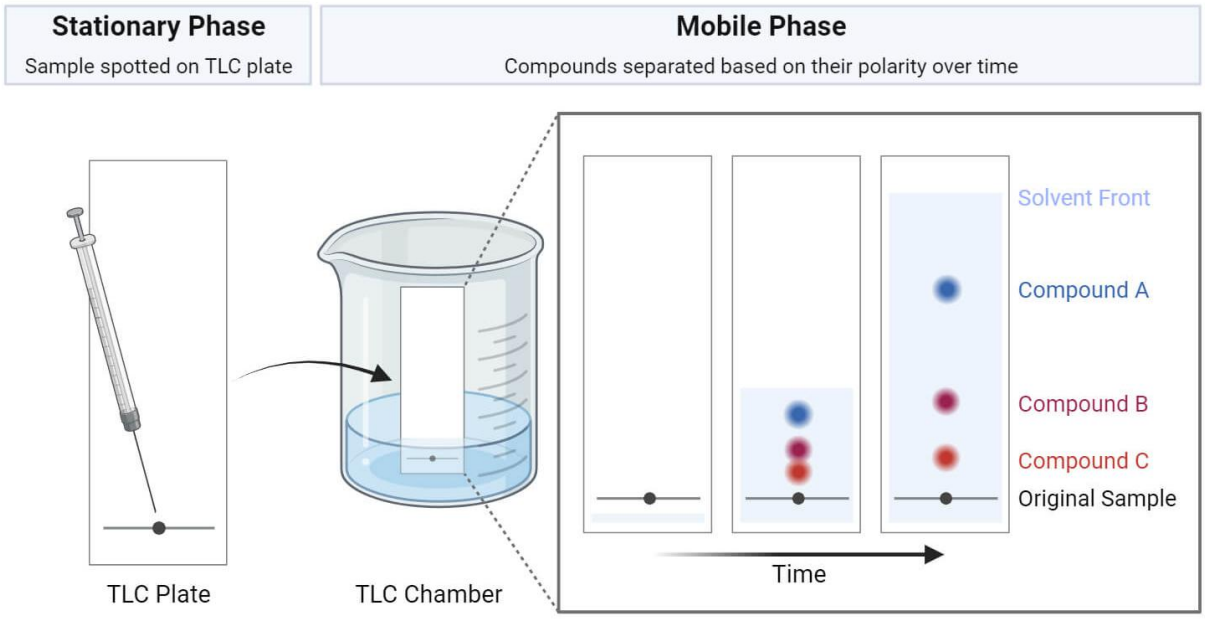

Figure 3 : Thin Layer Chromatography technic

Stationary Phase (Adsorbent)

The stationary phase is most often composed of silica gel (SiO_2) deposited on a rigid support made of glass, aluminum, or plastic. Each silica particle possesses silanol groups (Si–OH) on its surface, making silica a polar material (figure 4).

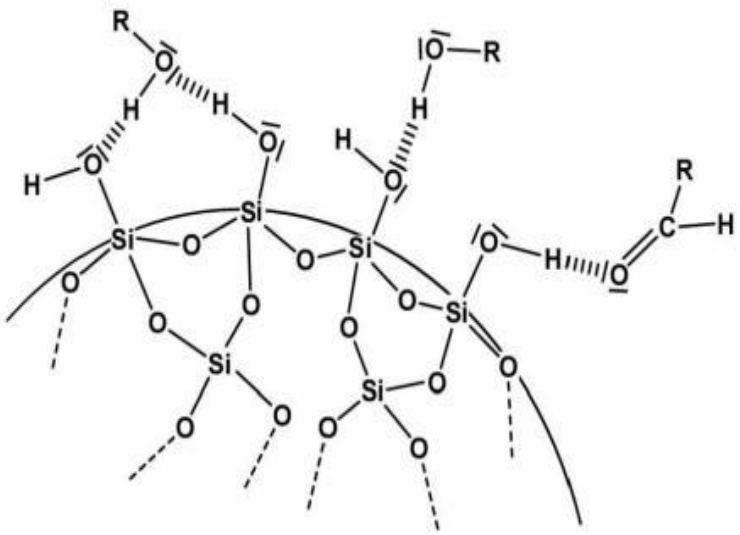

Figure 4: silica particle

Mobile Phase (Eluent)

An eluent is characterized by its polarity (figure 5). Comparing the polarity of different eluents is not always straightforward; therefore, an empirical polarity scale is generally used.

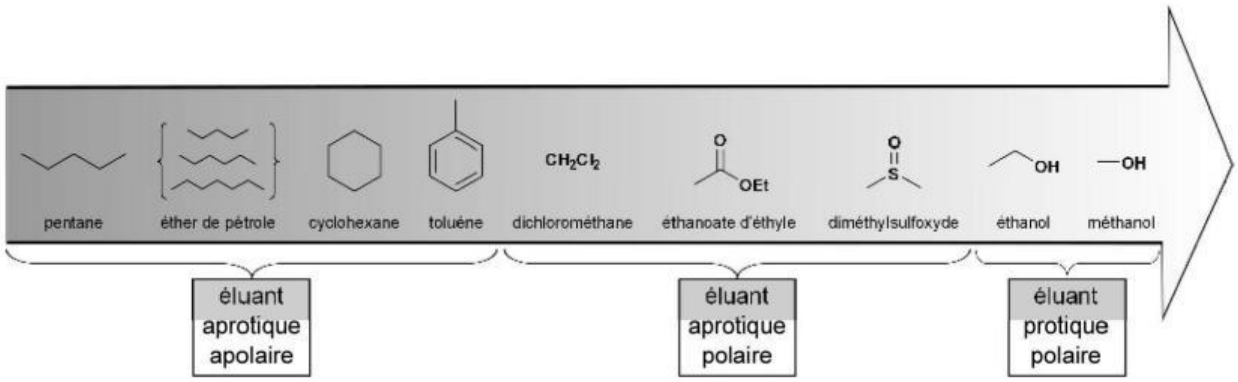

Figure 5: eluent and its polarity

Note: The eluent is often a mixture of solvents, allowing easy adjustment of its polarity by simply changing the proportions of the solvents.

4. Reagents and Equipment

4.1. Reagents Used

Solvent system 1 (v/v): Butanol, Acetone, Water (5:4:1) – 20 mL

Solvent system 2 (v/v): Methyl ethyl ketone, Acetic acid, Methanol (3:1:1) – 20 mL

Molisch reagent:

(0.25 g α-naphthol + 50 mL ethanol + 50 mL 20% H_2SO_4)

Honey

Glucose: 1 g

Fructose: 1 g

Sucrose: 1 g

Distilled water: 1 L

4.2. Equipment

TLC plate

4 TLC developing chambers or four 100 mL beakers

UV lamp

Micropipette or capillary tubes for sample application

Magnetic stirrer and stirring bar

Analytical balance, spatula, and weighing boat

Three 50 mL beakers

Five 50 mL volumetric flasks

Funnel

Hair dryer

Drying oven set at 100°C

5. Experimental Procedure

5.1. Preparation of the Eluent and Chromatographic Chamber

Use solvent system 1 (v/v): Butanol, Acetone, Water (5:4:1).

Solvent system 2 (v/v): Methyl ethyl ketone, Acetic acid, Methanol (3:1:1).

Pour the eluent into the TLC chamber or beaker to a depth of 5–8 mm.

Cover the chamber or beaker with a lid or watch glass to saturate the enclosed air with eluent vapors.

5.2. Preparation of the TLC Plate

Reactivate the plate by heating at 100°C for 30 minutes.

Lightly draw the starting line and application points approximately 2 cm from the lower edge of the plate using a graphite pencil.

5.3. Preparation of the Sample

Place 10 mL of honey into a 50 mL volumetric flask and dilute to the mark with distilled water to obtain diluted honey.

5.4. Preparation of Standard Solutions

Standard solutions of glucose, fructose, and sucrose are prepared at a concentration of 2.5 g/L.

Prepare these solutions by dissolving the sugars in distilled water in 50 mL volumetric flasks.

5.5. Application of Standards and Samples

Using a micropipette or capillary tubes, apply a very small drop of each standard solution (sucrose, glucose, and fructose) and the diluted honey sample onto the TLC plate according to the provided scheme.

Important: The spots obtained should have a diameter of approximately 3 mm. Handle the plate only by its edges to avoid damaging the silica layer.

Allow the spots to dry and repeat the application process to concentrate them, ensuring that the spots are superimposed accurately.

Perform three successive applications, allowing the spots to dry between each application.

5.6. Development of the Chromatogram

Place the silica plate vertically in the chamber or beaker and allow the eluent to migrate.

Note: Do not move the chamber during solvent migration.

Remove the plate when the solvent front is approximately 1 cm from the upper edge of the plate.

5.7. Visualization

Since the solutes are colorless, they must be visualized through a chemical reaction.

Molisch reagent is used because it produces a violet coloration upon reaction with carbohydrates.

Remove the plate and immediately mark the solvent front with a pencil.

Dry the plate, if necessary, using a hair dryer.

Under a fume hood, immerse the plate in Molisch reagent, allow it to dry, and then place it in an oven at 100°C for a few minutes.

Note: The spots may also be visualized under a UV lamp.

6. Results: Interpretation of the Chromatogram

Calculate the retention factor (Rf) for each sugar, defined as the ratio of the distance traveled by the sugar (measured from the center of the spot) to the distance traveled by the solvent front. This Rf value is characteristic for each sugar under the chromatographic conditions used.

By comparing the Rf values of the honey sample with those of the standards, identify and determine the sugar composition of the honey.

$$Rf = \frac{Distance\ traveled\ bythe\ substance\ (center\ of\ the\ spot)}{Distance\ traveled\ by\ the\ solvent\ front}$$

This practical session enables the qualitative identification of the major sugars present in honey, such as glucose, fructose, and sucrose, through comparison with reference standards.

Practical Work 8: Determination of the peroxide value of oils and fats

1. Introduction and objective

Lipids are susceptible to oxidation during processing and storage. The primary products of lipid oxidation are hydroperoxides and peroxides, which are formed by the reaction of unsaturated fatty acids with oxygen. These compounds are unstable and may further decompose into aldehydes, ketones, and other secondary oxidation products responsible for rancidity.

The peroxide value (PV) is one of the most important indicators of the oxidative state of fats and oils. It measures the amount of oxygen chemically bound in the form of peroxides and hydroperoxides and is widely used as a criterion for evaluating the freshness and quality of edible oils and fats. The aim of this practical work is to determine the peroxide value of an oil sample and assess its degree of oxidation.

3. Principle of the method

The oil sample is dissolved in a mixture of glacial acetic acid and iso-octane. In an acidic medium, the peroxides present in the sample oxidize potassium iodide (KI), releasing iodine (I_2).

The liberated iodine is then titrated with a standardized sodium thiosulfate solution in the presence of starch indicator. The amount of sodium thiosulfate consumed is proportional to the peroxide content of the sample.

The peroxide value is expressed as milliequivalents (meq) of active oxygen per kilogram of oil or fat.

4. Reagents and equipment

4.1. Reagents used

Demineralized water, boiled and cooled.

Glacial acetic acid.

Iso-octane.

Acetic acid/iso-octane mixture (60:40, v/v).

Potassium iodide (KI).

Saturated potassium iodide solution.

Sodium thiosulfate standard solution (0.01 N).

Sodium thiosulfate stock solution (0.1 N).

Starch indicator solution (1%).

Hydrochloric acid (4 mol/L).

4.2. Equipment

250 mL glass-stoppered Erlenmeyer flask.

Analytical balance (± 0.001 g).

Burette (10 or 25 mL).

Pipettes (0.5, 1, 10 and 100 mL).

Graduated cylinders.

Magnetic stirrer and magnetic bar.

Volumetric flasks.

Microwave oven (for melting solid fats, if necessary).

5. Experimental procedure

5.1. Preparation of the sample

Homogenize the oil or fat sample carefully.

Avoid exposure to air and direct sunlight.

For solid fats, gently heat the sample to approximately 10°C above its melting point.

If visible impurities are present, **filter** the sample before analysis, and must be mentioned in the report of the test.

5.2. Test portion

- Transfer **5 g** of sample into a clean, dry Erlenmeyer flask.

5.3. Dissolution of the sample

- Add **50 mL** of the acetic acid/iso-octane mixture (60:40, v/v).
- Swirl gently until the sample is completely dissolved.

  For fats with high melting points:
- Add **20 mL iso-octane** to the melted fat.
- Then immediately add **30 mL glacial acetic acid** while stirring gently.

5.4. Reaction with potassium iodide

- Add **0.5 mL of saturated potassium iodide solution**.
- Stopper the flask immediately.
- Mix using a magnetic stirrer or by manual shaking for exactly **60 seconds**, avoiding excessive aeration.

5.5. Addition of water

- Open the flask and immediately add **100 mL of demineralized water**.
- Rinse the stopper with a small amount of water.
- Mix thoroughly.

5.6. Titration

- Titrate immediately with **0.01 N sodium thiosulfate solution**.
- Continue titration until the solution changes from orange-yellow to pale yellow.
- Add **0.5 mL starch indicator solution**.
- Continue titration until the color changes from violet-blue to completely colorless.
- The endpoint is reached when the solution remains colorless for **30 seconds**.

5.7. Blank determination

- Perform a blank determination under the same experimental conditions using all reagents but without the oil sample.
- Record the volume of sodium thiosulfate consumed.

6. Results and calculations

Record the following data:

Parameter	Value
Mass of oil sample (g)	
Volume of $Na_2S_2O_3$ for sample (mL)	
Volume of $Na_2S_2O_3$ for blank (mL)	
Normality of $Na_2S_2O_3$	

The peroxide value is calculated using the following equation:

$$PV = \frac{(V - V0) \times N \times 1000}{m}$$

Where:

PV = Peroxide Value (meq O_2/kg oil)

V = Volume of sodium thiosulfate used for the sample (mL)

V_0 = Volume of sodium thiosulfate used for the blank (mL)

N = Normality of sodium thiosulfate solution

m = Mass of oil sample (g)

Interpretation of results

PV < 10 meq O_2/kg: Fresh oil with low oxidation.

PV between 10 and 20 meq O_2/kg: Moderate oxidation.

PV > 20 meq O_2/kg: Advanced oxidation; oil quality may be deteriorated.

Conclusion

The peroxide value is an important indicator of the oxidative stability and quality of edible oils and fats. By determining the concentration of hydroperoxides formed during lipid oxidation, it is possible to assess the freshness of the oil and its suitability for consumption or industrial applications.

Practical work 9: Determination of fat acidity in cereal products

1. Introduction and objective

During storage, lipids naturally present in cereal products undergo hydrolysis under the action of lipase enzymes, leading to the release of free fatty acids. The accumulation of these fatty acids is an indicator of product deterioration and loss of quality.

The determination of fat acidity provides a sensitive and reliable criterion for evaluating the storage condition and technological quality of milled cereal products. This method measures the amount of free fatty acids extracted with ethanol and quantified by titration with a standardized sodium hydroxide solution. The aim of this practical work is to determine the fat acidity of a cereal product and evaluate its storage quality by quantifying the free fatty acids released during storage.

3. Principle of the method

The free fatty acids present in the cereal product are extracted with 95% ethanol at room temperature. The mixture is then centrifuged, and an aliquot of the supernatant is titrated with a standardized sodium hydroxide solution using phenolphthalein as an indicator.

The amount of sodium hydroxide required to neutralize the extracted acids is proportional to the fat acidity of the sample. The result is expressed as milligrams of potassium hydroxide (KOH) per 100 g of dry matter.

4. Reagents and equipment

4.1. Reagents used

- Ethanol, 95% (v/v).
- Sodium hydroxide solution, 0.05 mol/L in 95% ethanol.
- Phenolphthalein indicator solution (1% in 95% ethanol).
- Distilled water.

4.2. Equipment

- Centrifuge tubes (45 mL) with airtight stoppers.
- Shaker (30–60 rpm).

- Analytical balance (± 0.01 g).
- Pipettes (20 mL and 30 mL).
- 250 mL Erlenmeyer flask.
- Microburette graduated to 0.01 mL.
- Laboratory grinder (for semolina and pasta samples).
- Sieves (160 µm, 500 µm and 1 mm mesh size).
- Orange filter for endpoint observation.

5. Experimental procedure

5.1. Preparation of the test sample

For flour samples

- Take approximately 50 g of flour.
- If necessary, pass the sample through a 1 mm sieve to break up agglomerates.
- Homogenize thoroughly before analysis.

For semolina and pasta samples

- Grind approximately 50 g of sample without significant heating.
- Continue grinding until at least 80% of the particles pass through a 160 µm sieve.
- Homogenize thoroughly before analysis.

5.2. Determination of moisture content

- Determine the moisture content of the sample according to the appropriate reference method (practical work 1).
- Record the moisture content for subsequent calculations.

5.3. Test portion

- Accurately weigh approximately **5 g** of the prepared sample into a centrifuge tube.

- Record the mass to the nearest 0.01 g.

5.4. Extraction of free fatty acids

- Add **30 ml of 95% ethanol** to the centrifuge tube.
- Close the tube tightly.
- Shake on a rotary shaker for **1 hour** at room temperature (20 ± 5°C).
- Centrifuge the mixture for **5 minutes at 2000 × g**.

5.5. Titration

- Transfer **20 ml** of the clear supernatant into a 250 ml Erlenmeyer flask.
- Add **5 drops of phenolphthalein indicator**.
- Titrate with standardized **0.05 mol/l sodium hydroxide solution** until a pale pink color persists for approximately **3 seconds**.
- Use an orange filter to facilitate endpoint detection.

5.6. Blank determination

- Perform a blank test under identical conditions.
- Replace the 20 ml aliquot of supernatant with **20 ml of 95% ethanol**.
- Record the volume of sodium hydroxide used.

6. Results and calculations

Record the following data:

Parameter	Value
Sample mass (g)	
Moisture content (%)	
Volume of NaOH for sample (mL)	
Volume of NaOH for blank (mL)	
Exact concentration of NaOH (mol/L)	

*The fat acidity (FA) expressed as **mg KOH per 100 g dry matter** is calculated using the following equation:

$$FA = \frac{8415(V1 - V0)c}{m} \times \frac{100}{100 - w}$$

Where:

- **FA** = Fat acidity (mg KOH/100 g dry matter)
- **V_1** = Volume of NaOH used for the sample (mL)
- **V_0** = Volume of NaOH used for the blank (mL)
- **c** = Exact concentration of NaOH solution (mol/L)
- **m** = Mass of sample (g)
- **w** = Moisture content of the sample (%)
- 8415 is the constant applicable to potassium hydroxide, corresponding to (56.1×1.5×100).

Interpretation of results

- Low fat acidity indicates good storage conditions and high product quality.
- High fat acidity indicates extensive lipid hydrolysis and possible deterioration during storage.
- Fat acidity is commonly used as an indicator of freshness and shelf-life of cereal products.

Conclusion

The determination of fat acidity is an important quality control test for cereal products. By measuring the concentration of free fatty acids released during storage, it is possible to assess the preservation state, freshness, and technological quality of flour, semolina, and pasta products.

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