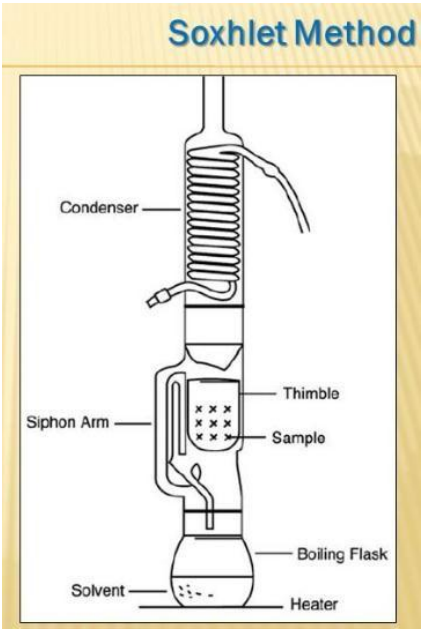
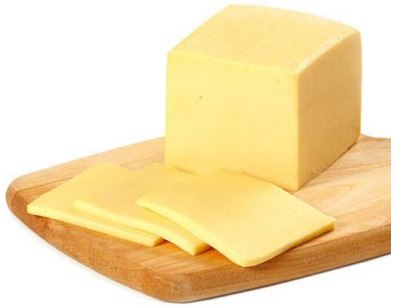
FE 271 FOOD CHEMISTRY LABORATORY

EXPERIMENT 2- FAT DETERMINATION

What is the aim of fat determination experiment?

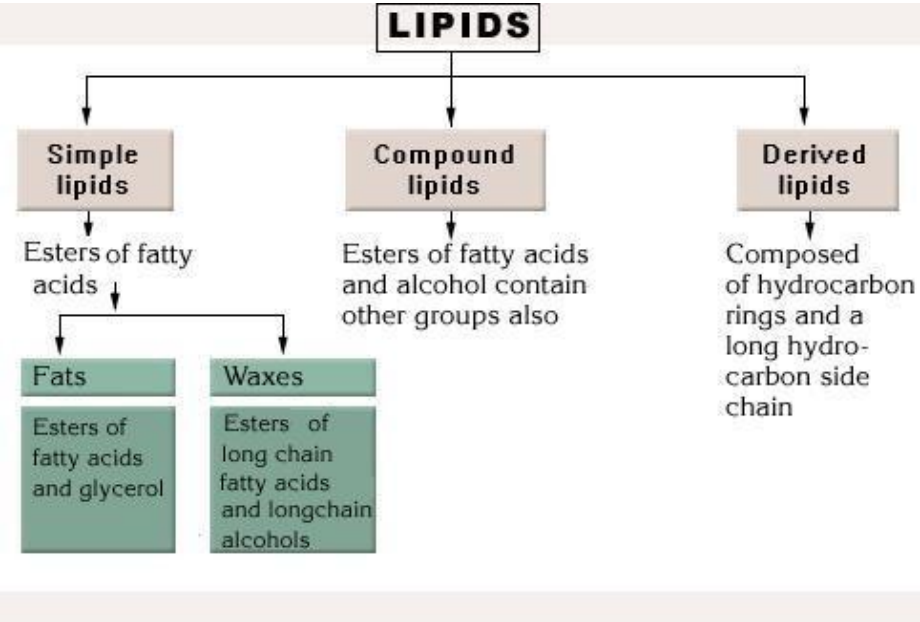
- To determine fat content of different food products by using Soxhlet method.

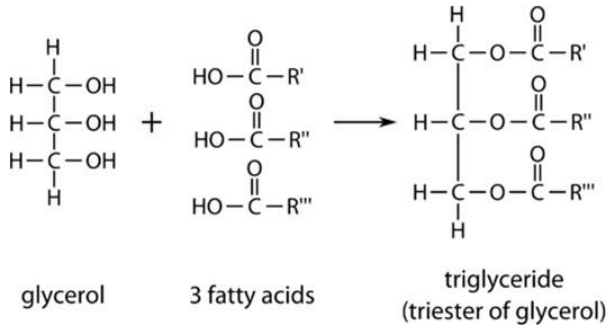


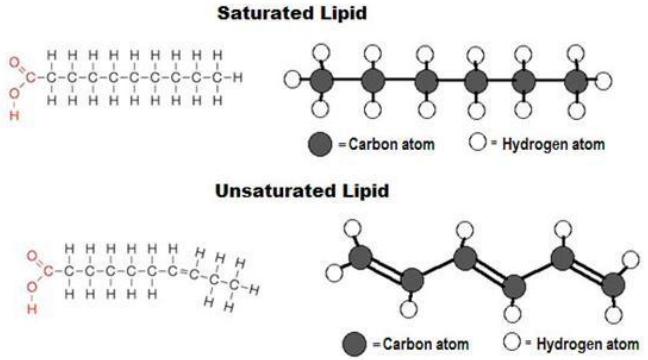


What are lipids?

- Lipids are food components that are soluble in organic solvent and insoluble in water.







Importance of Fat in Foods

- Nutrition
- Appearance
- Texture
- Flavor

The table below shows the fat content of some foods.

Lard, shortening, oils	almost 100% fat
Butter and margarine	81%
Salad Dressing	40-70%
Nuts	50-71%
Grains	3-5%
Raw Beef	25%
Eggs	12%
Milk	3.5%
Fruits and vegetables	very small quantities

FAT DETERMINATION METHODS

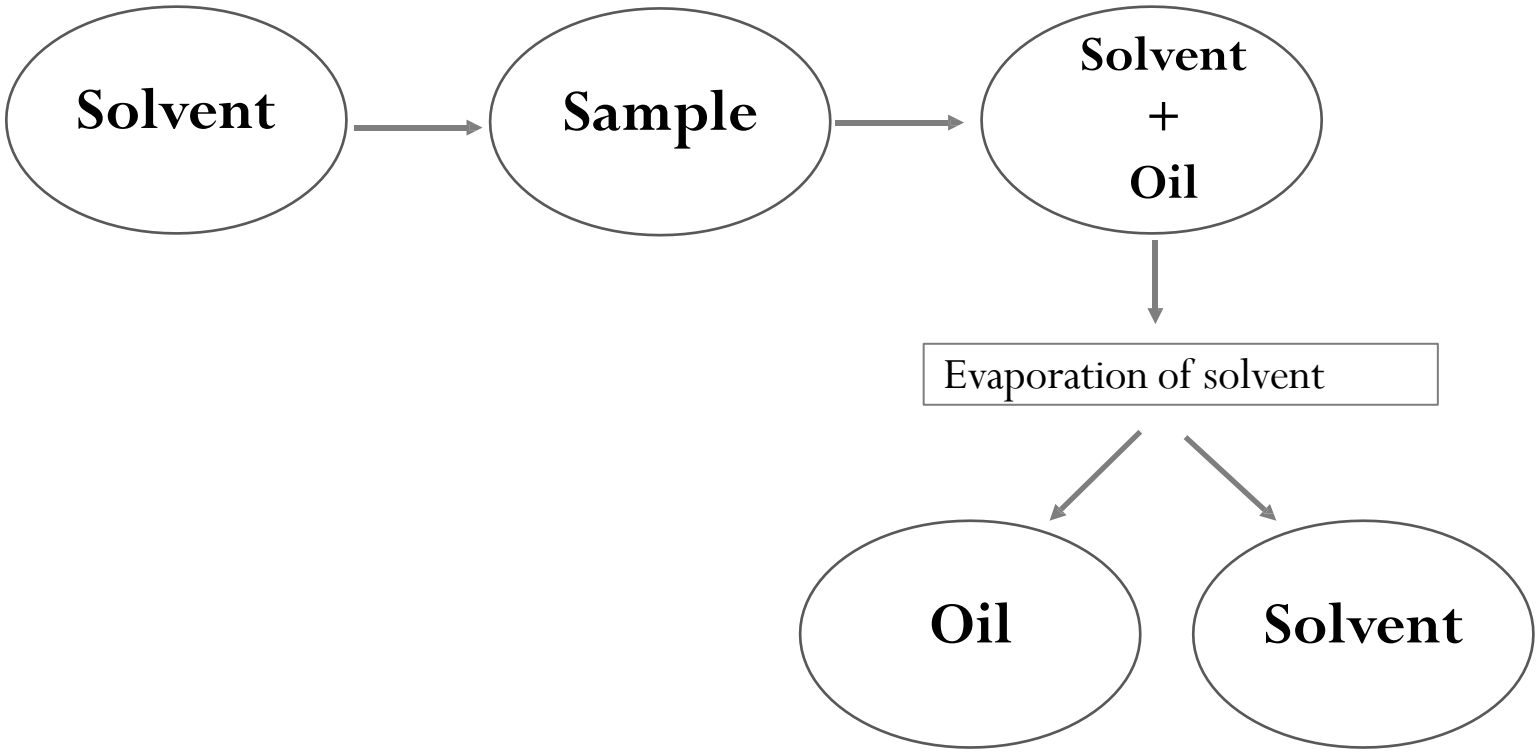
- Solvent extraction
  - continuous (e.g., Goldfish),
  - semicontinuous (e.g., Soxhlet),
  - discontinuous (e.g., Mojonnier, Folch)
- Non-solvent extraction (e.g. the Babcock or Gerber)
- Instrumental methods (e.g. NMR, infrared)

The method of choice depends on a variety of factors, including the nature of the sample, the purpose of the analysis and instrumentation available.

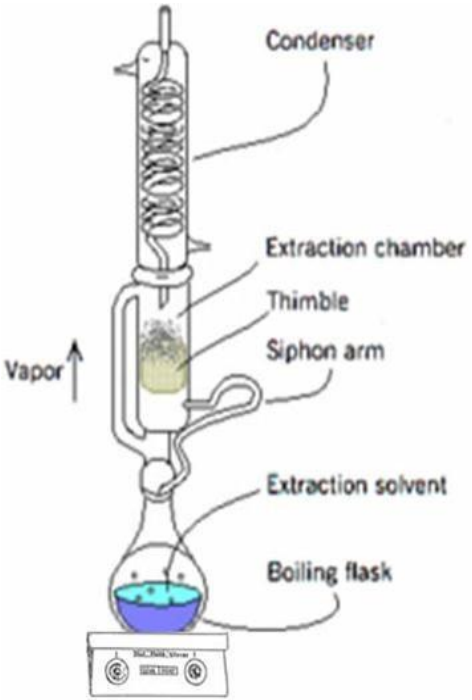
Importance of fat determination

- Economic (not to give away expensive ingredients)
- Legal (to conform to standards of identity and nutritional labeling laws)
- Health (development of low fat foods)
- Quality (food properties depend on the total lipid content)
- Processing (processing conditions depend on the total lipid content)

Solvent Extraction

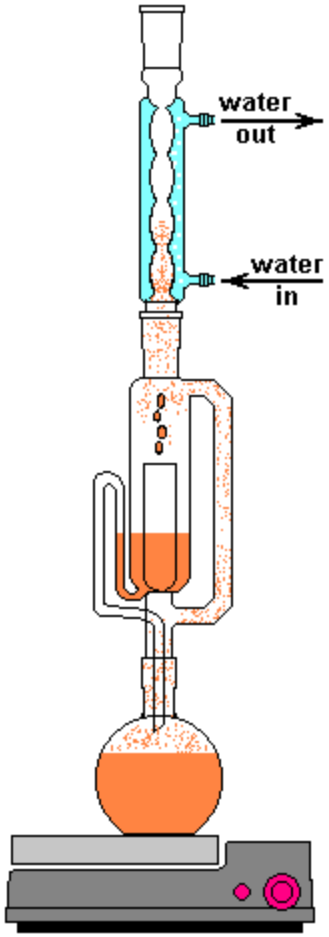


Traditional Soxhlet extraction working principle



- Sample is placed inside a thimble.
- The thimble is placed into the extraction chamber.
- The Soxhlet extractor is placed onto a flask containing the extraction solvent and then equipped with a condenser.
- The solvent is heated to reflux.
- The solvent vapour travels up a distillation arm, and floods into the chamber housing the thimble of solid.
- The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material.

Traditional Soxhlet extraction working principle



- The chamber containing the solid material slowly fills with warm solvent.
- Some of the desired compound will then dissolve in the warm solvent.
- When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask.
- This cycle may be allowed to repeat many times, over hours or days.
- After many cycles the desired compound is concentrated in the distillation flask.
- The flask containing solvent and oil is placed in rotary vacuum evaporator and solvent is evaporated. Finally, the oil in the flask is weighed.

Rotary vacuum evaporator

Automatic Soxhlet Extraction Machine

- **Immersion:** The sample in the thimble is immersed in the organic solvent. Extraction occurs in immersion step at 180°C for 90 min depending on the solvent and sample
- **Washing:** Trace amount of fats are washed in this step (180°C, 15 min)
- **Recovery:** Organic solvent is distilled and separated from the oil at 180°C for 30 min.

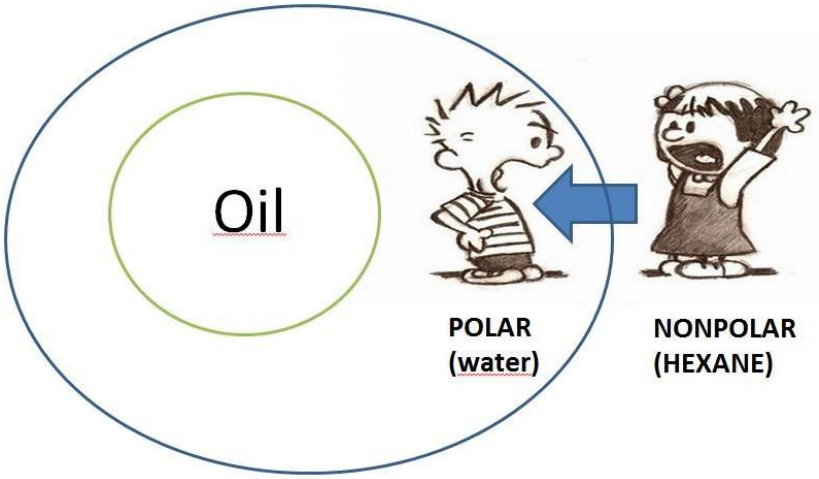
Sample preparation

- The sample preparation for lipid analysis depends on the type of food and the type and nature of lipids in the food.
- Several preparatory steps are common in lipid analysis. These act to aid in extraction by
  - removal of water (pre-drying),
  - reduction of particle size,
  - separation of the lipid from bound proteins and/or carbohydrates (by acid hydrolysis).

Sample preparation

Removal of water (pre-drying)

Organic solvent cannot easily penetrate the moist food tissues due to the hydrophobicity of the solvents. Drying the sample at high temperatures is undesirable because some lipids become bound to proteins and carbohydrates.



Sample preparation

Reduction of particle size

Decrease in particle size

↓

Increase in surface area

↓

Increase in solvent food contact area

↓

Solvent can easily penetrate into the sample

↓

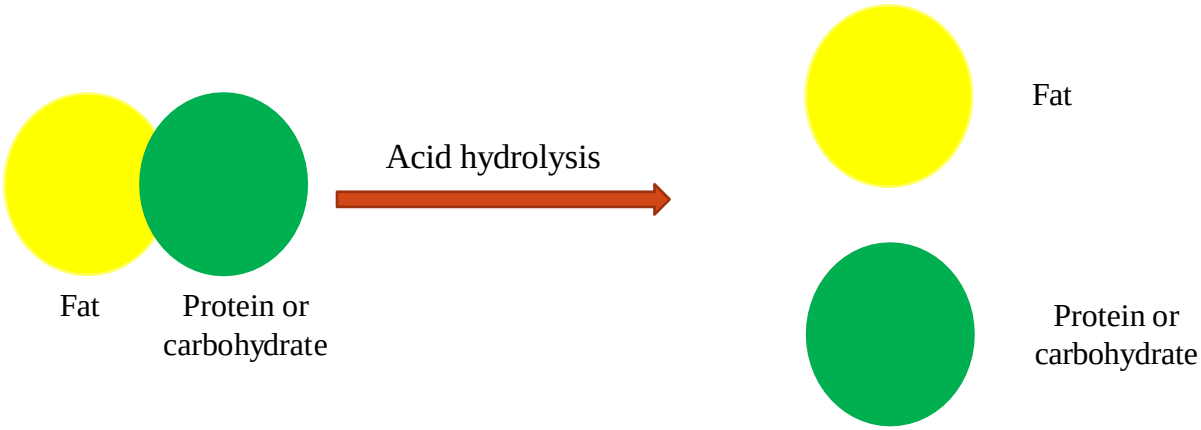
Efficient extraction

Sample preparation

Acid Hydrolysis

- A significant portion of the lipids in foods such as dairy, bread, flour, and animal products is bound to proteins and carbohydrates, and direct extraction with nonpolar solvents is inefficient.
- Such foods must be prepared for lipid extraction by acid hydrolysis.

- Acid Hydrolysis:
  - can break both covalently and ionically bound lipids into easily extractable lipid forms.
  - sample can be pre-digested by refluxing for 1 h with 3N hydrochloric acid.



Solvent Selection

Ideal solvents for fat extraction should

- have a high solvent power for lipids and low or no solvent power for proteins, amino acids, and carbohydrates
- evaporate easily and leave no residue,
- have a relatively low boiling point,
- be nonflammable and nontoxic in both liquid and vapor states.
- be inexpensive and nonhygroscopic.

It is difficult to find an ideal fat solvent to meet all of these requirements.

The most common solvents are ethyl ether, petroleum ether but pentane and hexane are also used to extract oil.

PROCEDURE

Sample preparation:

Sucuk
Kaşar

→ Cut into small pieces → Put into the oven for drying (~1 h)

Pistachio
Hazelnut
Potato chips

→ Grinding

PROCEDURE

For manual set-up

1

Measure and record the weight of flask and boiling chips

2

Weigh nearly 10 g of sample on the filter paper and record the value with four digits

3

Insert the sample in filter paper to the soxhlet apparatus (extractor), add 250 mL hexane and start the heating. Close the heater at the end of 2 h.

PROCEDURE

For manual set-up

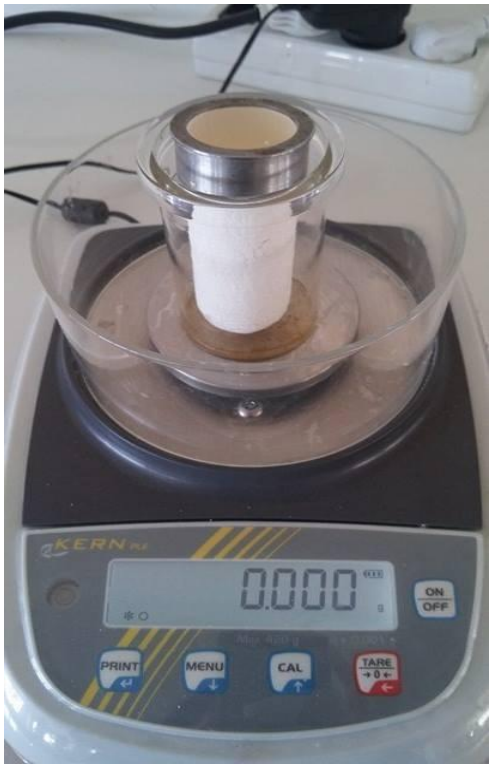
Evaporate the hexane using rotary vacuum evaporator. There will be only fat and boiling chips at the end of evaporation.

Measure and record the weight of flask containing oil and boiling chips

PROCEDURE

For machine

1. Record the weight of glass vial containing boiling chips with four digits.



2. Put the thimble on vial and press **Tare**.

3. Weigh your sample and record the value with four digits.

PROCEDURE

For machine

4. Insert the thimble into the machine.

5. Add hexane into the vial. Insert the vial on the machine. Start the programme.

6. End the programme. Wait for 10 min to cool down. Put the vial in desiccator and wait for 15 min. **Measure and record the weight of vial, oil and boiling chips.**

CALCULATION

$$Fat\% = \frac{[(wt\ of\ ext.\ flask + extracted\ fat) - (wt\ of\ ext.\ flask)]}{wt\ of\ sample} \times 100$$

Discussion:

- Give a brief information about your sample (e.g. its composition)
- Explain the reasons of pretreatment
- Compare your result with TSE or Codex. Explain if your result is in the range or not. If it is not in the legal range, explain the possible reasons of different finding.

DATA

Sample name:

Weight of flask:

Weight of sample:

Weight of flask and extracted fat:

Weight of extracted fat:

Submitted by:

Group:

Group members:

Submitted to: