

Lipids-II

Classification of Fatty Acids:

1. Saturated Fatty Acids:

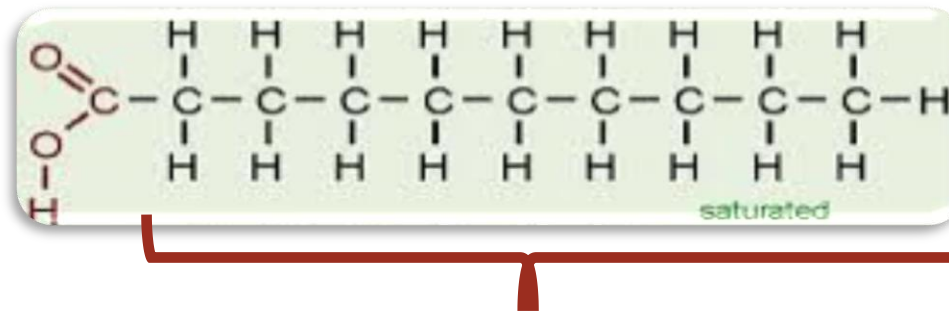
Fatty acids have **no double bonds**, side chain are (**alkane**).

a) Short chain:

From 4 to 10 Carbon atoms, and present as liquid in room temperature e.g butyric acid.

b) Long chain:

More than 10 Carbon atoms, present in solid at room temp. e.g. Palmatic (16) acid and Stearic (18) acid.

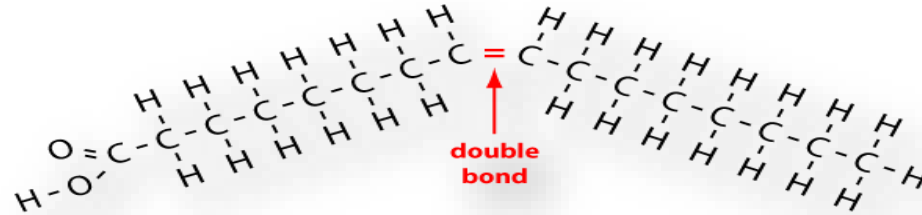


Single bond only

Classification of Fatty Acids cont':

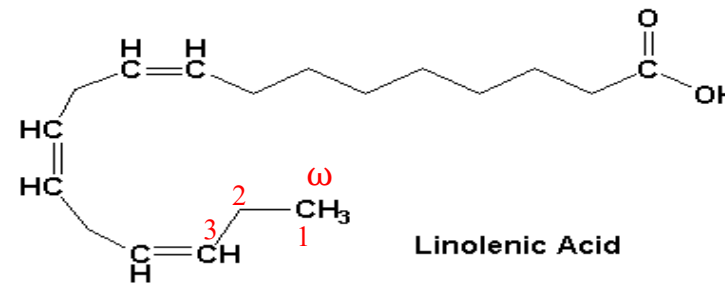
2. Unsaturated Fatty Acids:

Have one or more double bonds between carbon atoms (side chain has at least one double bond).



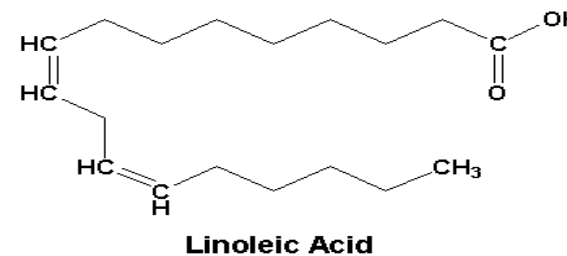
a) Essential Fatty acids:

- linolenic acid 18-C, 3 double bond (ω -3).
- Linoleic acid 18-C, 2 double bond (ω -6).



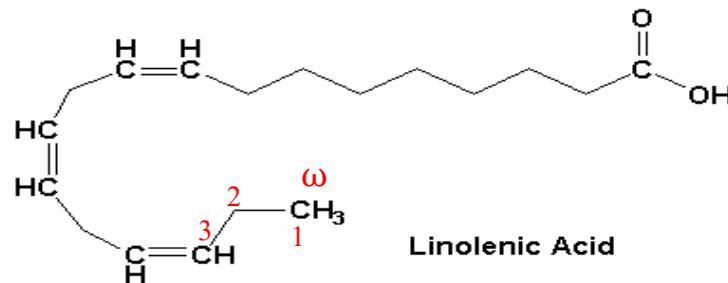
b) Non-essential Fatty acids:

- Can be synthesized in the body: Oleic acid.



Omega-3 fatty acids :

- Also called ω -3 fatty acids or n-3 fatty acids.
- Are **polyunsaturated fatty acids (PUFAs)** with a double bond(C=C) at the **third carbon** atom from the end of the carbon chain.
- The fatty acids have two ends, the carboxylic acid (-COOH) end, which is considered the beginning of the chain, thus "alpha", and the methyl (CH₃) end, which is considered the "tail" of the chain, thus "omega."
- The way in which a fatty acid is named is determined by the location of the first double bond, counted from the methyl end, that is, the omega (ω -) or the n- end.



Practical Part

Test of lipids:

- 1 Copper acetate test.
- 2 Liebermann - Burchard Test: For cholesterol.
- 3 Unsaturation Test.
- 4 Acrolein test: for glycerol or fats.

Experiment I: Copper acetate test

➤ **Objective:**

This test is used to distinguish between oil [neutral fat] and fatty acid [saturated and unsaturated].

➤ **Principle:**

- The **copper acetate solution** does not react with the oils (or fats), while **fatty acids** [saturated and unsaturated] react with copper acetate to form **copper salt**.
- Copper salt formed in the case of fatty acids can only be extracted by petroleum ether.

Experiment I: Copper acetate test

➤ Method:

1. Take two test tubes add 3 ml of petroleum ether and an equal volume of a solution of copper acetate.
2. Add 0.5 ml of each sample (olive oil, oleic acid) in each tube
3. Shake the tube and leave it for some time.

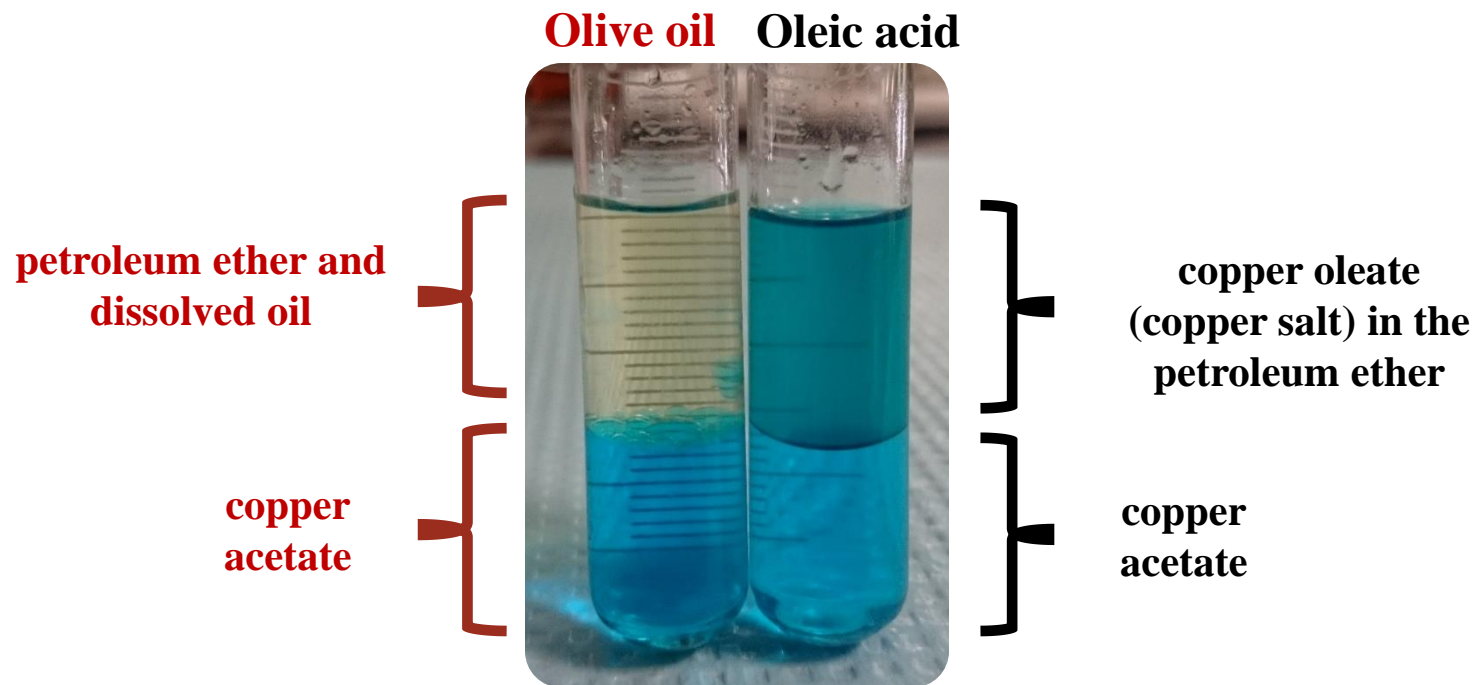
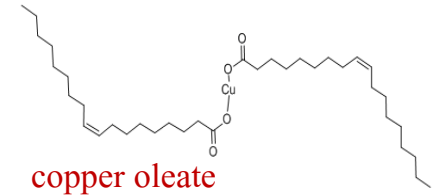
➤ Results:

Tube	Observation
Olive oil	
Oleic acid (F.A)	



Olive oil (-) Oleic acid (+)

- **Olive oil:** notice that petroleum ether **upper lay** containing the dissolved oil and appears colorless, aqueous solution remains blue in the **bottom**.
- **Oleic acid:** the **upper layer** of petroleum ether becomes green as a result of copper oleate (cupper salt). The **lower layer** becomes less in blue (become less concentrated).



Experiment 2 : Qualitative estimation of Cholesterol by Liebermann - Burchard Test

➤ Objective:

To detect the presence of cholesterol.

➤ Principle:

- Liebermann - Burchard test , is a chemical estimation of cholesterol, the cholesterol is react as a typical **alcohol with a strong concentrated** acids and the product are colored substances.
- Acetic anhydride are used as solvent and dehydrating agents.
- Sulfuric acid is used as dehydrating and oxidizing agent.
- A positive result is observed when the solution becomes **red** or **pink** , then **purple** , **blue**, and finally **bluish –green color**.

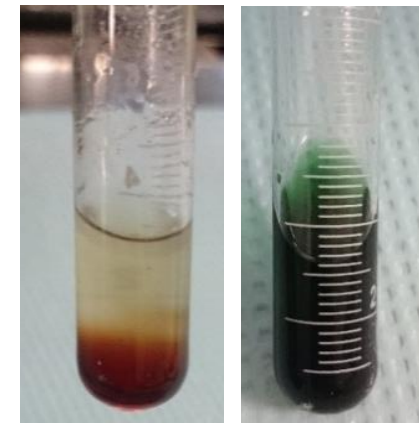
Experiment 2 : Qualitative estimation of Cholesterol by Liebermann - Burchard Test

➤ Method:

1. Dissolve a few crystals of cholesterol in 2 ml of chloroform in a dry test tube.
2. Now add 10 drops of acetic anhydride.
3. Add 2 to 3 drops of conc. sulfuric acid.
4. Repeat the reaction with olive oil.

➤ Results:

Tube	Observation
Olive oil	
Cholesterol	



Olive oil
(-)

Cholesterol
(+)

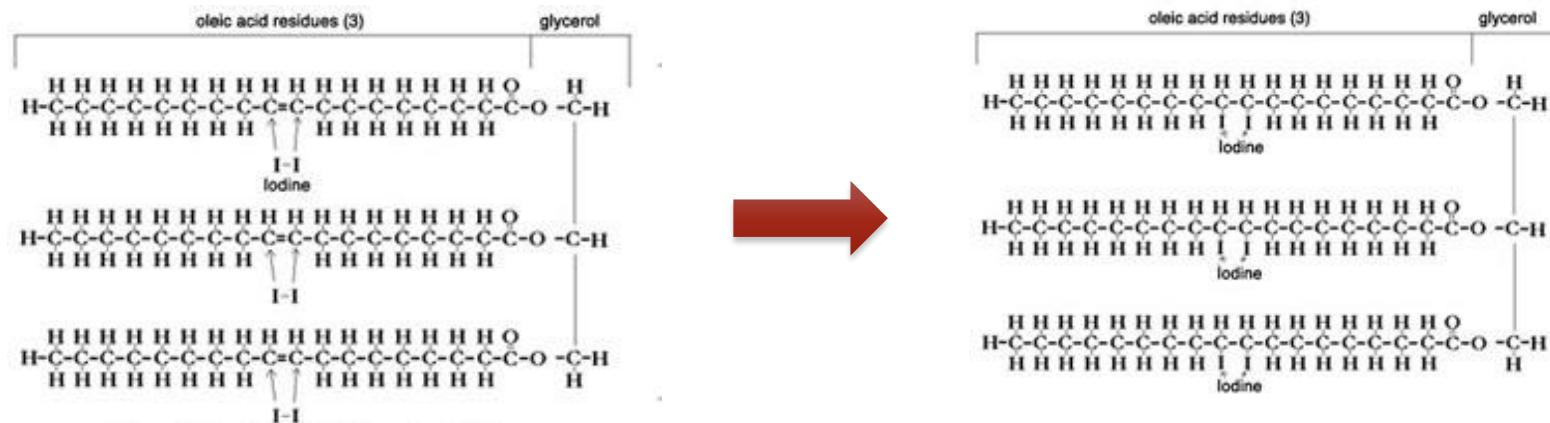
Experiment 3: Unsaturation Test

➤ Objective:

Determine the degree of saturation of different types oils.

➤ Principle:

- All neutral contain glycerides of some **unsaturated fatty acids**.
- These unsaturated fatty acids **become saturated by taking up iodine** → **If the fat contains more unsaturated fatty acids, it will take up more iodine.**
- Halogens (I, Br) will add across the double bonds and thus the decolorization of an iodine or bromine solution will indicate the presence of unsaturated fatty acids.



Experiment 3: Unsaturation Test

➤ Method:

1. Equally into 2 flask Add 10 ml of Chloroform then 10 drops of iodine reagent ,the chloroform shows **pink color due to presence of iodine.**
2. To one test flask add the oil sample drop by drop shaking the tube vigorously for about 30 seconds after addition of each until the **pink color is discharged** and count the number of drops. (**The pink color is discharged owing to the taking up of iodine by the unsaturated fatty acids of the oil**).
3. Repeat the experiment using butter.

➤ Results:

Tube	Observation
Olive oil	
Butter	

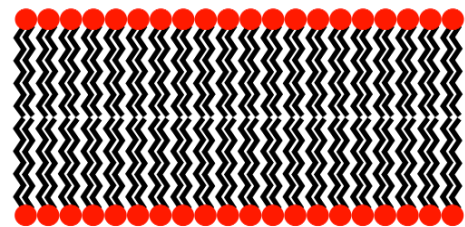


pink color formed



pink color discharged

- **Compare unsaturation** , it should be remembered that **more the number of drops of the sample (fat) required to discharge the pink color, the less the unsaturation (it is more saturated)**, because fat contains less unsaturated fatty acids (less double bonds) , it will take up **less iodine per drop** due to low number of double bonds (**so it will need more drops of fat sample to take up all iodine and decolorize the pink color**) and vice versa.
- There is an **inverse relationship** between number of fat drops required and number of double bonds.

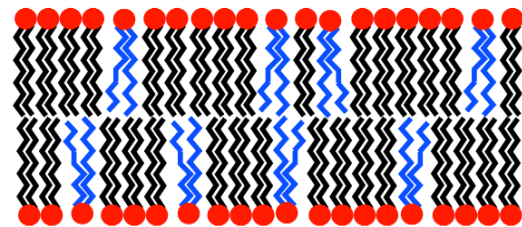
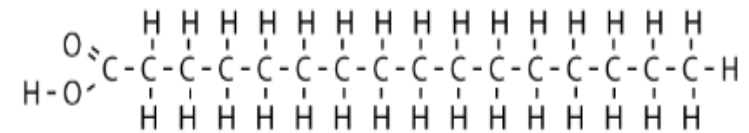


Saturated lipids only

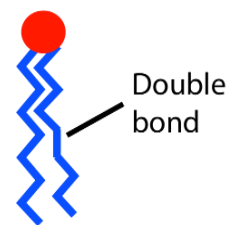


Saturated

saturated fatty acid

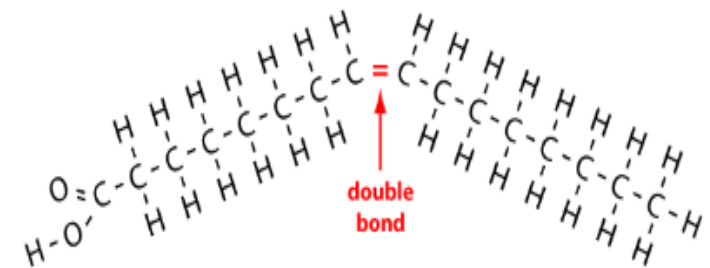


Mixed saturated and unsaturated



Monounsaturated

unsaturated fatty acid



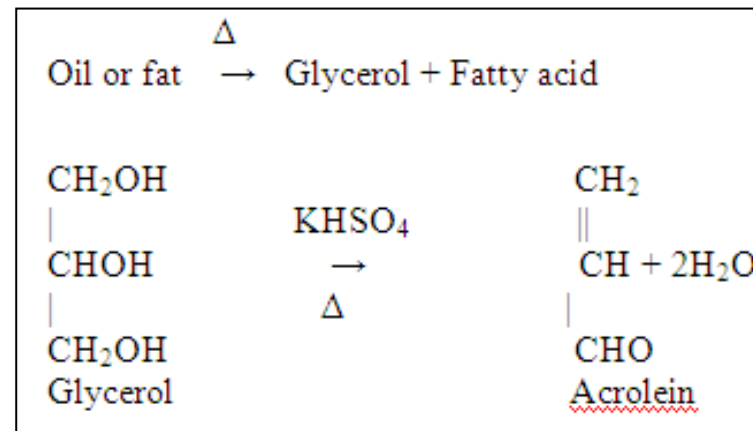
Experiment 4: Acrolein test

➤ Objective:

To detect glycerol or fats (Most lipid are found in the form of triglycerides, an ester formed from glycerol and fatty acids).

➤ Principle:

- When a fat is **heated strongly in the presence of a dehydrating agent** such as KHSO_4 [potassium bisulphate], the glycerol portion of the molecule is **dehydrated to form the unsaturated aldehyde, acrolein $\text{CH}_2=\text{CH}-\text{CHO}$** .
- Which can be distinguished by its irritating acrid smell and as burnt grease.



Another way to detect lipids :

- Other way to detect lipids is by dye **Sudan IV** (general dye for lipid), which produce **red color** with lipid.
- Its is fat soluble dyes used for biochemical staining of triglycerides, fatty acids, and lipoproteins.



Sudan IV (general dye for lipid)

Homework:

- Why iodine color discharged after addition of oil ?**
- Do you expect to get a positive result if you use free fatty acid like oleic acid or palmitic acid in acrolein test? and why ?**