

Lipids-II



Fatty Acids can be classified to:

A-Saturated Fatty Acids:

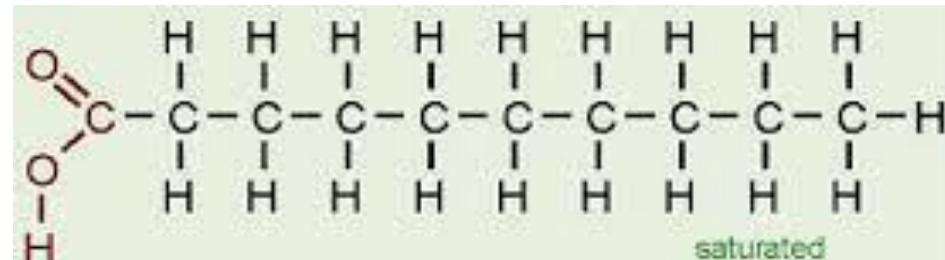
Fatty acids have no double bonds ,side chain are (alkane).

1) Short chain FA:

From 4 to 10 Carbon atoms ,and present as **liquid** in room Temp.

2) Long chain FA:

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



B-Unsaturated Fatty Acids:

have one or more double bonds between carbon atoms side chain.

[has at least one double bond].

1) Essential Fatty acids:

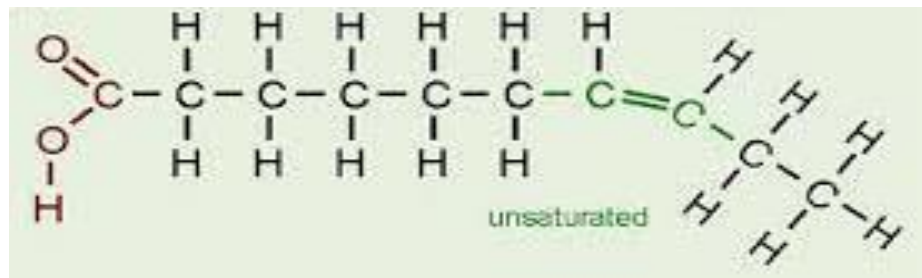
Can not be synthesized in the body

linolenic acid 18-C, 3 double bond (ω -3)

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

2) Non essential Fatty acids:

Can be synthesized in the body: Oleic acid



Practical Part

1-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**.

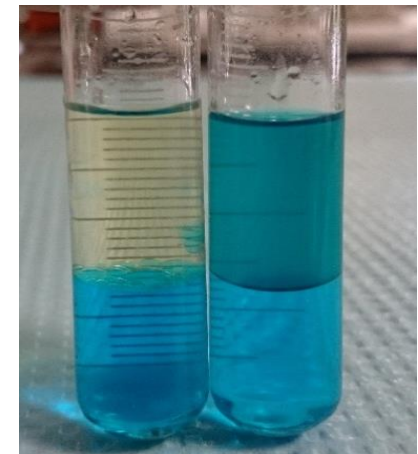
→ Copper salt formed in the case of fatty acids can only be extracted by petroleum ether.

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 in each tube
3. Shake the tube and leave it for some time.

Result :

	Observation	Comment
Olive oil		
Oleic acid		



olive oil:

notice that petroleum ether upper layer containing the dissolved oil and appears colorless.
aqueous solution remains blue in the bottom.

petroleum ether and dissolved oil



copper acetate



Oleic acid:

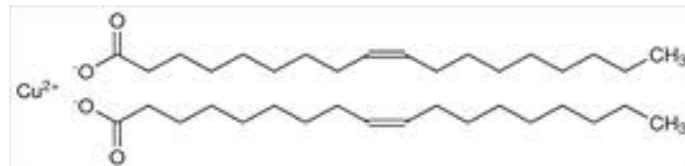
The upper layer of petroleum ether becomes green as a result of **copper oleate** [copper salt].

The lower layer becomes less in blue.

copper oleate in the petroleum ether



copper acetate



2-Liebermann - Burchard Test [acetic anhydride test] :

Objective:

To detect the presence of cholesterol.

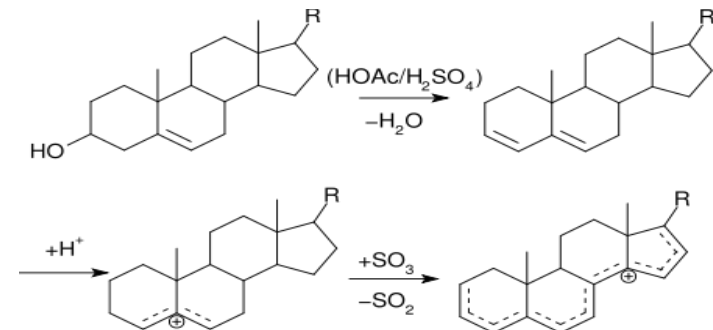
Principle:

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 **blue**, and finally **bluish –green** color.

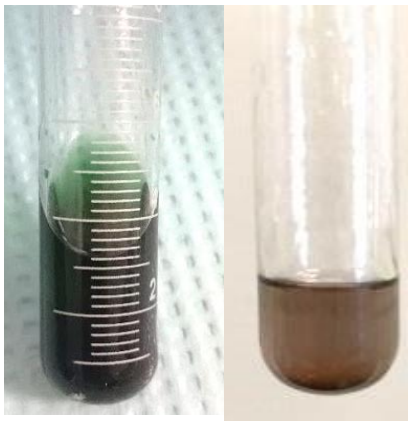


Method:

1. Dissolve a few crystals of cholesterol in 2 ml of chloroform in a dry test tube
2. add 10 drops of acetic anhydride
3. Add 2 to 3 drops of conc. sulfuric acid

Result :

Tube	Observation	Comment
cholestrol		
Olive oil		



cholestrol



Olive oil

3-Unsaturation Test:

Objective:

Determine the **degree of saturation** of different types of oils.

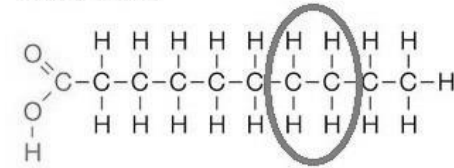
Principle:

All neutral contain glycerides of some unsaturated fatty acids.

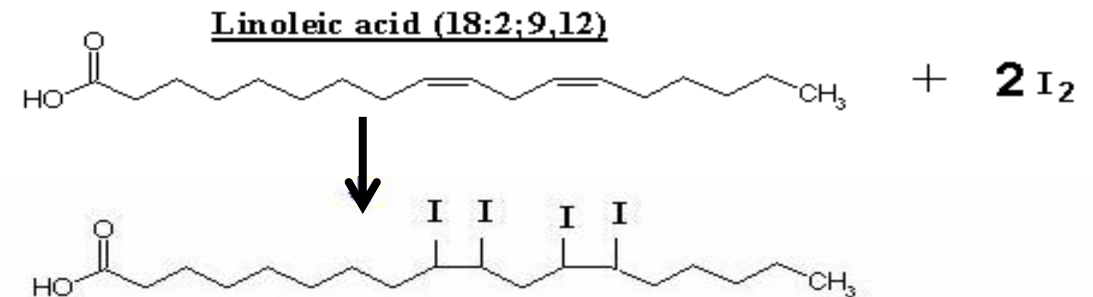
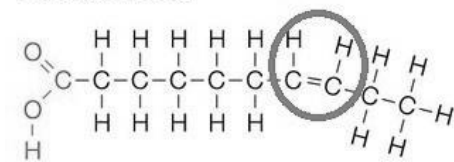
These unsaturated **fatty acids become saturated by taking up iodine.**

Halogens (I, Br) will add across the double bonds and thus the **decolorization** of iodine or bromine solution will indicate the presence of unsaturated fatty acids.

Saturated



Unsaturated

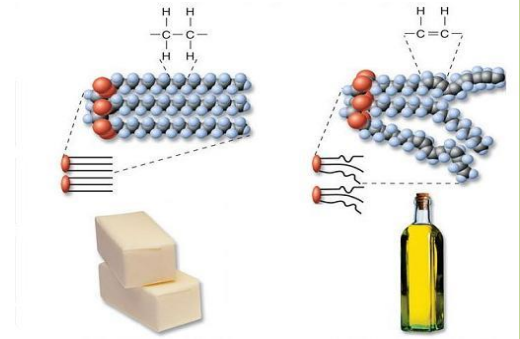


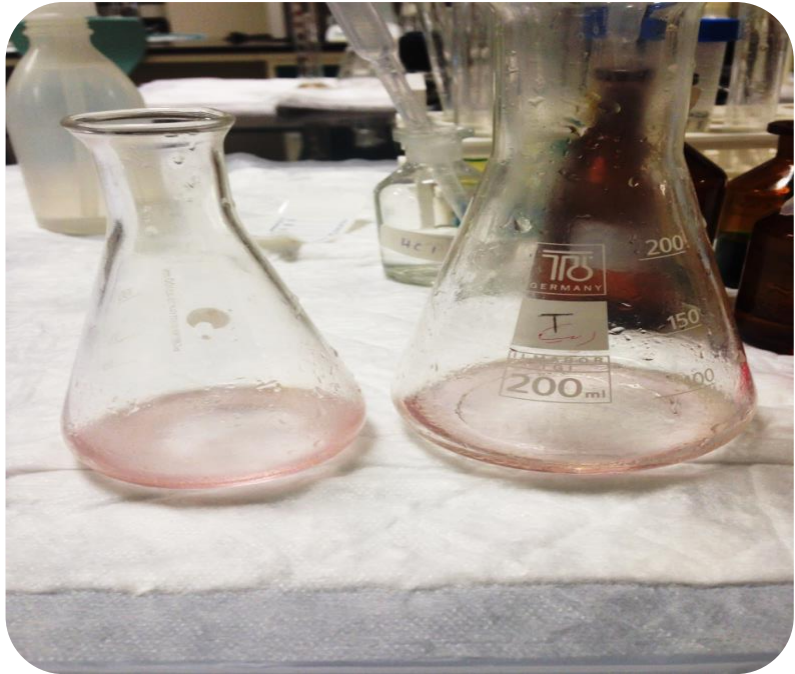
Method:

- Equally into 2 flask Add 10 ml of Chloroform then 10 drops of Hub's iodine reagent (alcoholic solution of iodine containing some mercuric chloride), **the chloroform shows pink color due to presence of iodine.**
- To one test flask add the **olive 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.** To the second flask add the **butter sample** by the same method.
- The pink color is discharged due to the taking up of iodine by the unsaturated fatty acids of the oil.

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 (saturated)**, because fat contains less unsaturated fatty acids, 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). Vice versa.

There is an **inverse relationship** between number of fat drops required and number of double bonds.





4-acrolein test:

Objective:

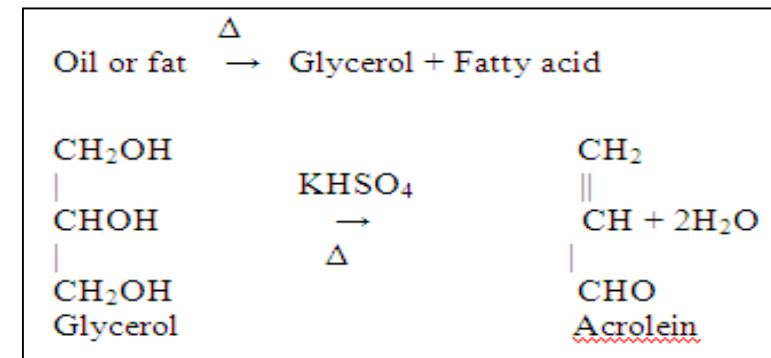
is used to detect glycerol or fats.

-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.



- Other way to detect lipids is by dye **Sudan IV** (general dye for lipid), which **produce red color** with lipid.



Sudan IV (general dye for lipid)

Questions:

Why olive oil does not form green color in copper acetate test ?

What do you expect if you used palmitic or linoleic acid in copper acetate test ?

Why acrolein test is used as a general test for oils and fats?

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 ?

Why iodine color discharged after addition of oil ?