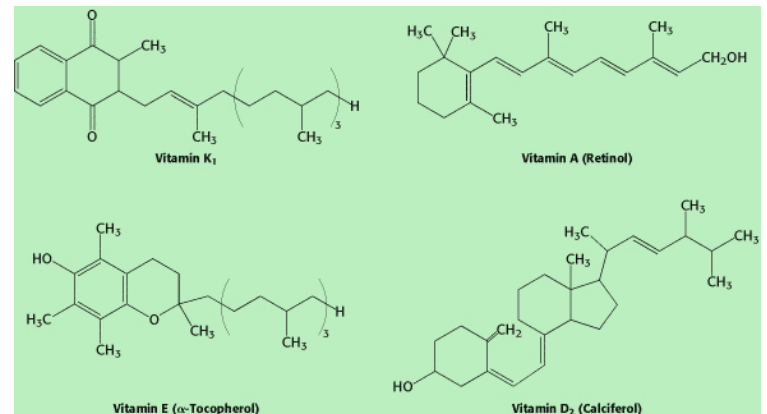
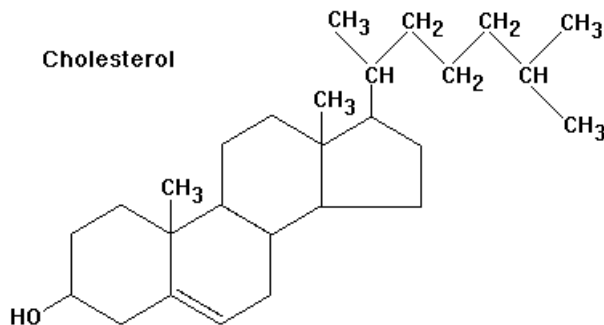


Qualitative tests of lipids 2



Derived lipids .

They are substances that are soluble in lipid or derived from the lipids by hydrolysis; for examples, cholesterol and fat soluble vitamins.



Fatty Acids Classification

α -saturated fatty acids

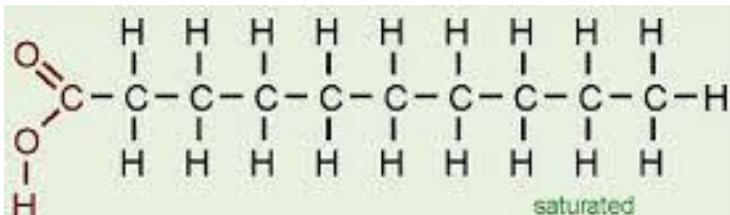
Saturated fatty acids have no double bonds, side chains are (alkane).:

a) Short chain

From 4 to 10 Carbon atoms, and present as liquid in room Temp. e.g. acetic (4) acid and butyric acid

a) Long chain:

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



Unsaturated fatty acids

have **one or more double** bonds between carbon atoms, side chain has at least one double bond.

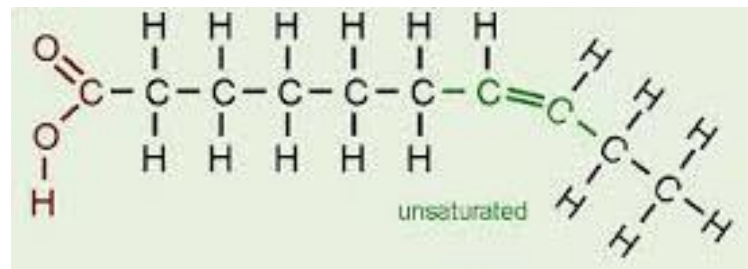
a) Essential Fatty acids:

linolenic acid 18-C, 3 bond (ω -3)

b) Non essential Fatty acids:

Linoleic acid 18-C, 2 bond (ω -6)

Can be synthesized in the body: Oleic acid



1-Copper acetate test

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

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

Copper acetate test

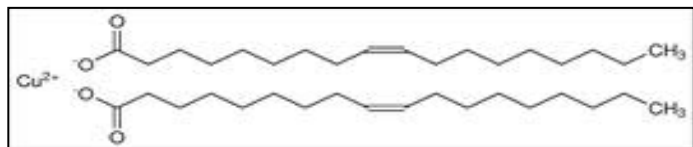
☞ **olive oil:** notice that petroleum ether upper lay containing the dissolved oil and appears colorless, aqueous solution remains blue in the bottom.

petroleum ether and dissolved oil

copper acetate



☞ **oleic acid** the upper layer of petroleum ether becomes green as a result of copper oleate. The lower layer becomes less in blue.



copper oleate in the petroleum ether

copper acetate



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.

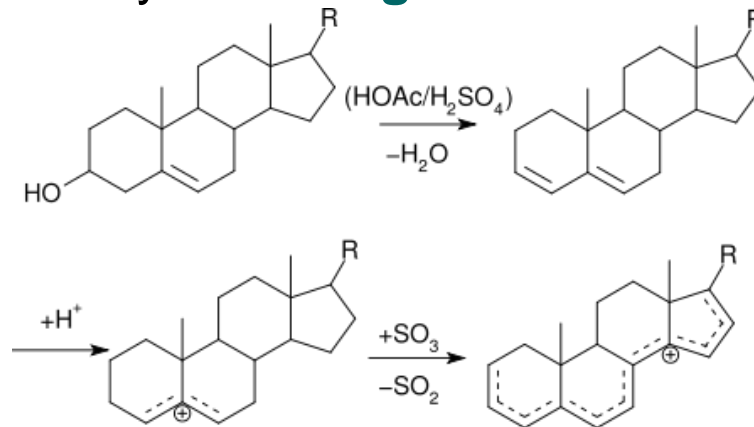
Result:

Tube	Observation	Comments
Olive oil		
Oleic acid		

2-Qualitative estimation of Cholesterol by Liebermann - Burchard Test

Principle:

- ∞ Liebermann - Burchard Test , is a chemical estimation of cholesterol, the cholesterol is react as a typical alcohol with a strong ,concentrated acids; the product are colored substances.
- ∞ Acetic anhydride are used as solvent and dehydrating agents, and the 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.



Method

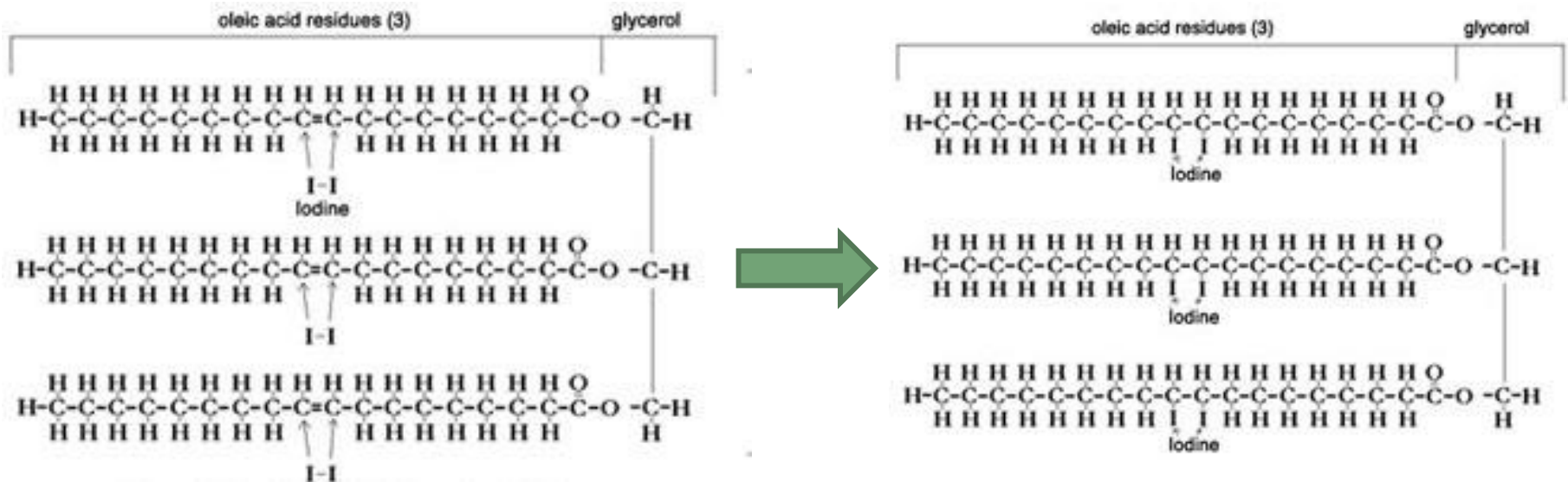
- ☞ Dissolve a few crystals of cholesterol in 2 ml of chloroform in a dry test tube
- ☞ Now add 10 drops of acetic anhydride
- ☞ Add 2 to 3 drops of conc. sulfuric acid
- ☞ Record your result

Tube	Observation	Comments
Cholesterol		
Olive Oil		

Unsaturation Test:

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.



Method:

- Equally into 4 flask Add 10 ml of Chloroform then 10 drops of Hub's iodine reagent ,the chloroform shows **pink color due to presence of iodine.**
- 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.
- Compare unsaturation , it should be remembered that more the number of drops required to discharge the pink color, the less is the unsaturation.**

Results:

Sample	Number of drops added from the sample	Conclusion
Mustard oil		
coconut oil		
olive oil		
saturated fat		

Acrolein test

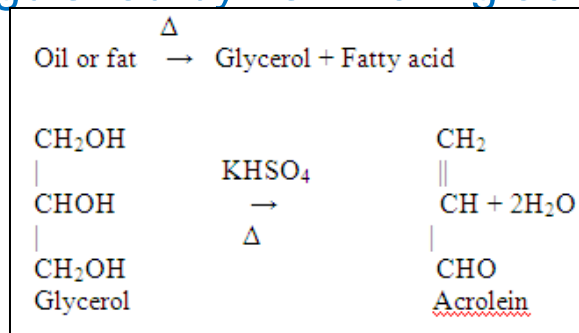
The "acrolein test" is used to detect glycerol or fats

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 , 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 acid 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.

