

# Lipids-II



## Fatty Acids can be classified to:

### A-Saturated Fatty Acids:

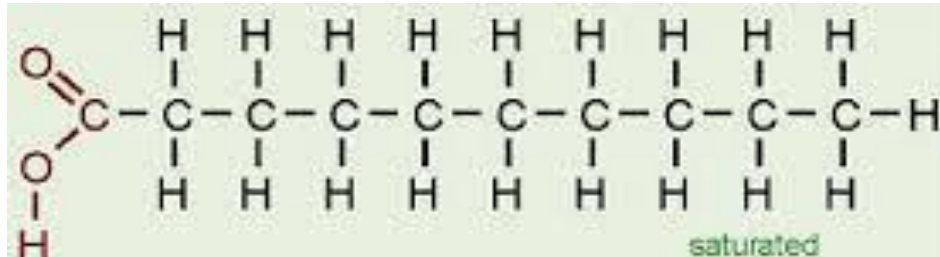
Fatty acids have no double bonds, side chain are (alkane).

#### 1) Short chain FA:

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

#### 2) Long chain FA:

More than 10 Carbon atoms, present in **solid** at room Temp. e.g. Palmitic (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:**

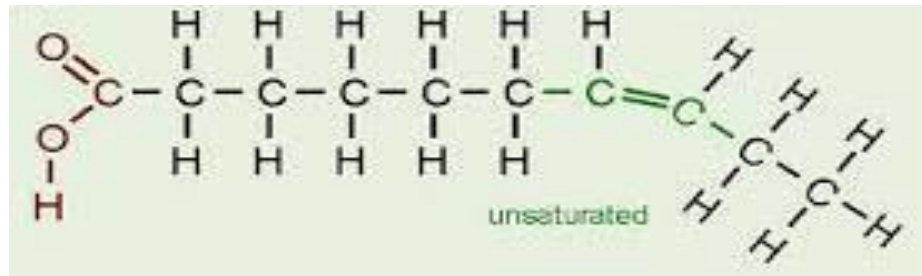
Can not be synthesized in the body

linolenic acid 18-C, 3 double bond ( $\omega$ -3)

Linoleic acid 18-C, 2 double bond ( $\omega$ -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**.

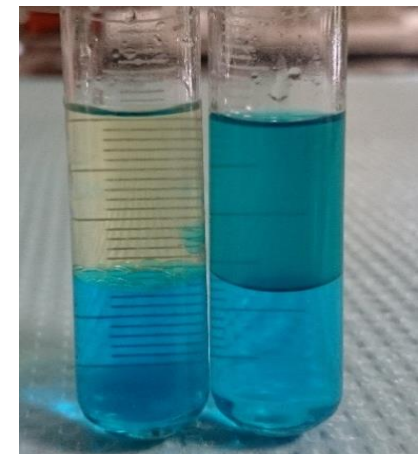
→ Copper salt formed in the case of fatty acids can only be extracted by petroleum ether.

## 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** →



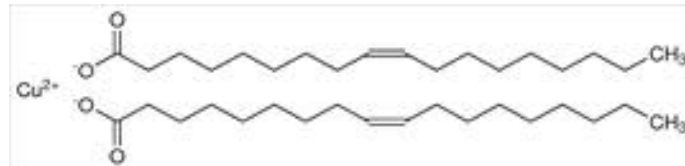
# Oleic acid:

- 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.

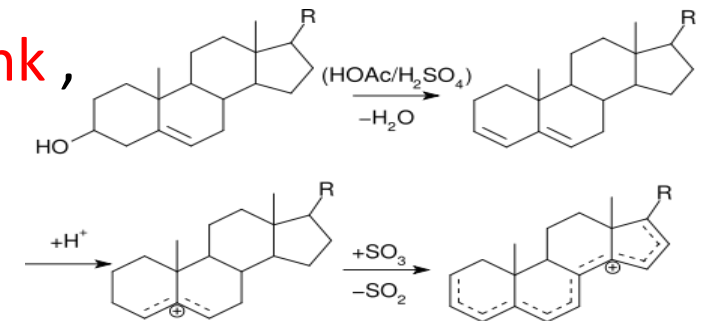
### Principle:

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
cholesterol		
Olive oil		



**cholesterol**



**Olive oil**

### 3-Unsaturation Test:

#### Objective:

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

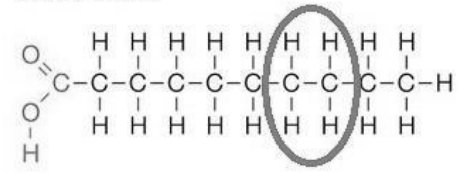
#### Principle:

- All neutral contain glycerides of some unsaturated fatty acids.

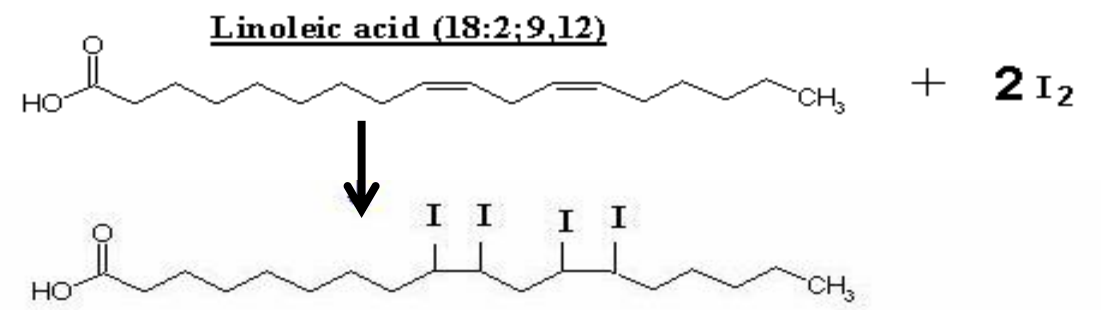
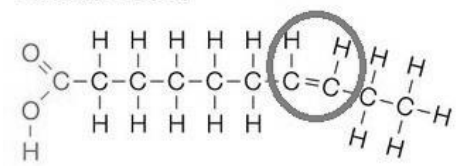
These unsaturated **fatty acids become saturated by taking up iodine.**

- 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



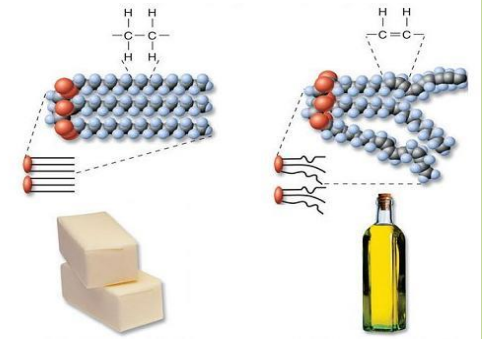
Unsaturated



## 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 of drops of the sample (fat) required to discharge the pink color less the unsaturation (saturated)**, because fat contains less unsaturated fatty acids. it will take up **less iodine per drop** di





# 4-acrolein test:

## Objective:

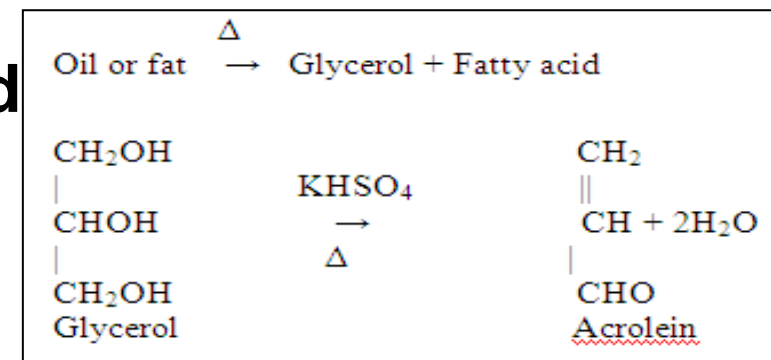
is used to detect glycerol or fats.

-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  $\text{KHSO}_4$  [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** 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 ?**