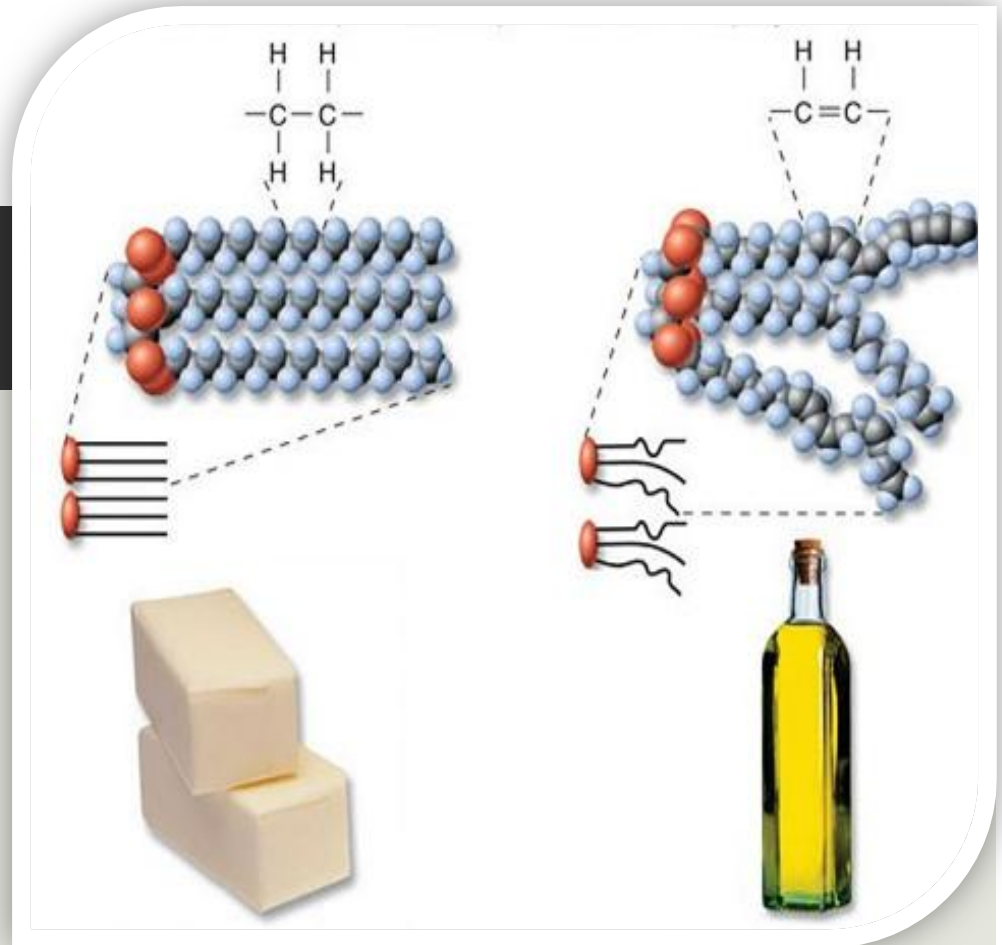


# Lipids

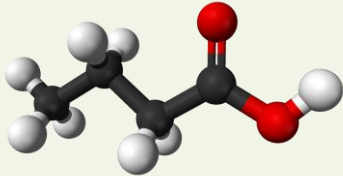
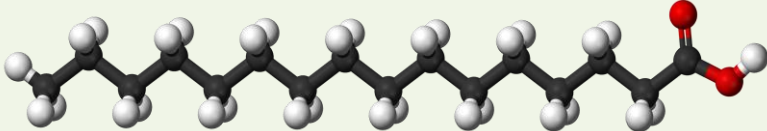
Qualitative test of lipids (II)



# Fatty Acids Classification

## 1. Saturated fatty acids:

- Have no double bonds ,side chain are (alkane).
- Depending on the side chain, it's divided to:

Short Side Chain	Long Side Chain
From <b>2 to 10</b> Carbon atoms, present as <u>liquid</u> in room temp.	More than <b>10</b> Carbon atoms, present in <u>solid</u> at room temp.
e.g. <b>butyric acid</b> (4C).	e.g. <b>Palmatic acid</b> (16C).
	

# Fatty Acids Classification

## 2. Unsaturated fatty acids:

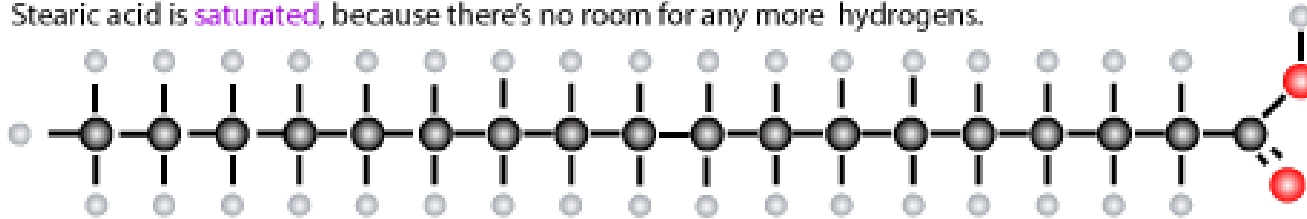
- Unsaturated fatty acids have **one or more** double bonds between carbon atoms, side chain has at least one double bond.
- Divided to:

Essential Fatty Acids	Nonessential Fatty Acids
Cannot be synthesized by the body	Can be synthesized in the body
<b>(Linoleic acid, Linolenic acid)</b>	e.g. <b>Oleic acid</b>

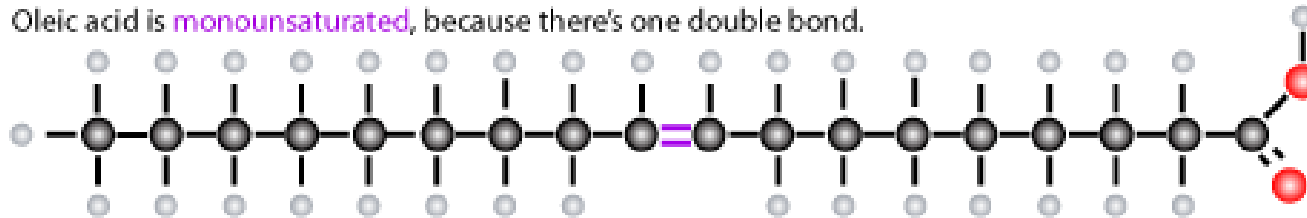
- ◆ Mammals lack the ability to introduce double bonds in fatty acids beyond carbon 9 and 10, because ...  
→ Humans lack the **(desaturase enzymes)** required for their production.

## Nonessential fatty acids (your body can make its own supply)

Stearic acid is **saturated**, because there's no room for any more hydrogens.

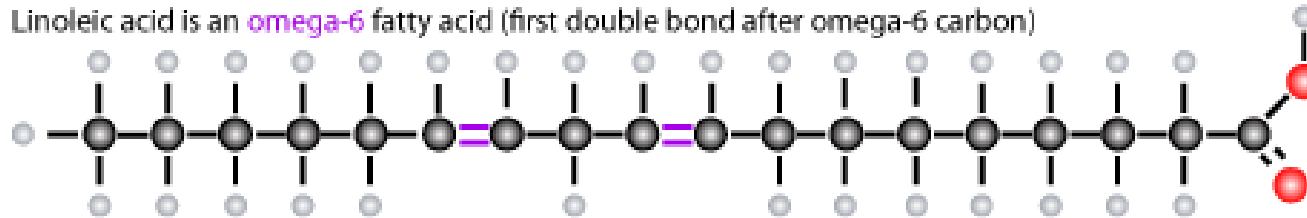


Oleic acid is **monounsaturated**, because there's one double bond.

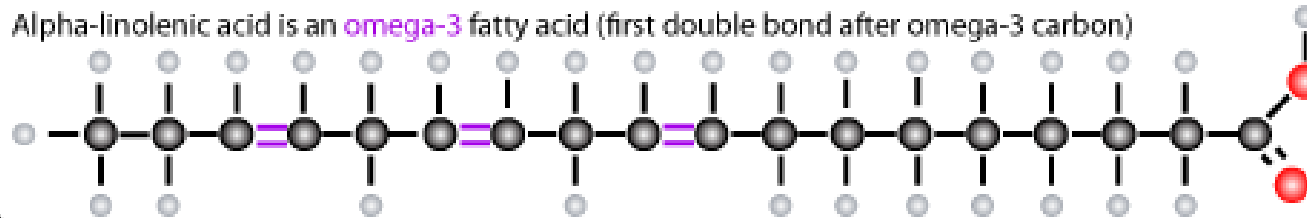


## Essential fatty acids (they have to come from your food)

Linoleic acid is an **omega-6** fatty acid (first double bond after omega-6 carbon)



Alpha-linolenic acid is an **omega-3** fatty acid (first double bond after omega-3 carbon)



Omega-1  
carbon

● = carbon   ● = oxygen   ● = hydrogen

# Qualitative tests of lipids II

1. Acrolein test.
2. Sudan IV Test.
3. Copper acetate Test.
4. Qualitative estimation of Cholesterol by Liebermann - Burchard Test.
5. Unsaturation Test.

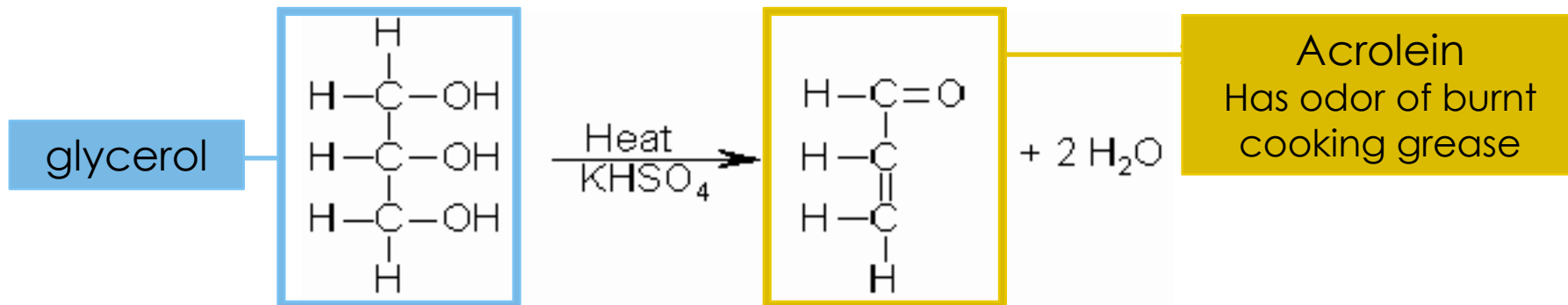
# 1<sup>st</sup> Acrolein test

## Objective:

- To detect presence of fats or glycerin.

## Principle:

- When a fat is heated strongly in the presence of a dehydrating agent such as potassium bisulfate (KHSO<sub>4</sub>), the glycerol portion of the molecule is dehydrated to form the unsaturated aldehyde, **acrolein (CH<sub>2</sub>=CH-CHO)**,
- which has the odor peculiar to burnt cooking grease.



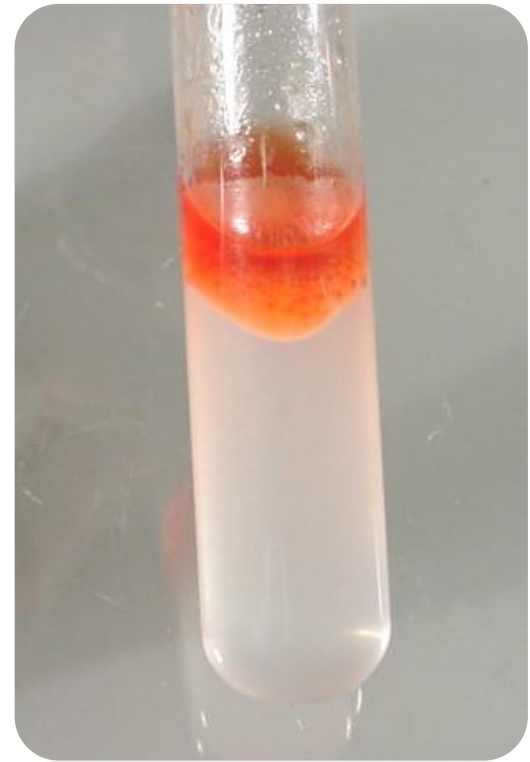
# 2<sup>nd</sup> Sudan IV test

- **Objective:**

- To detect presence of lipids.

- **Principle:**

- **Sudan IV is a non-polar stain** that readily stains lipids **red-orange**
- but does not stain **polar compounds** or aqueous solutions



# 3<sup>ed</sup> Copper acetate Test

## □ Objective:

- To **distinguish** between oil or neutral fat and fatty acid saturated and unsaturated.

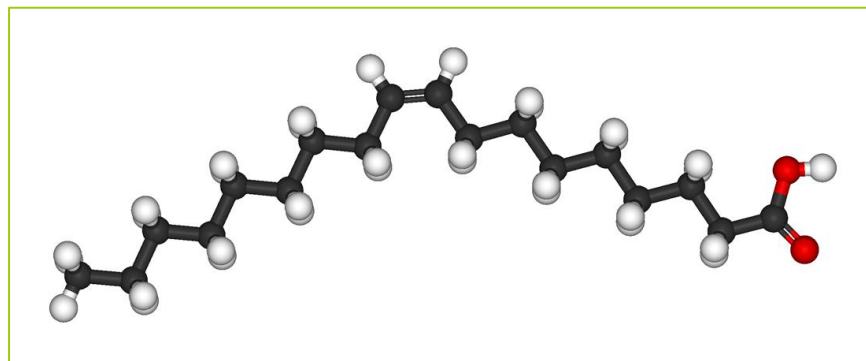
## □ Principle:

- The copper acetate solution does not react with the oils,
- while **saturated** and **unsaturated fatty acids** react with copper acetate to form **copper salt**.
- **unsaturated fatty** acids can only be extracted by petroleum ether.

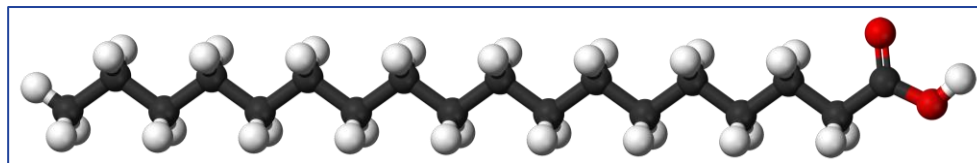


# 3<sup>ed</sup> Copper acetate Test

- In the case of **olive oil** notice that petroleum ether upper layer containing the dissolved oil and appears colorless, aqueous solution remains blue in the bottom.
- In the case of **oleic acid** the **upper layer** of petroleum ether becomes **green** as a result of copper oleate. The lower layer becomes less in blue.



- In the case of **stearic acid** notice that the petroleum ether upper layer remains colorless, while consists of **pale green precipitate** of copper stearate at the **bottom**.



# 3<sup>ed</sup> Copper acetate Test



petroleum ether  
and dissolved oil

copper acetate

Olive oil



copper oleate in  
the petroleum ether

copper acetate

Oleic acid

# 3<sup>ed</sup> Copper acetate Test

## □ Materials:

Olive oil , oleic acid, petroleum ether ,copper acetate solution (5%).

## □ Method:

□ Take two test tubes put 1 / 2 g of each sample and then added 3 ml of petroleum ether and an equal volume of a solution of copper acetate.

## □ Results:

Tube	Observation	Comments
Olive oil		
Oleic acid		

# 4<sup>th</sup> Qualitative estimation of Cholesterol by Liebermann - Burchard Test

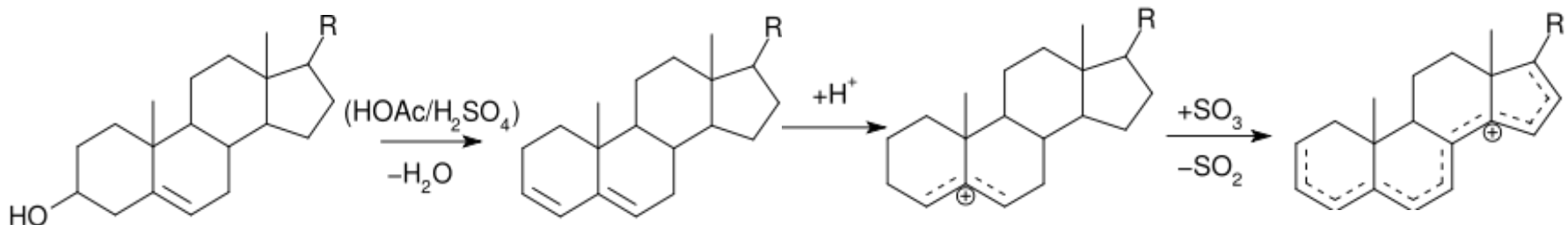
## Objective:

- To detect the presence of cholesterol.

## Principle:

- 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, and the sulfuric acid is used as dehydrating and oxidizing agent.
- A positive result is observed when the solution becomes ...

(red → blue, and finally → **bluish –green** color)



# 4<sup>th</sup> Qualitative estimation of Cholesterol by Liebermann - Burchard Test

## Materials:

Crystals of cholesterol, Acetic anhydride, Concentrated sulfuric acid, Chloroform.

## 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.

## Results:

Tube	Observation	Comments
Cholesterol		
Olive Oil		

# 5<sup>th</sup> Unsaturation test

## □ Objective:

- To indicate the amount of presence of double bonds in the lipid sample.

## □ Principle:

- All neutral fats 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.



# 5<sup>th</sup> Unsaturation test

## □ Materials:

Hubl's iodine reagent (alcoholic solution of iodine containing some mercuric chloride), Chloroform, Mustard oil, coconut oil, olive oil, saturated fat.

## □ Method:

- Equally into 4 flask Add 10 ml of Chloroform then 10 drops of Hubl'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.**

# 5<sup>th</sup> Unsaturation test

## □ Result:

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



# Comparing between content of fatty acid in different type of oils:

Type of oil	Capric (Decoic) C10	Palmitic C16	Stearic C18	Oleic (C18:1)	Linolenic (C18:3)	Linoleic (C18:2)	Arachidic C20	Any special fatty acid
<b>Coconut Oil</b>	6.0-10.0	8.0-11.0	1.0-3.0	5.0-8.0	----	0-1.0	0-0.5	Palmitoleic tr -2.5
Corn (Maize) Oil	7.0	8.0-12.0	2.0-5.0	19.0-49.0	----	34.0-62.0	----	----
Cotton Seed Oil	----	20	2.0	35	----	42	----	----
<b>Mustard Seed Oil</b>	----	1.5	0.4	<b>22</b>	6.8	<b>14.2</b>	----	Erucic 47.0
<b>Olive Oil</b>	----	7.0-16.0	1.0-3.0	<b>65.0-80.0</b>	----	4.0-10.0	0.1-0.3	----
Palm Oil	----	32.0-45.0	2.0-7.0	38.0-52.0	----	5.0-11.0	----	----
<b>Safflower Oil</b>	----	3.0-6.0	1.0-4.0	13.0-21.0	tr.	<b>73.0-79.0</b>	0.2	----
Sesame Oil	----	7.0-9.0	4.0-5.0	40.0-50.0	----	35.0-45.0	0.4-1.0	----
Soya Bean Oil	----	7.0-11.0	2.0-6.0	22.0-34.0	5.0-11.0	<b>43.0-56.0</b>	----	----
<b>Sunflower Seed Oil</b>	----	----	----	----	3.0-6.0	1.0-3.0	14.0-35.0	Behenic 0.8
<b>Wheat Germ Oil</b>	----	----	----	----	11.0-16.0	1.0-6.0	8.0-30.0	Tocopherol 0.3-0.5

**.. Now ..**

‘ Wear your gloves and lab coat  
And Act Like a biochemist ’

**Thank You**

