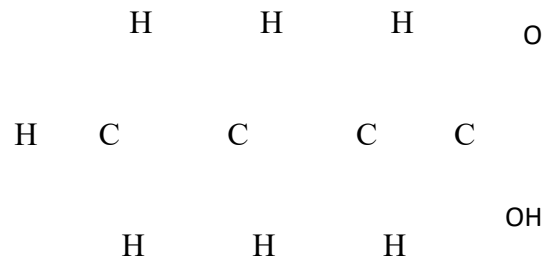


## Aim - Qualitative Test for lipids

**Lipid**, a [diverse](#) group of organic [compounds](#) including fats, oils, waxes, phospholipids, hormones, and certain components of membranes that are grouped together because they are insoluble in water but soluble in non-polar organic solvents. The [triglycerides](#), is sequestered as [fats](#) in [adipose cells](#) served as the energy-storage depot for organisms and also provide thermal insulation. Fats and oils are made from two kinds of molecules: glycerol (a type of alcohol with a hydroxyl group on each of its three carbons) and three fatty acids joined by dehydration synthesis and are known as triglycerides. The basic building blocks of lipids are fatty acids which are carboxylic acids with long hydrocarbon chains attached. Fatty acids consists of the elements carbon (C), hydrogen (H) and oxygen (O) arranged as a carbon chain skeleton with a carboxyl group (-COOH) at one end. The terms saturated, mono-unsaturated and poly-unsaturated differ in the number of hydrogen attached to the hydrocarbons tails of the fatty acids as compared to the number of double bonds between carbon atoms in the tail. The triglycerides containing the maximum possible amount of hydrogen (presence of number of carbon-carbon double bonds), are called saturated fats. Carbons sharing double bonds and are not bonded to as many hydrogen are called unsaturated fats. Monounsaturated fatty acids (MUFAs) have only one double bond. Polyunsaturated fatty acids (PUFAs) have more than one double bond. The hydrocarbon chains in saturated fatty acids are, thus, fairly straight and can pack closely together, making these fats solid at room temperature. Because of the *cis* double bonds in the hydrocarbon tails, unsaturated fats can't pack as closely together, making them liquid at room temperature. Some of qualitative tests are described below.

The general structure of a lipid molecule is:

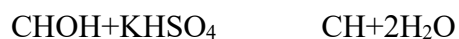


### (a) Acrolein Test for Glycerol

**Principle:** Acrolein test is used to detect the presence of glycerol or fat. When fat is treated strongly in the presence of a dehydrating agent like potassium bisulphate ( $\text{KHSO}_4$ ) the glycerol portion of the molecule gets dehydrated to an unsaturated aldehyde called acrolein which can be identified by its characteristic pungent smell.



Heat



Glycerol

Acrolein

### Materials:

- Lipid samples
- Anhydrous potassium hydrogen sulphate
- Potassium bisulphate or conc.  $\text{H}_2\text{SO}_4$
- Dry test tube

### Method:

- Take approximately 1.5g of anhydrous potassium hydrogen sulphate in a test tube.
- Add 5 drops of oil into the test tube.
- Add 1ml of conc.  $\text{H}_2\text{SO}_4$  or  $\text{KHSO}_4$ .
- Heat the tube with the contents slowly
- Note the characteristics pungent smell for acrolein.

### (b) Test for the Presence of Fatty Acids

**Principle:** The free fatty acids (non-esterified) present in a given sample of fat or oil can be

determined by titrating it against an alkali.

**Materials:**

- Lipid samples
- 0.1N NaOH
- Phenolphthalein indicator: 1% solution in alcohol

**Method:**

- Take 10 ml of 0.1N NaOH in a conical flask
- Add few drops of phenolphthalein indicator till it give a permanent pink colour
- Add test solution drop by drop with constant shaking of the flask
- Observe for the disappearance of pink colour indicating the presence of free fatty acids in the test sample.

**(c) Test for Unsaturation of Fatty Acids**

**Principle:** Fatty acids in animals usually contain more saturated fatty acids whereas fats of those aquatic animals and vegetables are generally unsaturated containing one or more double bonds. Since, halogens are readily added across the double bonds and this reaction is observed by decolourisation of bromine or iodine solution will indicate the presence of unsaturated fatty acids.

**Materials:**

- Lipid samples
- Bromine water: 5% solution is prepared in distilled water and is kept in dark bottle

**Method:**

- Take 5ml test solution in a test tube.
- Add bromine water to it drop by drop with shaking till the bromine solution retains its colour.
- Relative degree of unsaturation of various lipids is assessed from the amount of bromine water used (decolourised).