Qualitative tests of lipids

Lipids

Lipids are a heterogeneous group of compounds, including fats, oils, steroids, waxes, and related compounds, which are related more by their physical than by their chemical properties. Lipids are a class of compounds distinguished by their insolubility in water and solubility in non polar solvents. Lipids are important in biological systems because they form the cell membrane, a mechanical barrier that divides a cell from the external environment. Non polar lipids, such as triglycerides, are used for energy storage and fuel. Polar lipids, which can form a barrier with an external water environment, are used in membranes. Polar lipids include glycerophospholipids and sphingolipids. Fatty acids are important components of all of these lipids.

Lipids have the common property of being relatively insoluble in water and soluble in nonpolar solvents such as ether and chloroform. They are important dietary constituents not only because of their high energy value but also because of the fat-soluble vitamins and the essential fatty acids contained in the fat of natural foods.

A. Solubility test

1. Principle.

This test is used to know the solubility of lipids in some solvents, according to polarity feature lipids are insoluble in polar solvents because lipids are non polar compounds, thus lipids are soluble in non polar solvents like chloroform, benzene and boiling alcohol.

1. Procedure.

1. In two clean dry test tubes add 1 ml of olive oil for each tube.
2. Add for the first one 1 ml of chloroform.
3. Add for the second tube 1 ml of distilled water.
4. Shake both tubes vigorously for 2 minutes.
5. Allow the tubes to stand and note the formation of homogenous solution with chloroform indicating that the lipid is dissolved and the formation of two layers with water indicating that the lipid is insoluble in water.

B. Saponification test

1. Principle.

This test is used to form soap by saponification process which includes the hydrolysis of fat or oil with alkaline to yield glycerol and salt of fatty acid (soap).

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\text{Triglyceride} + \text{KOH} \rightarrow \text{Glycerol} + \text{Soap}
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2. Procedure.

1. In clean dry large test tubes add 2 ml of olive oil.
2. Add 4 ml of 20% alcoholic potassium hydroxide.
3. Shake well and boil the mixture in boiling water bath for 5 minutes.
4. Make sure after the five minutes that the soap is formed, all of the fat is converted to soap and an alcohol is evaporated. If not continue the boiling.
5. The solid material formed is soap.

C. Iodine test

1. Principle.

This test is used to test the degree of unsaturation of fatty acids. Fatty acids in animal fats are usually saturated, whereas those in vegetable oils are generally unsaturated. Halogens like iodine or bromine when added to unsaturated fatty acid the double bond will be
saturated and decolorize the iodine or bromine, the decolorization indicates the presence of unsaturated fatty acids. Iodine test is used for distinguish between saturated and unsaturated fatty acids as well as between oils and fats.

2. Procedure.

1. In clean dry test tube add 1 ml of oleic acid (unsaturated).
2. In other test tube add 1 ml of stearic acid (saturated).
3. Add for each tube 5 drops of hubl’s reagent.
4. Observe that the color of halogen in the reagent will be disappeared when it is added to the oleic acid until we reaches a certain point after which the color of the reagent will persist indicating that all of the double bonds are saturated by halogens.
5. Observe that the color of halogen in the reagent does not changed when it is added to stearic acid because this fatty acid is saturated.