Experiment 7

QUALITATIVE TESTS FOR LIPIDS

Structure

7.1 INTRODUCTION

In the previous experiments (5 & 6) we have studied about various qualitative tests routinely used to identify the presence or absence of carbohydrates, amino acids, proteins. In this experiment we'll know about qualitative tests used to identify lipids in a given test sample.

Lipids being insoluble in water known as hydrophobic biomolecules, but they are soluble in organic solvents like benzene, chloroform, hexane and diethyl ehter.Recall the properties of lipids which we have studied in block-3 of BBCCT-101. It is well known fact that lipids are high energy compounds and are classified as simple, conjugated and derived lipids. Apart from acting as energy source, lipids have diverse functions ranging from plasma membrane formation, micelle formation, hormonal action and thermal insulator etc.

However the availability of free floating lipids in blood and their accumulation in various vital parts of our body is one of the major causes for life style disorders like obesity and cardiovascular diseases. However in this experiment we will be studying about the different tests performed to identify the chemical nature of lipids i.e., saturated or unsaturated along with presence or absence of sterols. For better understanding of this experiment, it is advised to go through the physical and chemical properties of lipids discussed in Unit 9 of BBCCT-101.

The tests conducted to identify lipids are specific to the chemical nature and functional groups present on them. Qualitative analysis of lipids has a significant contribution in identifying the adulteration of edible oils and hence has vital role in industry, as well as in health sector. Before performing the experiment watch the video available at the given YouTube link: https://www.youtube.com/watch?v=l2QOi9mZoFc

Expected Learning Outcomes

After performing this experiment, you should be able to:

- ❖ explain the principle behind the specific test;
- ❖ identify specific fatty acid in a given solution;
- ❖ distinguish between identification and conformation tests; and
- ❖ enlist various tests used for identification of lipids.

7.2 MATERIAL AND PRINCIPLES

Materials Required:

Glassware: 10 mL boiling test tubes, pipettes (1-10 mL), dropper, glass rod and watch glass.

Equipment and accessories: Water bath, test tube holder, spatula and blotting paper.

Test sample: The commercially available oil samples like Coconut oil, Ghee, Palm oil, Butter and cholesterol can be used.

Reagents and Chemicals:

Alcoholic-KOH (2% w/v KOH in ethyl alcohol): Dissolve 2 g of potassium hydroxide (KOH) in 100 mL of ethyl alcohol.

Dilute potassium permanganate solution (0.05% w/v): Dissolve 50 mg of potassium permanganate in 100 mL of distilled water.

Hubl's Iodine Solution: To prepare this (a) dissolve 2.6 g of iodine in 40 mL of ethanol (95% v/v), (b) dissolve 6.0 g of mercuric chloride in 40 mL of ethanol (95% v/v). Transfer both solutions 'a' and 'b' into a beaker and make up the volume to 100 mL with same ethanol.

Potassium bisulphate (solid)

Bromine water

Acetic anhydride and Conc. sulphuric acid

Solvents: Chloroform, ether, benzene, carbon tetrachloride, hexane,

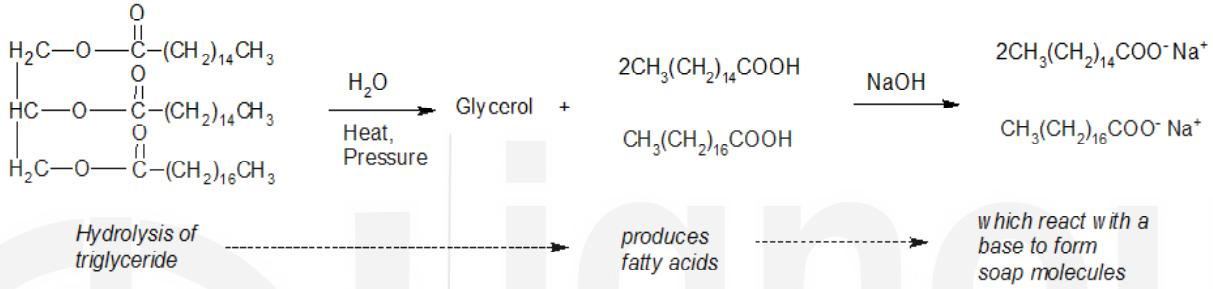
PRINCIPLES

Solubility test: This is a primary step to know the chemical nature of the given test sample

Principle: Due to hydrophobic of nature of lipids they are insoluble in water and are soluble in organic solvents.

Saponification test:

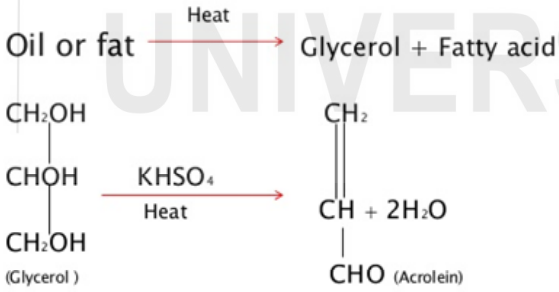
Principle: Lipids upon alkaline hydrolysis release glycerol and fatty acids. Later sodium (Na^+) or potassium (K^+) ions combines with fatty acids to form "soap" (foam). Hence, this is known as saponification reaction.



Reaction showing formation of soap molecule.

Acrolein test:

Principle: Formation of acrolein or acrylic aldehyde that has characteristic pungent odor is the key principle. In general lipids upon heating with potassium bisulphate produce acrolein.



Reaction showing formation of acrolein.

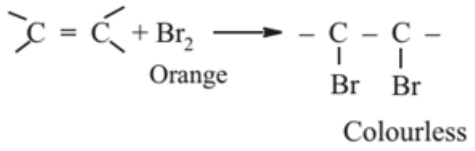
Hubl's iodine test (Tests for unsaturation):

This test is mainly helpful to assess the unsaturation in a given oil sample. The two principles that will explain the reaction mechanism are given below.

Principle

i) Bromine or iodine upon reacting with unsaturated fatty acids produce di-halo adducts. Consumption of more bromine indicates the higher percentage of unsaturation.

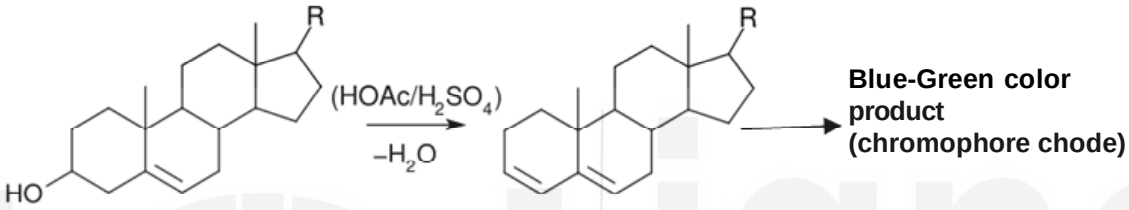
ii) Decolorization of alkaline potassium permanganate is also an indirect measure of unsaturation in fatty acids, where unsaturated fatty acids undergo incomplete oxidation.



Reaction showing formation of di-halo adduct.

Salkowski test and Liebermann-Burchard test (Test for cholesterol):

Principle: Cholesterol in the presence of concentrated sulphuric acid and acetic anhydride undergo dehydration producing colored product which is blue-green in color.



Reaction showing formation of blue-green chromophore.

7.3 PROCEDURE FOR QUALITATIVE ANALYSIS OF LIPIDS

Table 7.1: Qualitative analysis of lipids

Sl. No.	Test	Observation	Inference
1	**Solubility test:** To small quantities of fat/ fatty acid taken in a separate test tube add the following organic solvents separately: 1. a) Chloroform, b) ether, c) benzene, d) hexane (3 mL/ test tube) 2. Distilled water	i) Completely soluble in all four solvents *a - d*. ii) Found small floating droplets on water.	i) Indicates presence of fat or oil in the given solution ii) Indicates presence of fat or oil in the given solution.

2	**Saponification test:** Take approximately 100 mg of oil or fat in a test tube. Add 3 mL of alcoholic-KOH and mix well. Place the tube in a boiling water bath for 15-20 min.	Appearance of foamy solution	Indicates the presence of oil or fat
3	**Acrolein test:** Take approximately 100 mg of test sample in a test tube and add a pinch of potassium bisulphate and mix well. Heat the mixture over a Bunsen burner for 1-2 minutes.	Release of pungent odor	Indicates Presence of glycerol
4	**Tests for unsaturation:** **i) Bromine water test:** Take 1 mL of oil or 100 mg of fat in a test tube add 2 mL of non-polar organic solvent (Refer solubility test) and dissolve completely. To this add potassium permanganate solution or bromine water drop by drop.	i) Decolourisation of potassium permanganate or bromine water is observed ii) No decolorisation observed.	i) Indicates presence unsaturated fats or fatty acids ii) Indicates presence of saturated fats and fatty acids
	ii) Hubl's iodine test: Take 1 mL of oil or 100 mg of fat in a test tube add 2 mL of non-polar organic solvent (Refer solubility test) and dissolve completely. Add Hubl's reagent drop wise mixing the contents.	i) Decolourisation of iodine solution is observed ii) No decolrisation of Iodine is observed.	i) Indicates presence of unsaturated fats ii) Indicates absence of unsaturated fats or oils or presence of saturated fats or oils.

5	**Test for cholesterol:** 1. **Salkowski test:** Take 1 mL of oil or fat sample in a test tube and dissolve in 1 mL chloroform to this add equal volume of concentrated sulphuric acid along the walls of the tube. (Do not mix the contents).	Observes formation of blue-green color at the junction of two liquids	Indicates presence of cholesterol
	2. **Liebermann-Burchard test:** Take approximately 100 mg of fat or 1 mL of oil in a test tube, dissolve by adding 1 mL of chloroform add equal volumes of acetic anhydride followed by drop wise addition (along walls) of concentrated sulphuric acid.	Formation of an emerald green color	Confirms the presence of cholesterol

Self-Assessment Questions

1. List the organic solvents used for solubility test?
2. What is the importance of Iodine test?
3. Name the test used to identify the presence of cholesterol in the test sample?
4. _____ test principle based on soap formation.

7.4 SUMMARY

- The solubility test gives us an idea about the nature of the sample i.e., polar or non-polar.
- Formation of foamy solution in saponification test indicates the presence of fat.

- Hubl's iodine test confirms the presence or absence of unsaturation (double bonds) in the given sample.
- Salkowski test and Liebermann-Burchard test indicates and confirms the presence of sterol ring containing molecule i.e., cholesterol.

7.5 FURTHER READING

1. Experimental Biochemistry: A student Companion. Beedu Sashidhar Rao and Vijay Deshpande. ISBN 81-88237-41-8, I.K. International Pvt. Ltd.
2. Practical Biochemistry: for medical, dental and allied courses. 2nd edition, Dr. G. Rajagopal and Dr.B.D. Toora.ISBN 81-901769-5-1, Ahuja publishing house.
3. Preparative Organic Chemistry CHE-08 (L), Chemistry Lab-III. ISBN 81-7263-333-5, Published by Indira Gandhi National Open University, 1993 (Reprint December-2006).
4. Segel, I.H. Biochemical Calculations. 2nd ed. John Wiley & Sons.Inc.New York (1976).
5. Laboratory manual of Microbiology and Biotechnology (second edition), K.R. Aneja.ISBN 978-93-87025-49-3.MEDTECH a division of Scientific international (Pvt. Ltd).