	YILDIZ TECHNICAL UNIVERSITY **BIOMEDICAL ENGINEERING DEPARTMENT** **BME2901- BIOCHEMISTRY COURSE** **2020-2021 FALL SEMESTER**

EXPERIMENT 8

LIPID EXTRACTION AND DETERMINATION

1. THEORETICAL KNOWLEDGE

Lipids are biomolecules having a diverse group of organic compounds that include fatty acids, waxes, phospholipids, glycolipids, and sterols. These compounds are insoluble or poorly soluble in water due to the presence of long hydrocarbon chains in their structures. They are soluble in organic solvents such as benzene, ether or chloroform.

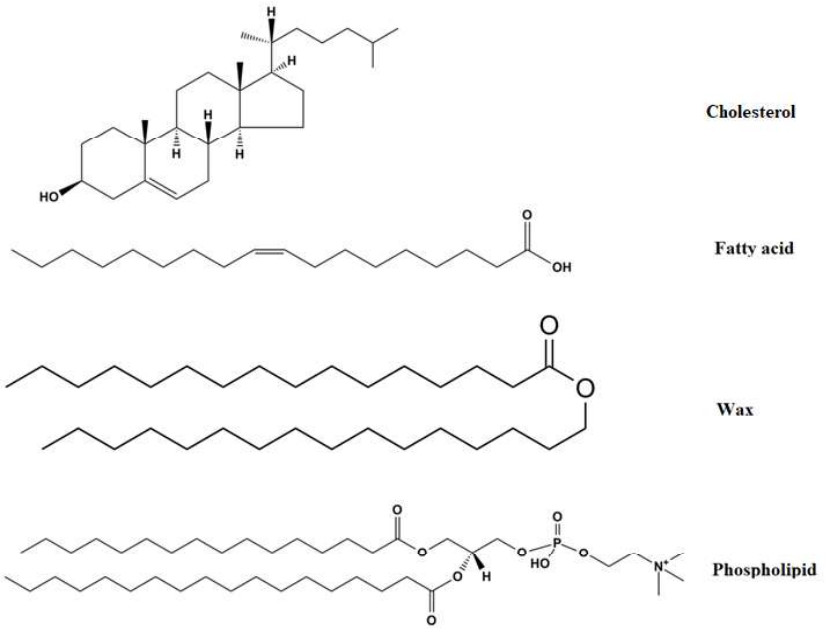

Figure 1. Structures of some common lipids.

Lipids perform a diverse range of functions within the cells of living organisms, such as 1) acting as a storage form of metabolic fuel or energy, e.g. fats 2) being an integral component

of the biological membrane, e.g. phospholipids and cholesterol 3) signaling molecule, e.g. hormones, acting as 4) cofactors, e.g. vitamins, 5) pigments, chlorophyll and 4) protective coat in bacteria, plants, insects, and invertebrates, e.g. waxes.

Cholesterol, the major sterol in animal tissues, is amphipathic, with a polar head group and a nonpolar hydrocarbon body. It has a short and rigid structure so that it fills the spaces between neighboring phospholipid molecules making the membrane less flexible. Similar sterols are found in other eukaryotes: stigmasterol in plants and ergosterol in fungi, for example. Bacteria cannot synthesize sterols; a few bacterial species, however, can incorporate exogenous sterols into their membranes.

The principle physicochemical characteristics of lipids used to distinguish them from the other components in foods are their solubility in organic solvents, immiscibility with water, low density, unique physical and spectroscopic properties. The analytical techniques based on these principles can be categorized into three different classes; solvent extraction, non-solvent extraction and instrumental methods.

Solvent extraction procedures are one of the most commonly used methods of isolating lipids from any working material, such as food, animal and plant tissues, bacterial cells, fungi and yeast cells. The fact that lipids are soluble in organic solvents, but insoluble in water, provides the analyst with a convenient method of separating the lipid components in the working material from water soluble components, such as proteins, carbohydrates and minerals.

The ideal solvent for lipid extraction would completely extract all the lipid components from the material, while leaving all the other components behind. In practice, the efficiency of solvent extraction depends on the polarity of the lipids present compared to that of solvent. Polar lipids (glycolipids or phospholipids) are more soluble in polar solvents than in non-polar solvents. On the other hand, non-polar lipids (triacylglycerols) are more soluble in non-polar solvents than in polar ones. In addition to the above considerations, a solvent should also be inexpensive, have a relatively low boiling point (so that it can be easily removed by evaporation), be non-toxic and be nonflammable.

Folch method, is one of the standard procedures to isolate total lipid fractions from biological matrices based on a solvent system consisting of chloroform/methanol/water. It is important that the ratio of chloroform, methanol and saline solution in the final mixture be close to 8:4:3. On the other hand, some modifications in the approach to reach the optimum

concentrations can be applied. In this method, the endogenous water in the tissue was considered as a component of the extraction system.

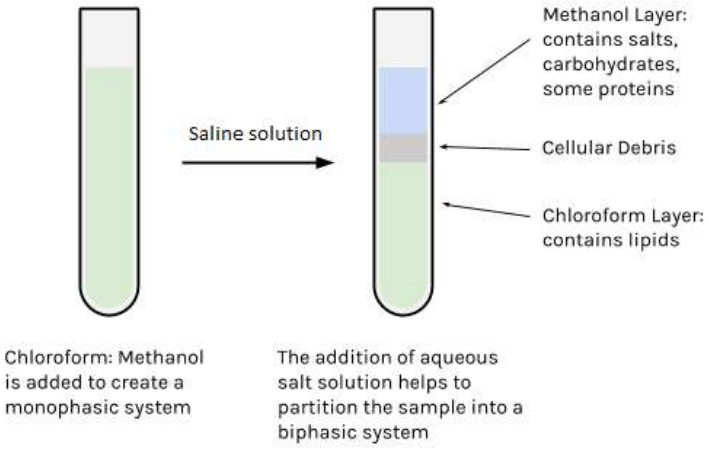

Figure 2. Folch extraction method.

Salkowsky experiment is a test which is applied for the qualitative analysis of cholesterol in a given liquid sample. It depends on the red coloring of the sterols with concantrated sulfiric acid as a result of the unsaturated lipid branches in their structures.

2. MATERIALS

- Egg
- Butter
- Olive oil
- Saline solution
- Methanol
- Chloroform
- Concentrated sulfuric acid
- Beakers
- Centrifuges tubes
- Centrifuge
- Magnetic stirrer

3. METHOD

a) Lipid Extraction

1. Test tubes 1-6 are weighed out and marked.
2. The egg is cracked open and the yolk is placed into a 100 ml beaker while the white is placed into another 100 ml beaker. Each part is homogenized by using a magnetic stirrer.
3. 1 ml of the yolk is measured out into test tube 1 by using the 10 ml graduated cylinder. This process is repeated with the white and placed into test tube 2. Each of these is diluted with 4 ml of saline.
4. 1 ml of each of two diluted solutions is measured out, the yolk being placed into the test tube 3 and white into test tube 4.
5. 1 ml of methanol and 2 ml of chloroform are added into both of the tubes 3 and 4. Tubes are centrifuged for 5 min at 2000 RCF.
6. After centrifugation, the bottom layer which is composed of lipids and chloroform is placed into test tube 5 (coming from test tube 3- the yolk) and test tube 6 (coming from test tube 4- the white).
7. These tubes are allowed to evaporate to ensure total evaporation.

b) Salkowsky Experiment for Lipids

Name four test tubes as positive control (butter and olive oil), negative controls (distilled water), sample 1 (egg yolk) and sample 2 (egg white). Transfer 1 ml of each sample into their named tubes. Then add 1 ml of chloroform into each test tube and add 500 µl of concentrated sulfuric acid. Mix the tubes and detect red-purple color appearance.