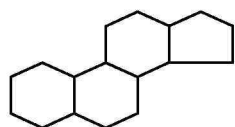


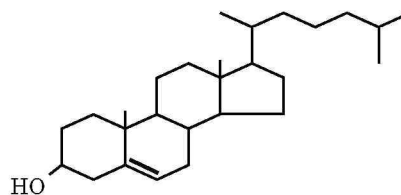
Analysis of lipids

Lipids are chemically heterogeneous mixtures. The only common property they have is their insolubility in water. We can test for the presence of various lipids by analyzing their chemical constituents. Foods contain a variety of lipids, most important among them are fats, complex lipids, and steroids. Fats are triglycerides, esters of fatty acids and glycerol. Complex lipids also contain fatty acids, but their alcohol may be either glycerol or sphingosine. They also contain other constituents such as phosphate, choline, or ethanolamine or mono- to oligo-saccharides. An important representative of this group is lecithin, a glycerophospholipid, containing fatty acids, glycerol, phosphate, and choline. The most important steroid in foods is cholesterol. Different foods contain different proportions of these three groups of lipids.

Structurally, cholesterol contains the steroid nucleus that is the common core of all steroids.



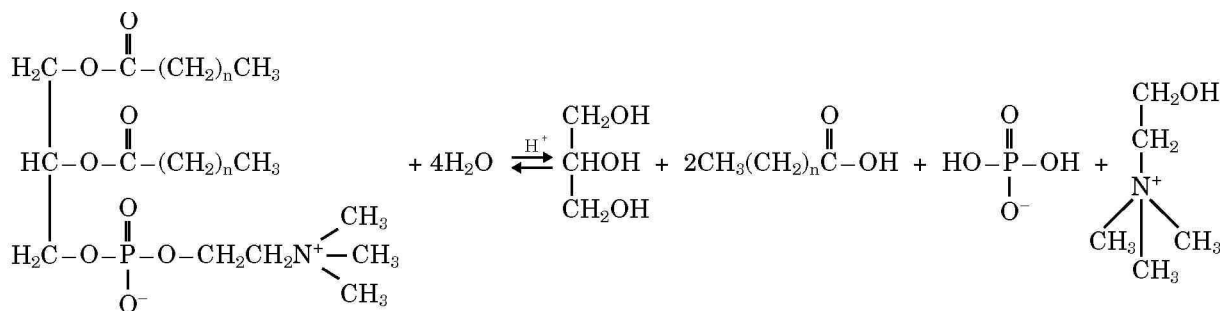
Steroid nucleus



Cholesterol

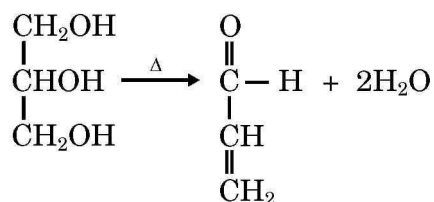
There is a special colorimetric test, the Liebermann-Burchard reaction, which uses acetic anhydride and sulfuric acid as reagents, that gives a characteristic green color in the presence of cholesterol. This color is due to the OH group of cholesterol and the unsaturation found in the adjacent fused ring. The color change is gradual: first it appears as a pink coloration, changing later to lilac, and finally to deep green.

When lecithin is hydrolyzed in acidic medium, both the fatty acid ester bonds and the phosphate ester bonds are broken and free fatty acids and inorganic phosphate are released. Using a molybdate test, we can detect the



presence of phosphate in the hydrolysate by the appearance of a purple color. Although this test is not specific for lecithin (other phosphate containing lipids will give a positive molybdate test), it differentiates clearly between fat and cholesterol on the one hand (negative test), and phospholipid on the other (positive test).

A second test that differentiates between cholesterol and lecithin is the acrolein reaction. When lipids containing glycerol are heated in the presence of potassium hydrogen sulfate, the glycerol is dehydrated, forming acrolein, which has an unpleasant odor. Further heating results in polymerization of acrolein, which is indicated by the slight blackening of the reaction mixture. Both the pungent smell and the black color indicate the presence of glycerol, and thereby fat and/or lecithin. Cholesterol gives a negative acrolein test.



Objectives

To investigate the lipid composition of common foods such as corn oil, butter, and egg yolk.

Procedure

Use six samples for each test: (1) pure cholesterol, (2) pure glycerol, (3) lecithin preparation, (4) corn oil, (5) butter, (6) egg yolk.

Phosphate Test

CAUTION!

6 M nitric acid is a strong acid. Handle it with care. Use gloves.

- 1- Take six clean and dry test tubes. Label them. Add about 0.2 g of sample to each test tube.
- 2- Hydrolyze the compounds by adding 3 mL of 6 M nitric acid to each test tube.
- 3- Prepare a water bath by boiling about 100 mL of tap water in a 250-mL beaker on a hot plate.
- 4- Place the test tubes in the boiling water bath for 5 min. Do not inhale the vapors.
- 5- Cool the test tubes. Neutralize the acid by adding 3 mL of 6 M NaOH.
- 6- Mix. During the hydrolysis, a precipitate may form, especially in the egg yolk sample. The samples in which a precipitate appeared must be filtered. Place a piece of cheese cloth on top of a 25-mL Erlenmeyer flask. Pour the turbid hydrolysate in the test tube on the cheese cloth and filter it.
- 7- Transfer 2 mL of each neutralized (and filtered) sample into clean and labeled test tubes. Add 3 mL of a molybdate solution to each test tube and mix the contents.
- 8- Heat the test tubes in a boiling water bath for 5 min. Cool them to room temperature
(Be careful. The molybdate solution contains sulfuric acid.)
- 9- Add 0.5 mL of an ascorbic acid solution and mix the contents thoroughly. Wait 20 min. for the development of the purple color. Record your observations on the Report Sheet. While you wait, you can perform the rest of the colorimetric tests.

The Acrolein Test for Glycerol

1. Place 1 g of potassium hydrogen sulfate, KHSO_4 , in each of seven clean and dry test tubes. Label them. Add a few grains of your pure preparations, lecithin and cholesterol, to two of the test tubes. Add a drop, about 0.1 g, from each, glycerol, corn oil, butter, and egg yolk to the other four test tubes. To the seventh test tube add a few crystals of sucrose.

2. Set up your Bunsen burner in the hood. It is important that this test be performed under the hood because of the pungent odor of the acrolein.
3. Gently heat each test tube, one at a time, over the Bunsen burner flame, shaking it continuously from side to side. When the mixture melts it slightly blackens, and you will notice the evolution of fumes. Stop the heating. Smell the test tubes by moving them sideways under your nose or waft the vapors. Do not inhale the fumes directly. A pungent odor, resembling burnt hamburgers, is the positive test for glycerol. Sucrose in the seventh test tube also will be dehydrated and will give a black color. However, its smell is different, and thus is not a positive test for acrolein. Do not overheat the test tubes, for the residue will become hard, making it difficult to clean the test tubes. Record your observations on the Report Sheet.

Lieberman-Burchard Test for Cholesterol

1. Place a few grains of your cholesterol and lecithin preparations in labeled clean and dry test tubes. Similarly, add about 0.1-g samples of glycerol, corn oil, butter, and egg yolk to the other four labeled clean and dry test tubes. (The next step should be done in the hood.)
2. Transfer 3 mL of chloroform and 1 mL of acetic anhydride to each test tube. Finally, add 1 drop of concentrated sulfuric acid to each mixture. Mix the contents and record the color changes, if any. Wait 5 min. Record again the color of your solutions. Record your observations on the Report Sheet.

NAME

SECTION

DATE

PARTNER
REPORT SHEET

GRADE

Tests	Cholesterol	Lecithin	Corn Glycerol	oil	Butter	Egg yolk	Sucrose
1. Phosphate a. Color							
b. Conclusions							
2. Acrolein a. Odor							
b. Color							
c. Conclusions							
3. Lieberman- Burchard a. Initial color							
b. Color after 5 min.							
c. Conclusion							