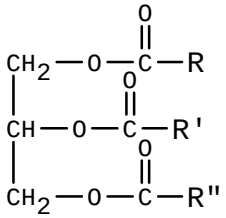
EXPERIMENT 7

LIPIDS: DETERMINATION OF FAT IN FRENCH FRIES

Materials Needed

French fries or potato chips
dichloromethane
2 Pasteur pipets
Br_2/ CH_2Cl_2 solution
1 10-mL beaker
3 small test tubes
1 capillary tube
boiling stones
1 applicator stick
1 small piece cotton
anhydrous Na_2SO_4(s)
small samples of some other fats; lard, peanut oil, linseed oil, palm oil, etc.



a fat molecule

Additional Reading Assignment

Denniston, Chapter 18

Purpose

In this experiment the percentage of fat in a commercial brand of French fries will be determined. The fat will be extracted from the French fries using dichloromethane (CH_2Cl_2) and the isolated fat will be tested for unsaturation using a Br_2/CH_2Cl_2 solution. You will also test the relative unsaturation levels of some other fats using Br_2/CH_2Cl_2.

Background

Of the three basic classes of food molecules (proteins, carbohydrates, and fats), fats have the highest energy value. As a general rule fats provide nine calories per gram whereas proteins and carbohydrates each provide only 4 cal/g. Therefore, one strategy often used for weight control is to limit one's intake of fat. In addition, fatty foods have been associated with cardiovascular diseases and other health problems. However, some fat in the diet is necessary to maintain proper metabolism and nutrition.

Ordinarily, potatoes contain virtually no fat. However, once cooked, potatoes (or any food) will retain some of the cooking fat. In this experiment the fat will be separated from French fries using a technique known as extraction. The French fries will be washed in a solvent (dichloromethane) in which the fats are soluble but the other constituents of the French fries (mainly carbohydrates, salt, and water) are insoluble. Separation of the solvent/fat solution from the undissolved solids, drying, and removal of the solvent by evaporation leaves the fat.

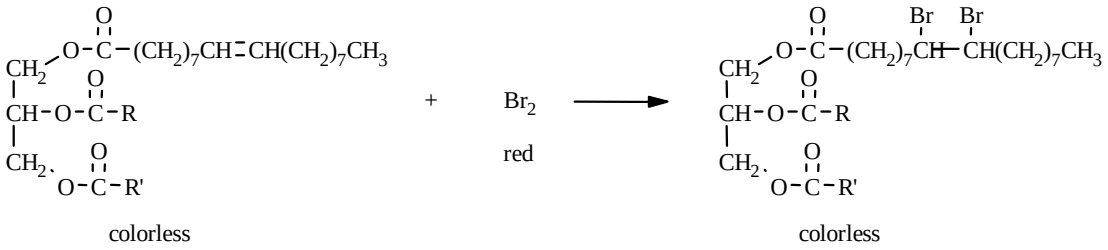
Structurally, fats are fatty acid triesters of glycerol (see structure at top of this page) and, therefore, a more precise term for them is "***triglyceride***". (They are also referred to as "triacylglycerols".) Triglycerides from plants are generally viscous liquids at room temperature and are, therefore, referred to as "oils". However, these "vegetable oils" should not be confused with oils from petroleum products (i.e., motor oil, mineral oil, etc.)

In addition to determining the amount of fat in the French fries, the isolated fat will also be tested for the presence of unsaturation (i.e., double bonds). The double bonds of an unsaturated fat are present in the fatty acid side chains (R groups). Vegetable oils generally have a fairly high content of unsaturated fatty acids while animal fats have a relatively higher content of saturated fatty acids. Large amounts of saturated fats in the diet have been linked to high blood levels of low-density lipoproteins (LDL, a.k.a. "bad cholesterol"), a condition often associated with atherosclerosis and heart disease.

Structural formulas of some common fatty acids, the building blocks of fats are shown below.

Saturated Fatty Acids:		Unsaturated Fatty Acids:
$CH_3(CH_2)_nCOOH$	n = 10 lauric acid	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$
	n = 12 myristic acid	oleic acid (monounsaturated)
	n = 14 palmitic acid	$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$
	n = 16 stearic acid	linolenic acid (polyunsaturated)

The presence of unsaturated fatty acids in a fat sample is easily tested for using a solution of bromine (Br_2) in dichloromethane (CH_2Cl_2). If the fat is unsaturated then the bromine reacts quickly with it by adding to the carbon-carbon double bonds (C=C) in the unsaturated fatty acid chains (as in the equation below). Because bromine is red in color and the addition products are colorless, the reaction is easily observed - the red color of the bromine solution disappears when it is added to the unsaturated fat. However, after just enough bromine has been added to completely



react with all of the double bonds present in the fat sample, the next drop of bromine solution no longer can react and the red color persists. Therefore, the number of drops necessary to reach this point can be used as a gauge of the level of unsaturation in the fat.

Procedure

SAFETY - Dichloromethane is harmful to breathe. Carry out all operations in a fume hood.

1. Weigh out about 1 gram of French fries onto a piece of weighing paper using the top-loading electronic balance in the lab. Record the actual weight used to the nearest mg (0.001 g).

2. Cut the French fries into small pieces using a spatula. The pieces should be about 0.5 cm long. Transfer the French fry pieces into a medium size test tube.

3. Working in a fume hood, add 2 mL CH_2Cl_2 to the French fry pieces in the test tube. Stir the mixture for several minutes, carefully continuing to break up the French fry pieces.

4. Add a boiling chip to a dry, 10-mL beaker. Weigh the beaker and the boiling chip to the nearest mg.

5. Prepare a microscale filtration apparatus by placing a tiny piece of cotton inside a Pasteur pipet. Use a small spatula to add anhydrous sodium sulfate (Na_2SO_4) to the pipet to a height of about 1-2 cm. (See Figure 1.) (The anhydrous Na_2SO_4 is a drying agent. It serves to quickly dry the solution being filtered through.)

6. Remove the solution from the French fries using a Pasteur pipet. Filter the solution through the filtration apparatus prepared in step #5. (Figure 1) Collect the filtrate in the 10-mL beaker. Attach a pipet bulb to the filter pipet and squeeze it so as to force any liquid remaining in the cotton and Na_2SO_4 out of the filtration pipet and into the beaker.

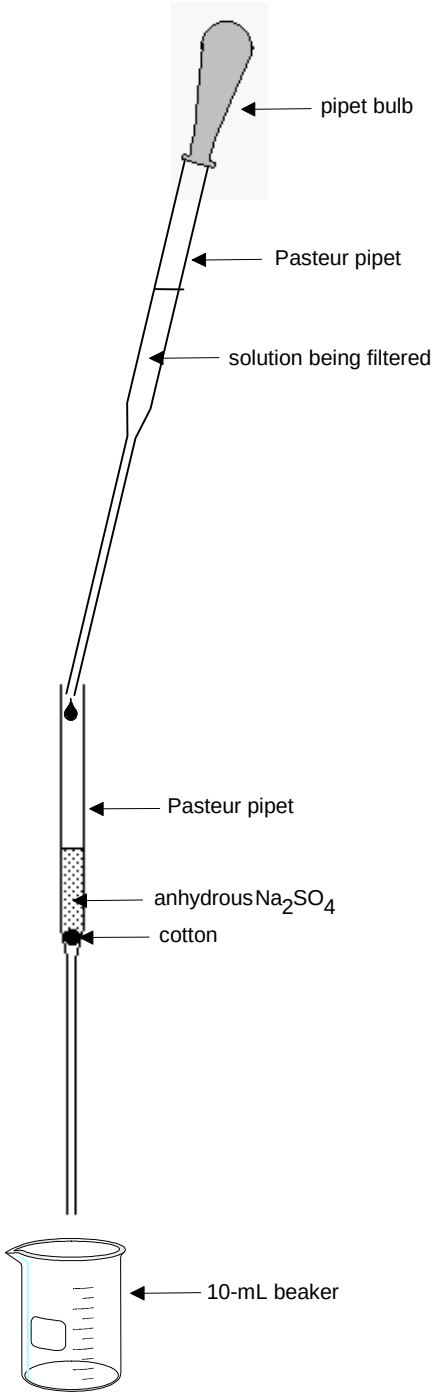
7. Add 2 mL additional CH_2Cl_2 to the French fry remnants in the test tube. Stir the mixture as in step #3 then repeat step #6, collecting the filtrate into the same 10-mL beaker containing the first filtrate.

8. Boil off the CH_2Cl_2 from the French fry extracts on a hot plate (set on low!). Remove the beaker from the hot plate when the liquid completely stops bubbling. As the amount of liquid gets very small it may be helpful to turn the beaker on its corner so that you can observe whether bubbles are rising from the boiling chip or not. A small amount of a thick oily liquid should remain. Allow the beaker to cool for a few minutes before carrying out a final weighing. Calculate the percent fat content of the French fries.

9. Test the French fry fat for unsaturation using the following procedure. Weigh a small test tube. Transfer four drops of the fat sample to the test tube and weigh it again. Next, add 1 mL of CH_2Cl_2 and shake/swirl to dissolve. Now add bromine/CH_2Cl_2solution to the fat in the test tube one drop at a time. Keep adding the bromine solution until the red color no longer disappears and note the number of drops necessary on the report sheet. Calculate the volume (in drops) of bromine solution per gram necessary to completely react with the double bonds in the fat.

10. Carry out unsaturation tests on two of the other fat samples available in the lab using the same procedures as in step #9. Note the number of drops necessary for persistence of the red bromine color on the report sheet. Calculate the drops per gram necessary to completely react with the double bonds in each of the fats. (For solid fats a 10% solution in CH_2Cl_2 will be provided. Carry out the tests in the same way but realize that the actual mass of fat being tested is 10 times less that the weight recorded in the table.)

Figure 1. Microscale filtration procedure



PRE-LABORATORY QUESTIONS

EXPERIMENT 7

LIPIDS: DETERMINATION OF FAT IN FRENCH FRIES

Name _____ Section _____ Date_____

1. Give the complete structure of a single fat molecule that contains saturated, monounsaturated, and polyunsaturated fatty acids.

2. Draw out the structure of the product of reaction of the fat given in response to question 1 with excess bromine. (React your fat molecule with excess Br_2 and give the structure of the product.

REPORT AND DATA SHEET

EXPERIMENT 7

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Names _____ Section _____ Date _____

Weight of French fries _____

Weight of 10-mL beaker with boiling chip _____

Weight of beaker plus fat _____

Weight of fat _____

% Fat by weight in French fries _____

Results of bromine tests:

Fat tested	test tube weight (g)	test tube weight + fat weight (g)	fat weight (g)	volume Br_2 soln needed (drops)	drops per gram
French fry fat					

Questions

1. How many grams of fat are in a 3-oz serving of the French fries you analyzed? (16 oz = 453.6 g) (Show calculation) Compare the result to the stated fat grams in a serving of McDonalds fries (this value can be found on the Internet).

2. Use your results to calculate the percent of calories from fat in the French Fries. Assume that French fries consist entirely of fat and carbohydrates, i.e that the rest of the weight of the fries other than the fat was due to carbohydrates. (Show you calculation below).

3. Use your results to order the fats you tested by degree of unsaturation (from most unsaturated to least unsaturated). Do the results agree with what would have been expected from a reading of textbook chapter? Explain.

4. What was the purpose of including anhydrous sodium sulfate in the pipet used for filtering the French fry extracts? Explain thoroughly.

5. (Extra Credit) French fries are not only high in fat content, recently it has been discovered that they also contain high levels of the probable human carcinogen, acrylamide. Give the structure of this compound and discuss how it gets into the French fries. List some other foods that contain high levels of acrylamide.