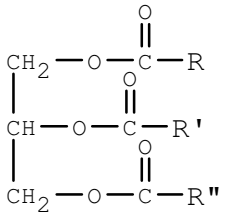
EXPERIMENT 9

LIPIDS: DETERMINATION OF FAT IN FRENCH FRIES

Materials Needed

French fries or potato chips	1 capillary tube
dichloromethane	boiling stones
2 Pasteur pipets	1 watch glass
Br_2/CCl_4 solution	1 small piece cotton
1 10-mL beaker	anhydrous Na_2SO_4(s)

small samples of some other fats; lard, peanut oil, linseed oil, etc.



a fat molecule

Additional Reading Assignment

McMurry, Chapter 24.

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 Br_2/CCl_4. You will also test the relative unsaturation levels of some other fats using Br_2/CCl_4

Background

Of the three basic classes of food molecules (proteins, carbohydrates, and fats), fats have the highest energy value; most possess approximately twice the number of calories per gram as do proteins or carbohydrates. Therefore, it is important that people engaged in dieting restrict their intake of fat. Also, foods that are high in fat content have been associated with cardiovascular diseases. 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***".

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:

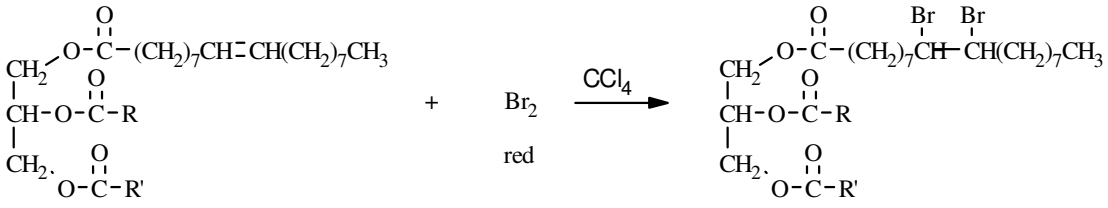
$CH_3(CH_2)_nCOOH$

n = 10 lauric acid
n = 12 myristic acid
n = 14 palmitic acid
n = 16 stearic acid

Unsaturated Fatty Acids:

$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$
oleic acid (monounsaturated)
$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$
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 carbon tetrachloride (CCl_4). 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 and carbon tetrachloride are harmful to breathe. Carry out all operations using them 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 few boiling stones to a dry, 10-mL beaker or small vial. Weigh the beaker/vial and boiling stones 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 8-1.)

6. Remove the solution from the French fries using a Pasteur pipet. Filter the solution through the filtration apparatus prepared in step #5. (Figure 8-1). Collect the filtrate in the 10-mL beaker. Use the pipet bulb to force any liquid remaining in the cotton and Na_2SO_4 out of the filtration pipet and into the beaker.

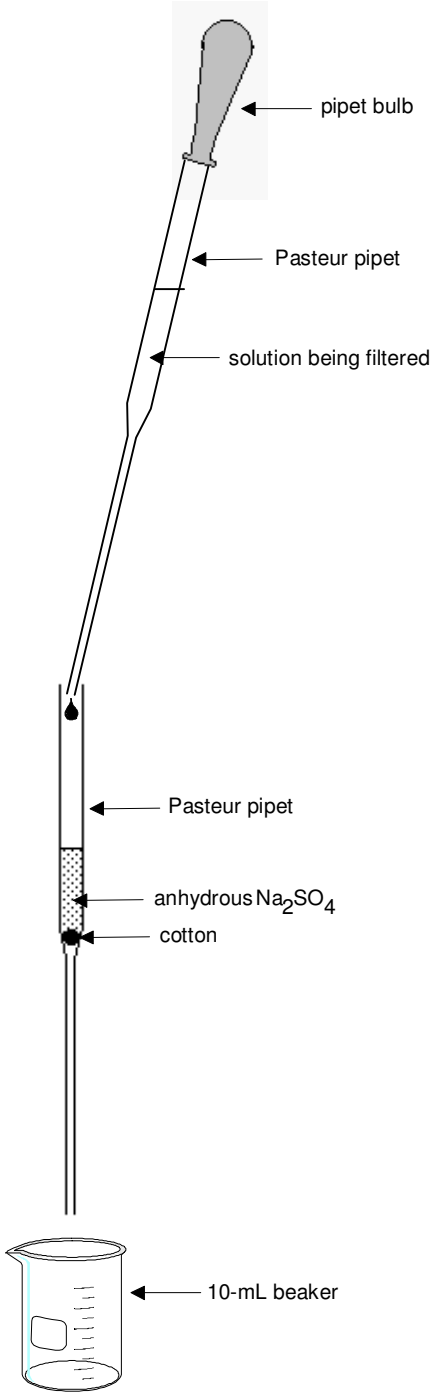
7. Add 2 mL additional CH_2Cl_2 to the French fry remnants in the test tube. Stir the mixture as in step #3 then repeat step #6, collecting the filtrate into the same 10-mL beaker containing the first filtrate.

8. Boil off the CH_2Cl_2 from the French fry extracts on a hot plate (set on low!) in the hood. Remove the beaker from the hot plate immediately when the liquid completely stops bubbling. 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/ CCl_4 solution to the fat in the watch glass 0.5 mL at a time. Keep adding the bromine solution until the red color no longer disappears and note the number of mL necessary on the report sheet. Calculate the volume of bromine solution per gram necessary to completely react with the double bonds in the fat.

10. Carry out unsaturation tests on the other fat samples available in the lab using the same procedures as in step #9. (For solid fats melt them carefully first so that you can measure out four drops.) Note the number of mL necessary for persistence of the red bromine color on the report sheet. Calculate the mL per gram necessary to completely react with the double bonds in each of the fats.

Figure 8-1. Microscale filtration procedure



PRE-LABORATORY QUESTIONS

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Name _____ Section _____ Date_____

1. Give the complete structure of a single fat molecule which 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

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LIPIDS: DETERMINATION OF FAT IN FRENCH FRIES

Name _____ **Section** _____ **Date** _____

Partners _____

Weight of French fries _____

Weight of 10-mL beaker with boiling stone _____

Weight of beaker plus fat _____

Weight of fat _____

% Fat by weight in French fries _____

Results of bromine tests:

Fat tested	watch glass weight (g)	watch glass + fat weight (g)	fat weight (g)	volume Br_2 soln needed (mL)	mL 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)

2. 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 the additional reading assignment? Explain.

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