Lipids: Determination of Fat in French Fries

Goals
- To determine the fatty acid content of some edible lipids
- To rank some edible lipids for degree of unsaturation
- To determine the percentage of fat in a commercial brand of French fries and test it for level of unsaturation

Introduction
Lipids are naturally occurring compounds that vary considerably in structure but share a common feature of being soluble in nonpolar solvents but not in water. There are many subclassifications such as fatty acids, waxes, triacylglycerols, steroids, protaglandins, etc.

This experiment will focus on the chemistry of the triacylglycerols, commonly known as fats and oils or triglycerides. In terms of the functional groups present, triacylglycerols are triesters; three ester functional groups are present. An ester is a compound produced from the reaction of an alcohol with a carboxylic acid. The alcohol involved in triacylglycerol formation is always glycerol (1,2,3-propanetriol), a three-carbon alcohol with three hydroxyl groups. Fatty acids are the carboxylic acids involved in triacylglycerol formation. A typical triacylglycerol is shown below:

\[
\begin{align*}
\text{CH}_2\text{O} & \text{C}-(\text{CH})_{16}-\text{CH}_3 \\
\left| \right| \\
\text{CH}_2\text{O} & \text{C}-(\text{CH})_{12}-\text{CH}_3 \\
\left| \right| \\
\text{CH}_2\text{O} & \text{C}-(\text{CH}_2)_{6}-(\text{CH}_2-\text{CH}==\text{CH}_2)_{3}-\text{CH}_2-\text{CH}_3
\end{align*}
\]

In a simple triacylglycerol, the three fatty acids are identical. A mixed triacylglycerol, like the example above, contains more than one kind of fatty acid. The fatty acids differ in the number of carbon atoms in the chain and the number of double bonds present.

Of the three basic classes of food molecules (proteins, carbohydrates, and fats), fats have the highest energy value. As a general rule, fats provide 9 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.
Background to the Procedure

A fat is a triacylglycerol mixture that is a solid or a semi-solid at room temperature (25°C). Generally, fats are obtained from animal sources. An oil is a triacylglycerol mixture that is a liquid at room temperature (25°C). Generally, oils are obtained from plant sources.

Because they are mixtures, no fat or oil can be represented by a single specific chemical formula. Many different fatty acids are represented in the triacylglycerol molecules present in the mixture. The actual composition of a fat or oil varies even for the species from which it is obtained. Composition depends on both dietary and climatic factors.

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.

The presence of unsaturated fatty acids in a fat sample is easily tested for using a solution of bromine (Br₂). If the fat is unsaturated, then the bromine reacts quickly with it by adding to the carbon-carbon double bonds 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 can no longer 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.
Experimental Procedures

**Wear your safety goggles and gloves!**

Organic chemicals often have strong odors and are usually volatile and flammable, so avoid inhaling the vapors and keep them away from open flames.

Bromine is a particularly corrosive element. You must wear gloves while working with this reagent, and you must wear goggles throughout the experiment.

**A. The Relative Iodine Value of Edible Fats and Oils**

**Materials:** six fats or oils (butter, coconut oil, lard, olive oil, peanut oil, and sunflower oil), small- or medium-sized test tubes, methylene chloride (IUPAC name is dichloromethane, CH$_2$Cl$_2$), bromine solution, acetone, 150 mL beaker, 1 mL disposable pipets, parafilm (or corks for the test tubes)

Methylene chloride is harmful to breathe. Carry out this procedure in a fume hood!

Unsaturated hydrocarbons react with iodine, undergoing conversion of the double bonds to single bonds. The *iodine number* is the number of grams of iodine that react with 100 g of a lipid (fat or oil). The reaction also occurs with chlorine or bromine. We will use bromine to determine the relative iodine value of a series of edible fats and oils.

A.1 Place 3.0 mL of methylene chloride in a test tube. This will be the control. You will be able to use the same control for each fat studied. Add one drop of the bromine solution to the test tube, mix well, and note the color. This is the end color you are looking for. When your reaction test tube reaches this color, you are finished with that particular fat analysis. Put a piece of parafilm over the test tube to prevent evaporation of the solvent.

A.2 Weigh an empty test tube in a 150 mL beaker; then place in it about 0.25 mL of one of the fats (or oils) to be investigated, and weigh the test tube again. Calculate the mass of the fat used. This flask will be your reaction test tube.

Some of the fats may be solid. In that case, place a small chunk in the test tube—a piece about half the size of a penny. You will need to get all your sample into the test tube, not stuck at the lip. Dissolve your sample in 3.0 mL of methylene chloride.

A.3 Add a drop of the bromine solution to the reaction test tube and swirl the mixture gently. As the reaction between bromine and the double bonds of the fat occurs, the bromine color should disappear. Continue adding bromine reagent, with swirling, counting the number of drops needed to complete the reaction of the fat (until the color of the reaction test tube matches the color of the control test tube). Record the number of drops needed.

A.4 Repeat the analysis (steps A.2 and A.3) with the other fats assigned to you.

A.5 Calculate the relative *iodine number* by dividing the number of drops of reagent by the grams of fat for each fat studied. Report your answer with two significant figures. Rank the fats in increasing order of iodine number: \(1 = \text{most saturated}, \ 8 = \text{most unsaturated}\).

A.6 Pour the contents of the reaction test tubes into the waste container provided. **Save the control test tube; you’ll need it for Part B.** Rinse the test tubes first with water and then with acetone.
Determination of Fat in French Fries

Materials: French fries, spatula, test tube, methylene chloride (IUPAC name is dichloromethane, CH₂Cl₂), 10 mL beaker, filtration apparatus (see next page)

Methylene chloride is harmful to breathe. Carry out this procedure in a fume hood!

B.1 Weigh out about 2 grams of French fries onto a piece of weighing paper or a weighing boat. Record the actual weight used as accurately as possible.

B.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.

B.3 Working in a fume hood, add 4 mL methylene chloride to the French fry pieces in the test tube. Stir the mixture for several minutes, carefully continuing to break up the French fry pieces.

B.4 Add a boiling chip to a dry, 10 mL beaker. Record the mass of the beaker and the boiling chip.

B.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 (Na₂SO₄) to the pipet to a height of about 1–2 cm. (See Figure 1.) The anhydrous Na₂SO₄ is a drying agent. It serves to quickly dry the solution being filtered through.

B.6 Remove the solution from the French fries using a Pasteur pipet. Filter the solution through the filtration apparatus prepared in step B.5 (Figure 1). Collect the filtrate in the previously weighed 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 Na₂SO₄ out of the filtration pipet and into the beaker.

B.7 Add 2 mL additional methylene chloride to the French fry remnants in the test tube. Stir the mixture as in step B.3, then repeat step B.6. Note the color of the solution before you squeeze it into the filtration apparatus. Collect the filtrate into the same 10 mL beaker containing the first filtrate. If the color of the solution was clear, go to the next step. If the color was slightly yellowish, repeat step B.7 one more time with 2 mL of methylene chloride.

B.8 Boil off the methylene chloride 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 thick oily liquid should remain. Allow the beaker to cool for a few minutes before carrying out a final weighing.

B.9 Calculate the percent fat content of the French fries as follows:

\[
\% \text{ fat} = \frac{\text{mass of oily liquid}}{\text{mass of French fries}} \times 100
\]

B.10 Test the French fry fat for unsaturation using the procedure from Part A. You may not have 0.25 mL of French fry fat. In that case, use what you have. What’s important is that you record the mass of the fat used. Record the number of drops of bromine solution needed to react with all the double bonds. Calculate the relative iodine number of the French fry fat.
Figure 1. Microscale filtration procedure
Pre-Lab Study Questions

1. Draw the structure of tripalmitin, the triacylglycerol made from glycerol and palmitic acid.

\[
\text{glycerol} \quad \begin{array}{c}
\text{CH}_2\text{OH} \\
\text{CH}\text{OH} \\
\text{CH}_2\text{OH}
\end{array} \quad \text{palmitic acid} \quad \begin{array}{c}
\text{C} \\
\text{O} \quad \text{C} \\
\text{O} \quad \text{OH}
\end{array}
\]

2. Show the products for the reaction of linolenic acid with excess bromine, Br\(_2\).

\[
\text{linolenic acid} \quad \begin{array}{c}
\text{O} \\
\text{C} \quad \text{O} \quad \text{OH}
\end{array}
\]

3. Three fats, labeled X, Y, and Z, were analyzed for double bond content and found to have “iodine numbers” of 78, 147, and 180, respectively. Which of the fats was the most saturated?
### A. The Relative Iodine Value of Edible Fats and Oils

<table>
<thead>
<tr>
<th>Fat</th>
<th>Mass of test tube (g)</th>
<th>Mass of test tube + fat (g)</th>
<th>Mass of fat (g)</th>
<th>Drops of Br, needed</th>
<th>Drops per gram of fat</th>
<th>Rank in unsaturation</th>
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<tbody>
<tr>
<td>Butter</td>
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<td>Coconut oil</td>
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<td>Lard</td>
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<td>Olive oil</td>
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<td>Peanut oil</td>
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<tr>
<td>Sunflower oil</td>
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</tbody>
</table>

Q.1 Coconut oil is composed of primarily saturated fatty acids, and yet, it is classified as an oil because it has a melting point below (or right at) room temperature. Why does it have such a low melting point even though it has primarily saturated fatty acids, which typically have higher melting points?

Q.2 Polyunsaturated oils are more healthy than using saturated fats for cooking. What does polyunsaturated mean? Does a polyunsaturated oil have a high or low relative iodine value?
B. Determination of Fat in French Fries

Calculation of Percent Fat in French Fries

Mass of French fries _________________ g
Mass of beaker + boiling chip _________________ g
Mass of beaker + boiling chip + oily liquid _________________ g
Mass of oily liquid _________________ g
Percent fat _________________ %

Calculation of Relative Iodine Value in French Fries

Mass of test tube _________________ g
Mass of test tube + oil _________________ g
Mass of oil _________________ g
Drops of Br₂ needed _________________
Drops per gram of oil _________________

Q.3 On the McDonald’s web site, you can find nutritional information for their food products: http://nutrition.mcdonalds.com/nutritionexchange/nutritionfacts.pdf

Using values for the serving size and total fat, calculate the percent fat for a large French fries. How does this value compare with the value you obtained experimentally?

Q.4 The McDonald’s nutritional information also indicates that the French fries are low in saturated fats. Therefore, the fries should have a relatively high iodine number. Do your results support this conclusion?