Procedure:

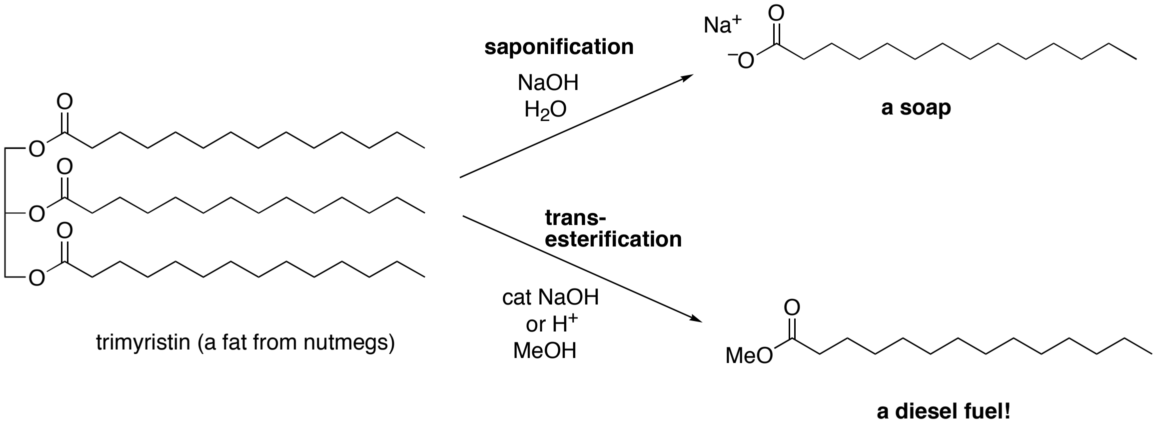
In a 250 mL Erlenmeyer Flask with a magnetic stir bar placed 0.35 g of anhydrous sodium hydroxide and dissolved it with 20 mL of methanol. The mixture was stirred until all of the sodium hydroxide was dissolved.

In a 400 mL beaker placed 100 mL of pure vegetable oil. The oil was heated to $60^{o}C$. Added all the dissolved sodium hydroxide into the heated oil, immediate the mixture becomes cloudy. Allow to stir for 30 minutes on high.

Transfer contents of beaker into a 250 mL separatory funnel. Allow the mixture to separate for 1 hr. Transfer the glycerol (bottom layer) into a beaker from bio-diesel.

Biodiesel

Many of the foods that we eat are rich in triacylglycerols— i.e. fat. The ancient process of saponification turns fat into soap. Triacylglycerols also can undergo a process known as trans-esterification, by which a catalytic amount of base or acid is used to exchange three equivalents of an alcohol for glycerol. The resulting esters make excellent fuels for diesel engines. The concept is illustrated below for trimyristin, which is a major constituent of nutmegs.



While a nutmegmobile may not be the solution to the national energy crisis, many less expensive oils— such as soybean, corn and safflower oils, are starting to become valid options as fuels. The advantage of using biodiesel is that it can be used in diesel engines without modification. Moreover, if you buy a used diesel engine automobile, then you can run your car for next to nothing using waste vegetable oil. In this lab, we demonstrate a simple procedure for preparing biodiesel, and in the second section of lab we will compare spectral data of biodiesel generated from local restaurants, and demonstrate the use of the biodiesel in a real engine.

Procedure: Day 1

Prior to lab, your TA will have prepared a solution of 5.0 mL of sulfuric acid (conc) in 250 mL of methanol. Each student should obtain 60 mL of this solution.

Pour 50 mL of the oil into a 125 mL separatory funnel, followed by 60 mL of the acidic methanol. The separatory funnel was capped and inverted once. The separatory funnel is then vented. This process is repeated until no gas is given off. Finally, the separatory funnel is shaken vigorously at least 10 times. The mixture was allowed to separate. The aqueous layer will contain the glycerin and the organic layer will contain the desired bio-diesel. Wash the organic layer with distilled water to neutralize the acid (again, carefully vent the separatory funnel as described above). Wash several times until the aqueous layer is neutral (test it with pH paper). Dry the bio-diesel with $MgSO_4$ or $NaSO_4$. Filter. Characterize your sample by IR to ensure that your diesel is free from water. Also, analyze your IR spectrum for to qualitatively test for unsaturation (double bonds). Then give your biodiesel to your TA, who will combine all of the samples prepared in your lab section, and will analyze the biodiesel by ^{1}H NMR (and return a spectrum to you at the start of the next lab period). In the next period, you will analyze your NMR spectrum, and your lab's biodiesel will be tested in the engine.

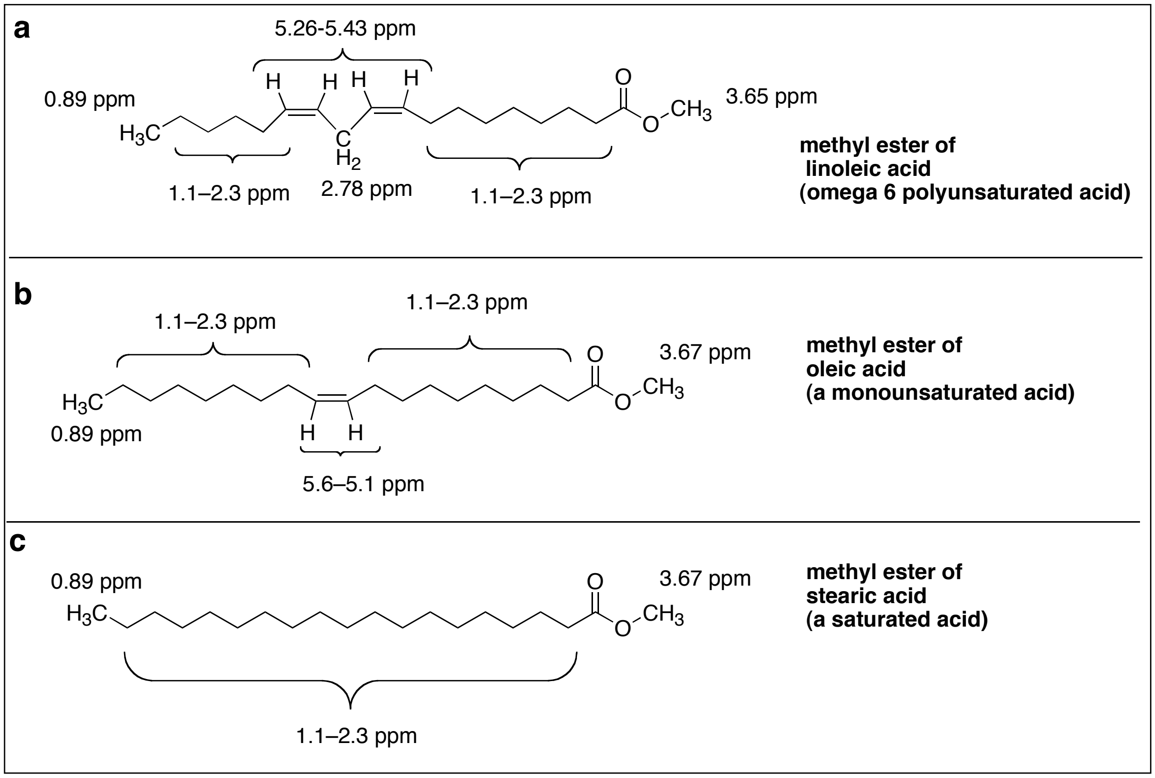
Using biodiesel

Your TA will demonstrate use of the biodiesel in a model airplane engine. The biodiesel will be mixed as follows:

% by volume: Ether 40%, Bio-Diesel 40%, Castor Oil 20%

Interpreting NMR spectra

Most of us are aware that all fats are not created equally. In general, we have probably heard that polyunsaturated fats are particularly good for us, and that saturated fats are particulary bad. In terms of chemical structure, this is illustrated for methyl esters of: (a) linoleic acid, which is derived from a polyunsaturated fat, (b) oleic acid, derived from a monounsaturated fat, and (c) stearic acid, from a saturated fat.



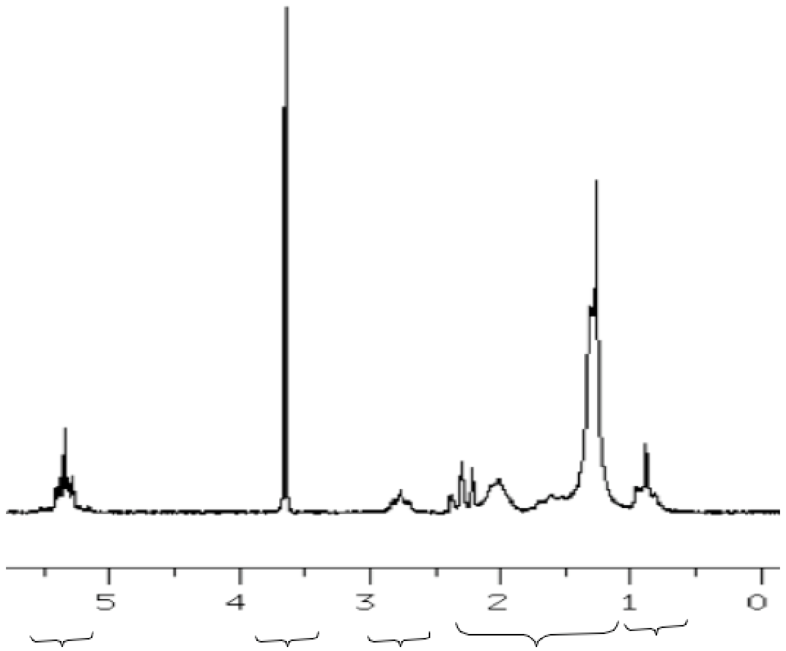
Our knowledge of NMR spectroscopy allows us to distinguish these different types of fats. Thus, both poly- and mono-unsaturated fats possess resonances at 5.6-5.1 ppm in the ^{1}H NMR spectrum, attributable to the alkene protons. However, only the polyunsaturated fats will possess peaks at 2.6-2.8 ppm, attributable to ‘doubly allylic’ protons.

Worksheet: analysing oil from different sources

1) **Determine the average number of carbons and hydrogens.** This can be done by standardizing your NMR integration to the methyl ester resonance. We know that this resonance is equal to 3 hydrogens. For example, if our crude integrals measure 0.91, then we can estimate that an integration of 0.3 corresponds to 1 hydrogen. We can then fill in the number of hydrogens associated with the other peaks. This illustrated by the example below, where we find ~30 hydrogens.

   Now, we can approximate the number of carbons by assuming that the peaks from 5.1-5.6 ppm are methine resonances, that the peaks from 1.0-3.0 ppm are methylene resonances, and that the peaks at ~3.6 ppm and ~0.9 ppm are methyl resonances. For the spectrum below, we find an average of ~15 carbons. Now, remember that we also need to add in the carbon from the carbonyl, bringing our total to 16 carbons.

   Thus, we extimate an average formula of $C_{16}H_{30}O_2$.



0.51	0.91	0.24	5.1	0.96	**Total**
~1.7H	**3H**	~0.8H	~22H	~3H	~ 30H
~1.7C	~1 C	~0.4 C	~11C	~1C	~15 C

2) Approximate the average IHD.

For our example above, $C_{16}H_{30}O_2$, the IHD is 2. Since one degree of unsaturation comes from the carbonyl, this means that each molecule contains an average of one alkene.

3) **Calculate the ratio of polyunsaturated to monounsaturated fats**

This can be done by comparing the integrals of the peaks at 2.6-2.8 ppm (doubly allylic) and at 5.1-5.6 ppm (alkene). We need to double the integral of the peak at ~2.8 ppm, because there are twice the number of alkene hydrogens as allylic hydrogens.

Thus, for our example:

0.51 (total alkene integral) – [2 x 0.24(polyunsaturated integral)] = 0.03 (monounsaturated integral)

• Now, we can calculate the ratio of poly/monounsaturated fat by simply dividing the polyunsaturated integral by the monounsaturated integral. We do this for our example as follows:

0.24/0.03 ~ 8

Thus, we estimate that the ratio of polyunsaturated to monounsaturated fat for our sample is 8:1. This sample did not come from Burger King!