Chemistry 365 Biochemistry
Individual Lab #6
Gas Chromatographic Analysis of Triacylglycerols

References:

Heinzen, H.; Moyna, P.; Grompone, A. *J. Chem. Educ.* **1985**, *62(5)*, 449-450.

Introduction:

Vegetable oils and animal fats are energy storage molecules for organisms, and are composed primarily of a mixture of triglycerides or triacylglycerols. Triacylglycerols are tri-esters formed by the condensation of three fatty acid molecules and one molecule of glycerol, a tri-alcohol. Fatty acids are long-chain organic acids having an even number of carbon atoms. These fatty acids may have no carbon-carbon double bonds (saturated fats) or one or more cis, non-conjugated double bonds (unsaturated fats). Vegetable oil triacylglycerols are composed primarily of four fatty acids: palmitic acid, stearic acid, oleic acid and linoleic acid. Hydrolysis of these triacylglycerols with subsequent formation of the methyl ester derivatives of the fatty acids allows analysis of the percent composition of fatty acids in the oils. Individual oils or mixtures may be identified by this procedure. Percent compositions of several common edible vegetable oils, determined in this laboratory, are shown below.

Oil	Palmitate	Stearate	Oleate	Linoleate **(Percents)**
Corn	11.0	2.0	25.0	62.0
Olive	16.0	1.0	67.0	16.0
Peanut	12.0	3.0	56.0	29.0
Sunflower	6.0	3.0	25.0	66.0

Solutions and Reagents:

1) Diethyl ether
2) 0.5 M KOH in methanol
3) 0.1 M HCl

Instrumentation:

Hewlett-Packard 5890A Gas Chromatograph
Hewlett-Packard 3396A Integrator
Capillary column (polar, Carbowax, 15m)
Thermal Conductivity Detector

Injector and Detector T: 230^{o}C
Initial T: 180^{o}C Final T: 220^{o}C Ramp= 5^{o}C/min

Laboratory:

1) Prepare your oil sample for GC analysis. In a test tube, mix 10 drops of oil, 3 mL of ether and 3 mL of KOH/MeOH. Mix well and let stand for at least 10 minutes. Add 3 mL of 0.1 M HCl. You will inject one □L from the ether layer.

2) While you wait for your time to run the GC, calculate the Average TAG MW (ATM) and Degree of Unsaturation (DOU)(average moles C=C/mole TAG) for one oil and one 50:50 mixture. Show the results to the GC starter before running your chromatograph.

Report:

1) Draw the structures of each of the four fatty acids being determined and show their molecular weights in your notebook.

2) Show the sample calculations of ATM and DOU for one oil and a 50:50 mixture in your notebook.

3) Set up a spreadsheet to calculate the fatty acid percents, ATMs and DOUs for the four vegetable oils and all possible 50/50 mixtures of those four oils.

4) Also on the spreadsheet, from your chromatograph, use the appropriate peak areas to calculate the percent fatty acid composition of your unknown oil, the ATM and the DOU.

5) Determine the identity or possible identities of your unknown oil by comparing the GC results to the results in the spreadsheet table above.

6) In your notebook, write an abstract summarizing your analysis and result.