Analysis of Essential Depot Seed Oils Using Gas Chromatography-Mass Spectrometry

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Abstract

This research observed the concentration of fatty acid methyl esters(FAME) in various seed oils including Meadowfoam, Baobab, Hempseed, and Olive oil. The samples used for this research were supplied by Essential Depot, and the results of this research will be reported to them. When the samples are first received, they are composed of triglycerides which must be broken down to form FAMEs. This process must be done because triglycerides are not volatile, which is not ideal for analysis using GC: MS. FAME concentration does not tell one which triglycerides are present, however, it does show the concentration of each fatty acid that makes up the triglycerides. Chromatographic peaks are assigned by comparing retention times of known FAME retention times to assign FAME peaks in samples. Additionally, this research looks at a FAME standard containing the most common FAMEs to show the exact retention times in the H-SC GC: MS column. Once chromatographic peaks are assigned, they are integrated to show the concentration of FAME in a sample. These percentages are then compared to known literature values of FAME concentration for each type of seed oil, in addition to identical seed oils from competitors such as Plant Therapy, Eden's Garden, and Green Tidings. If the correct types and concentrations of FAMEs are present in the sample, it can be considered authentic, but if the inverse is true, one will be able to tell which FAME was used to adulterate the sample.

Introduction

My first goal during the semester was to make samples that are able to be injected into the GC: MS. Triglycerides are large nonpolar molecules that are composed of three bonded glycerides that have fatty acid chains attached to them. In a triglyceride molecule, the three attached fatty acids do not need to be the same, but there must always be three of them. The seed oils being analyzed in this research are composed of these triglyceride molecules, however, because of the size of these molecules, they are not readily able to be vaporized. In order to analyze seed oils using GS: MS, triglycerides are formed into FAMEs. This means one is not able to tell which triglycerides are present in a seed oil but is now able to tell the fatty acids that make up those triglycerides. For each different type of seed oil, there will be different types and concentrations of FAMEs present, and this is used as a signature to identify Hempseed oil from baobab oil for example. The characterizing feature of FAMEs, and fatty acids in general, is the number of carbons in the fatty acid chain and the degree of saturation(number of double bonds). Additionally, the concentrations and types of FAMEs in a certain seed oil should be the same regardless of the actual seed they came from. So two different samples of the same seed oil should have roughly the same in both type and concentration of FAME.

FAME samples are injected into the GS: MS where they are vaporized and separated into each type of FAME in the sample. This process results in a chromatograph where peaks are then assigned and integrated to show concentrations. This data is compared to known literature values and authenticity is accessed. If there are signs of adulteration, the adulterant can be identified through analysis of the chromatograph.

To improve the accuracy of this research a standard containing various concentrations of common FAMEs was injected into the GC: MS. This was done to analyze how these FAMEs samples reacted with the column being used for this research. The chromatograph received from the standard was compared to the literature associated with the standard and gave insight into how to better assign FAME peaks.

The main goal behind this research is to report to Essential Depot the authenticity of their oils. One of the statements on all of their products is they provide completely unadulterated oils. This research will provide validation to these claims that are being made to their customers, and inform Essential Depot on the integrity of their suppliers.

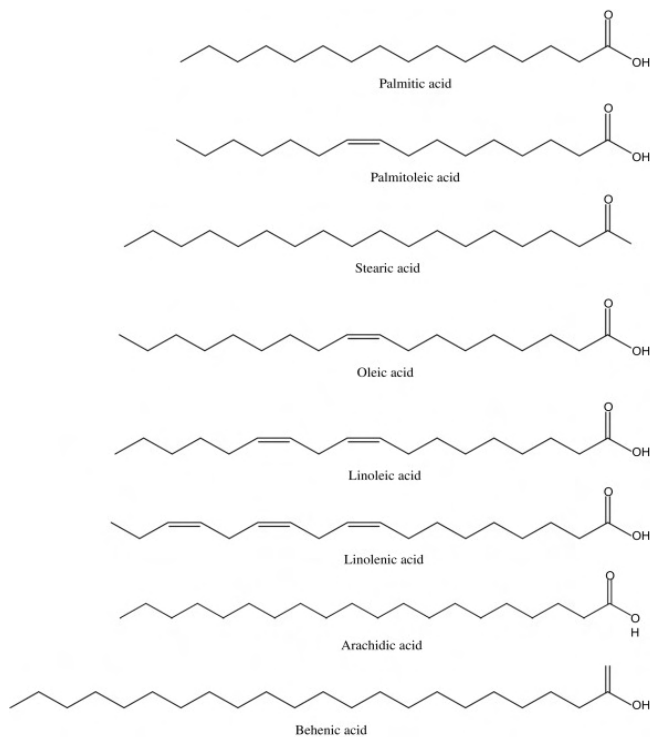

Figure 1: Names of Common Fatty Acids

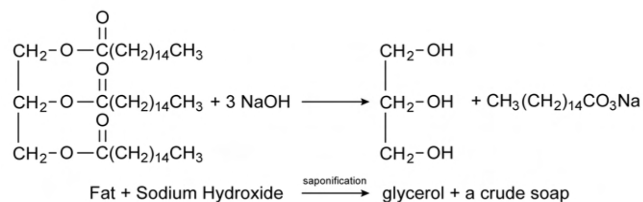

Figure 2: Saponification Reaction

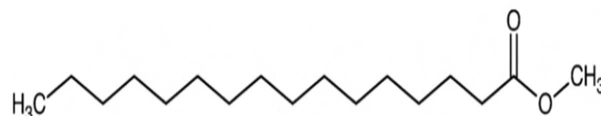

Figure 3: Palminitc Acid FAME

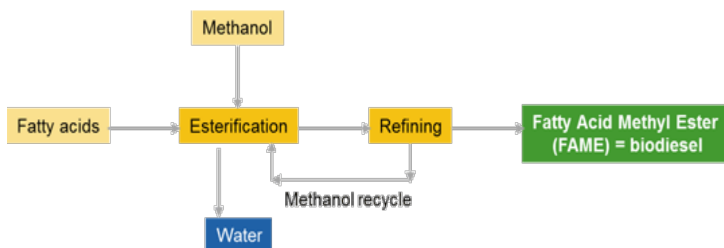

Figure 4: Esterification Reaction

Experimental

Saponification and Esterification

In order to break apart the triglyceride molecules, one must first use a process called saponification. In this process, a triglyceride is cleaved by either KOH or NaOH in a solution with methanol. For this research, .5 M NaOH in methanol was used. To start this procedure, 100 mg of the seed oil is placed into a round bottom flask, where it is heated and a condenser is attached. Then, 5 mL of the .5 M NaOH solution is added to the round-bottom flask and heated until the seed oil globule dissolves. The next part of this procedure is esterification where the methyl group of the methanol is attached to the fatty acid. This occurs by the addition of a solution of boron trifluoride in methanol to the round-bottom flask. The Born trifluoride acts as a catalyst that allows the methyl group to bind, forming the FAME. To retrieve the FAMEs from the solution a hexane solvent is added along with saturated saltwater. This allows the solution containing the hexane and FAME to separate from the unwanted chemicals. This occurs because the FAMEs and hexane are nonpolar. The FAME solution obtained is readily able to be injected into the GC: MS after it is washed with water and slightly diluted with more hexane solvent. The success of this procedure in this research can be found in the 13C spectra.

Gas Chromatography

In gas chromatography, the first step is to have a sample that is able to be easily vaporized and has no water present. If a sample is not volatile it will not be fully vaporized in the inlet port and an accurate judgment on chemical composition can not be made. This can also affect future injections using the same inlet port. Likewise, if water is present in the sample it has a strong affinity to stick to the column, therefore affecting the results. Once an appropriate sample is obtained and injected it is vaporized and passed through a column where it is separated. While in the column, the sample is in the mobile phase where an inert gas, such as helium, carries the sample through the column. As the sample is carried through the column, the compounds that make up the sample are separated based on how they interact with the stationary phase of the column which causes them to travel at different speeds.

Mass Spectrometry

After a sample has been chemically separated by the gas chromatograph, the compounds hit a detector called the mass spectrometer. This instrument will break each separated compound coming from the gas chromatograph into ionized

fragments using a high-energy beam of electrons. Each charged fragment will have a certain mass which is then divided by its charge to receive a mass to charge ratio. Following this, the fragments go through a process of acceleration and deflection while being exposed to a magnetic field and traveling through a short tunnel. Finally, the fragments reach a detection plate at the end of the tunnel where mass to charge and relative abundance are calculated.

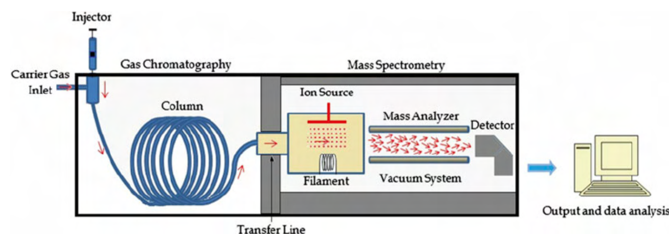

Figure 5: GC: MS Instrumentation

Results and Discussion

Of the four Essential Depot oils that were analyzed, the most authentic oils were Meadowfoam, Baobab, and Olive oil. The FAMEs present in these samples were authentic in not only type but also in concentration. In some instances, the concentration of a FAME that should be present does not occur, but this is most likely a product of the type of GC column being used. Likewise, small percentage discrepancies in FAME concentration when compared to the Firestone norms are a product of integration choices made during post-run analysis. The Essential Depot oils also appear to be keeping up with the competitors. Hempseed oil was the most ambiguous of the four oils. This sample contained the correct FAMEs, but they were not in the correct concentrations. This is evidence of possible adulteration or dilution by another FAME, like linoleic acid for example. One redeeming factor of Hemp Seed oil is that when compared to its competitors, it is no more adulterated than they are.

The injection of the Restek standard solution also gave some valuable results. It showed that the H-SC column was competent enough to accurately analyze these samples, although there were some other conclusions. The first of which is that in the HS-C column, the fatty acid methyl esters containing eighteen carbons do not come out in the anticipated order. The Restek literature had the carbons coming out by order of increasing unsaturation, however, in the H-SC column, the polyunsaturated FAMEs hit the detector before the less saturated ones. This was observed in all other oils containing eighteen carbon-long chains, and this pattern occurred for other long carbon chains. Additionally, the standard showed that even in columns made for this research eighteen carbon-long fatty acid FAMEs are hard to separate, although the order in which they come out can be helpful in identification.

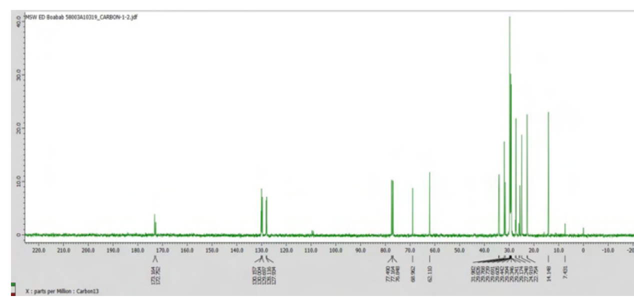

Figure 6: ^{13}C NMR Spectrum Before Saponification and Esterification

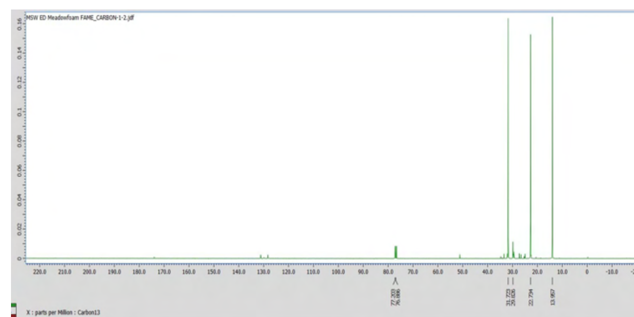

Figure 7: ^{13}C NMR Spectrum After Saponification and Esterification

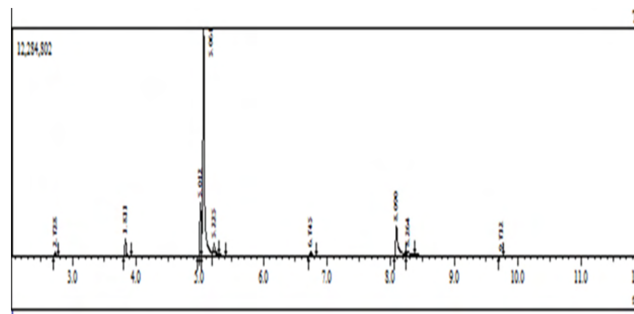

Figure 8: Chromatograph of Restek Standard

	Restek Norms	Experimental
Myristic Acid FAME (14:0)	1.0	0.8
Palmitic Acid FAME (16:0)	4.0	3.8
Stearic Acid FAME (18:0)	3.0	2.4
Oleic Acid FAME (18:1)	45.0	65.7
Linoleic Acid FAME (18:2)	15.0	11.7
Linolenic Acid FAME (18:3)	3.0	-
Arachidic Acid FAME (20:0)	3.0	1.4
Behenic Acid FAME (22:0)	3.0	2.2
Erucic Acid FAME (22:1)	20.0	11.8
Lignoceric Acid FAME (24:0)	2.0	0.3

Table 1: Restek Norms Compared to Experimental Data

	Firestone Norms	Essential Depot	Plant Therapy	Eden's Garden
Oleic Acid FAME(18:1)	1-3	-	1.2	1.0
Eicosenic Acid FAME(5C-20:1)	58-77	83.9	74.5	63.1
Docosenoic Acid FAME(22-1)	8-24	9.0	13.2	17.3
Docosadeinoate Acid FAME(22-2)	7-15	5.1	11.1	17.4

Table 2: FAME Concentration of Meadowfoam Oil

	Firestone Norms	Essential Depot	Eden's Garden	Green Tidings
Palmitic Acid(16:1)	25-46	21.6	24.7	26
Palmitoleic Acid FAME(16:1)	0.3-1.7	-	0.4	-
Stearic Acid FAME(18:0)	0-4	4.9	3.5	3.8
Oleic Acid FAME(18:1)	21-59	59.3	38.4	38.1
Linoleic Acid FAME(18:2)	12-29	13.6	29.9	29
Eicosanoic Acid FAME(20:0)	0.5-1.0	-	0.5	-

Table 3: FAME Concentration of Baobab Oil

	Firestone Norms	Essential Depot	Plant Therapy	Eden's Garden	Green Tidings
Palmitic Acid FAME(16-0)	6-12	5.3	6.2	6.9	7.2
Stearic Acid FAME(18:0)	1-2	-	4.1	2.9	3.7
Oleic Acid FAME(18:1)	11-16	0.8	37.1	-	-
Linoleic Acid FAME(18:2)	45-65	40.0	48.7	53.1	53
Linolenic Acid FAME(18:3)	13-30	53.9	2.4	36.4	35.7
Eicosanoic Acid FAME(20:0)	2.0	-	0.4	0.7	0.4

Table 4: FAME Concentration of Hempseed Oil

	Firestone Norms	Essential Depot
Palmitic Acid FAME(16:0)	7.5-20	10.9
Stearic Acid FAME(18:0)	0.5-5.0	1.5
Oleic Acid FAME(18:1)	55-83	83.4
Linoleic Acid FAME(18:2)	3.5-21	4.2

Table 5: FAME Concentration of Olive Oil

Figures five and six show evidence of the success of saponification and esterification. In figure five, the two peaks between sixty and seventy refer to the three carbons linking the triglyceride molecule. There are only two peaks present because two of the carbons in the triglyceride molecule are identical. The result is one large peak that represents the identical carbons, and one smaller peak that represents the middle unique carbon. In figure six these two peaks disappear and one small peak between sixty and seventy appears, showing that a methyl ester has formed. The peaks present in figure six are small, and there are large hexane peaks that are a product of the saponification and esterification procedures, but this is solid evidence that a FAME was formed.

Figure seven shows the Restek FAME standard which was injected into the H-SC GC: MS column and table one shows the experimental values compared to the norms provided by Restek. This standard contained all of the FAMEs that were present in the seed oils that were analyzed, although the concentrations of each FAMEin the standard when compared to each oil may have been different. The result of this data was validation that my identification of the FAMEs in the seed oil samples was correct.

The other tables provided show the FAME concentrations of the seed oil samples from Essential Depot and their competitors. As previously mentioned, Meadowfoam, Baobab, and Olive oil were the most authentic of the seed oils analyzed. They contained the correct concentrations of the most abundant FAMEs that should be present. Some of the spaces in the table were blank, but this was a product of integrating choices and lack of sensitivity in the column. That being said, the chromatographic data was sufficient to claim that these oils were authentic, and this was further proven by comparisons to competitor oils. For Hemp seed oil there is evidence that this sample was adulterated. This is shown by a very low concentration of oleic acid and a very high concentration of linolenic acid. When compared to competitor oils they showed similar levels of adulteration but with different fatty acids, and of all the Hempseed oils Green Tidings was the most authentic.

Conclusion

For future work on this project, more FAME samples of Essential Depot seed oils should be synthesized to make sure that the samples that I analyzed were not outliers in a greater batch. This is especially true for Hempseed oil since my results showed evidence of adulteration. Furthermore, a GC column that is better suited for FAMEs could improve the accuracy of results. Literature has shown that FAME affinity for a column can affect the data. A common column that is used for this research is the

FameWax column made by Restek. This column could aid in better separation of peaks and correct some tailing tendencies of the peaks found in the current H-SC GC column. While this type of column would certainly improve FAME analysis, it may not be suitable for other research that takes place in the H-SC GC column.

I would like to acknowledge Dr. Kevin M. Dunn for advising this project. His style of mentorship provided me with the fundamental knowledge required for this research in addition to the opportunity to make this project my own. Furthermore, this research would not be possible without Essential Depot. Reporting my findings to them is the purpose of this research and they have ensured that the Hampden-Sydney Chemistry Department is equipped with the necessary equipment to make these reports.

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