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GC-MS Analysis of Synthesized Biodiesel

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GC-MS Analysis of Synthesized Biodiesel

by

Courtney Whetstine

An Abstract of a Project
in
Forensic Science

Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Master of Science

December 2019

State University of New York
College at Buffalo
Department of Chemistry

Abstract

GC-MS Analysis of Synthesized Biodiesel

Biodiesel is an alternative energy resource that can replenish depleting natural petroleum storage. Synthesis of biodiesel is cost-efficient as it can be chemically converted from renewable wastes, such as vegetable oil or animal fat. These fuels are also biodegradable and the exhaust emissions from biodiesel are non-toxic and environmentally-friendly. Biodiesel is a stable fuel that performs reliably in all diesel engines. Biodiesel is also easy to synthesize, safe to handle, and works with diesel fuel storage and pumping systems.

The United States has an abundance of vegetable oils that have not been widely used to our advantage. Additionally, fast-food restaurants across the United States produce over 3 billion gallons of used cooking oil each year. These restaurants pay to dispose of the used oil into landfills. In this project, we collected the used vegetable oil from three local restaurants and chemically converted them into biodiesel through transesterification. Furthermore, we successfully developed the GC-MS method that can identify the chemical composition of the biodiesel as well as quantify the esters of fatty acids that are present. Based on the GC pattern, we successfully confirmed the origin of the two samples are soybean and sunflower oils. Interestingly, we found both biodiesels contain the same four fatty acid methyl esters, but in different concentrations. To validate our scientific results, we synthesized biodiesel from cooked/uncooked oil and confirmed our results without any ambiguity. Finally, we studied the physical properties of our biodiesel and compared that with the pure natural diesel as well as biodiesel and diesel mixture.

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College at Buffalo
Department of Chemistry

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List of Abbreviations

°C/min = Degree Celsius per minute

AG Diesel Fuel = Agricultural Diesel Fuel

ASTM = American Society for Testing and Materials

AVG = Average

DHA = Docosahexaenoic Acid

DPA = Docoosapentaenoic Acid

DSQ = Dual Stage Quadrupole

EPA = Eicosapentaenoic Acid

FAME = Fatty Acid Methyl Ester

GC-MS = Gas Chromatograph – Mass Spectrometer

IUPAC = International Union of Pure and Applied Chemistry

R^2 = Linear Regression

m/z = Mass to Charge Ratio

mm^2/s = Millimeters Squared per Second

MW = Molecular Weight

µL = Microliter

NREL = National Renewable Energy Laboratory

PA = Peak Area

RT = Retention Time

SD = Standard Deviation

Std = Standard

TIC = Total Ion Chromatogram

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I. Introduction

1.1 Diesel Fuel

Diesel Fuel, also known as petroleum distillate fuel, is sold commercially for the use in motor vehicles that have diesel or compression ignition engine. It was named after inventor Rudolf Diesel. Diesel fuel is refined from crude oil at petroleum refineries. United States petroleum refineries produce an average of 11 to 12 gallons of diesel fuel from each 42-gallon barrel of crude oil. Diesel fuel is used in the diesel engines found in most freight and delivery vehicles, trains, buses, and military vehicles *(5)*.

Diesel engines can ignite the fuel by elevated temperature within the air in the cylinder by the mechanical compression. In gasoline engines a spark plug is used to ignite the air-fuel mixture with a spark, whereas in diesel engines, the air compression increases the air temperature inside the cylinder to a high degree that atomizes the diesel fuel injected into the combustion chamber ignites spontaneously. The combustion and expansion activate a piston within the engine. This converts chemical energy stored in the fuel into mechanical energy, which is then used to power diesel engines. The principal drawback of diesel engines is their emission of air pollutants. These engines typically discharge high levels of soot (particulate matter), reactive nitrogen compounds, and odor *(7)*.

Different types of diesel fuel grades can be used in a diesel engine to run the vehicles. The efficiency of such diesel fuels is often rated by their cetane number, in contrast to gasoline which is rated by octane number. The cetane number measures the ignition and burning efficiency of the diesel fuel while incorporated into the diesel engine. The cetane number can be measured using an Ignition Quality Tester (IQT). This method measures the time delay between the start of fuel injection and the start of significant combustion through auto-ignition of a pre-measured amount of diesel fuel in a constant volume chamber *(21)*. The higher the cetane number, the more volatile the fuel. Typically, most diesel vehicles use fuel with a cetane rating between 40 to 55 depending on the type of diesel fuel being used. Standard diesel fuel comes in two grades, diesel #1 (DG1) and diesel #2 (DG2). DG1 is the most expensive grade of diesel, mainly because the paraffin or wax is removed from the fuel. This prevents the fuel from gelling and clogging the fuel filters during cold weather. This grade is preferable for those who use a diesel engine at constant cold temperatures. A disadvantage, however, is that this grade has fewer energy components that make the fuel performance less efficient than other grades and is more expensive per gallon. DG2 is the most readily available fuel. DG2 comprises the highest energy components and offers the best fuel performance as well as lubrication properties. The downside of this diesel fuel grade is the presence of paraffin. Paraffin allows fuel to thicken and gel and this clogs the fuel filters at colder weather. A winterized diesel can be created by mixing diesel grades #1 and #2. It has the optimized properties of both diesel grades, the ability to handle low temperatures due to lower paraffin than in grade #1 and the high energy components and fuel performance as in grade #2. The fourth type of diesel fuel is Agricultural Diesel (AG) which is available for off-road vehicles. Red dye is added to the diesel fuel to differentiate AG diesel fuel from other diesel fuel grades. Since it is not subjected to tax like other diesel fuel grades, it is less expensive *(6).* Apart from these fuels, more environment-friendly fuel can be created from the used cooking oil, which is also known as biodiesel fuel.

1.2 Biodiesel Fuel

Biodiesel is a type of alternative renewable diesel fuel that can be derived from vegetable oils or animal fats. Biodiesel is defined as "the mono-alkyl esters of long-chain fatty acids derived from renewable lipid feedstock, such as vegetable oils and animal fats for use in compression ignition engines". This definition was adopted by the American Society for Testing and Materials (ASTM) Biodiesel Task Force. The mono-alkyl ester definition eliminates pure vegetable oils as well as monoglycerides and diglycerides from consideration as biodiesel *(9)*.

Due to widespread availability and low cost of petroleum diesel fuel, vegetable oil-based fuels were not popular, except in times of high oil prices and shortages. World War II and the oil crises of the 1970s peaked some interest, but the newer diesel engines at the time could not run on traditional vegetable oil, due to the high viscosity of vegetable oil. A Belgian inventor discovered transesterification to convert vegetable oils into fatty acid methyl esters and use them as a diesel fuel replacement *(11)*. Biodiesel was first manufactured commercially in the U.S. in 1991 in Kansas City, Missouri. In 1995, the University of Idaho provided biodiesel to Yellowstone National Park, which used the fuel in a bus that was driven several hundred thousand miles without damage to the engine and is still in use today. As a result, other national parks began using biodiesel in their vehicles *(12)*. The biodiesel industry became a household name in the U.S. after the terrorist attacks of 9/11/01, which resulted in historically high oil prices and an increased awareness of energy security *(11)*.

Alternative fuel refers to substances that can be used as fuels. Due to the energy crisis, the following factors have led to the increasing need for finding a feasible fuel alternative to conventional sources. Fossil sources are limited and will eventually dwindle out. Since only a handful of countries have useable fossil fuel reserves, this forces other nations to depend on them for energy. In 2018, the United States imported 11% of its petroleum, and transportation was responsible for nearly three-quarters of total US petroleum consumption. Since vegetable oils are used to make biodiesel, this helps eliminate the 3 billion gallons of used cooking wastes going to the landfill yearly in the United States. Biodiesel has a positive energy balance, meaning that biodiesel yields 4.56 units of energy for every unit of fossil energy consumed over its life cycle *(10).* Using biodiesel also reduces emissions because the carbon dioxide released from biodiesel combustion is offset by the carbon dioxide absorbed from growing the feedstock used to produce the fuel. Lastly, biodiesel causes far less damage than petroleum diesel if spilled or released into the environment because it is less combustible. Its flashpoint is 130°C compared to petroleum diesel which is 52°C *(10).* Therefore, biodiesel, in general, is safer to handle, store and transport compared to conventional diesel fuel.

Biodiesel can be used in its pure form as a fuel, or it can be blended with petroleum diesel fuel. The amount of biodiesel present in the petroleum diesel fuel is represented by a letter "B", which is followed by the percentage of biodiesel, as shown below in Table 1. For example, B5 indicates a blend containing 5% of biodiesel and 95% of petrol diesel fuel.

Table 1: Biodiesel Blends

	Biodiesel	**Petrol Diesel**
B2	2%	98%
B5	5%	95%
B20	20%	80%
B100	100%	0%

1.3 Vegetable Oils

Vegetable oils contain energy that comes directly from the sun. Vegetable oil crops such as peanuts, sunflowers and soybeans concentrate solar energy in their oils. Also, it takes as little as a couple of months to grow vegetable oil crops from seeds, while fossil oils demand years of metamorphosis of organic materials under intense heat and pressure. There are over 350 species of *oleaginous,* or oil-producing plants and thousands of sub-species, which makes vegetable oils more accessible from a wide variety of plant resource as well.

The soybean or soya plant has been cultivated in East Asia for the past 5,000 years. There are more than 2,500 known soybean species that are cultivated around the world and it is the most commonly grown crop in the United States. It makes approximately 345 lbs of oil/acre. The soybean is a high protein bean that includes 20% oil and 80% meal by weight. Therefore, soybeans are the most suited crop for oil production. Most of the biodiesel made within the United States is made from virgin soybean oil or used cooking soybean oil.

Sunflowers are mostly grown for aesthetic pleasing. They grow between 15 inches and 8 feet tall. Sunflowers yield approximately 720 lbs oil/acre. Sunflower oil is the second most important edible oil in the world and is used for cooking, margarine, salad dressing, soaps and lighting. Sunflower is the only major oil crop native to western North America *(3)*.

II. Background

2.1 Transesterification

Biodiesel is synthesized from vegetable oil by a process called transesterification. In the transesterification process, an existing ester moiety is replaced by another type using a chemical approach. In vegetable oil, a variety of fatty acids attaches to glycerol would make it a type of fat called triglyceride. Since 20% of a vegetable oil molecule is composed of glycerin, which makes the oil thick and sticky. During transesterification, glycerin is removed from the vegetable oil to make the oil thinner, or to reduce its viscosity.

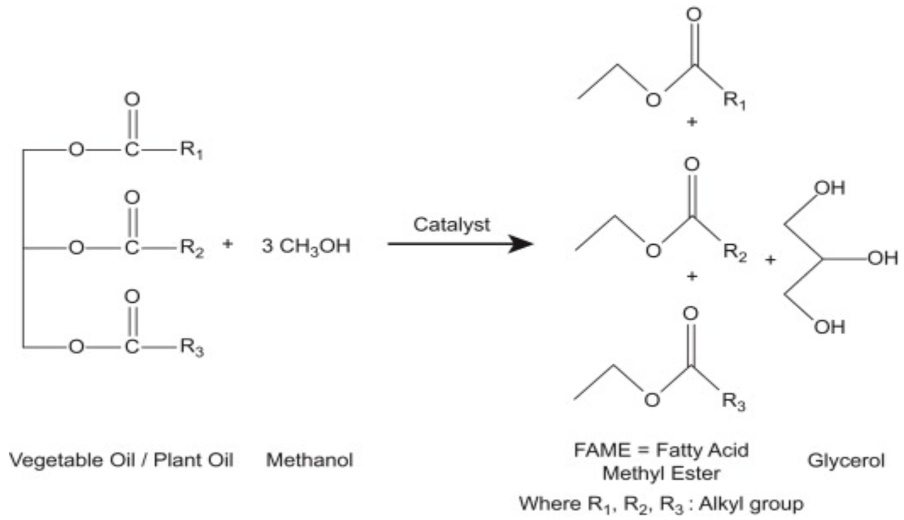


Figure 1: Transesterification Reaction [3].

During the transesterification process, the glycerin component of vegetable oil is replaced with alcohol, as illustrated in Figure 1. For this, methanol is preferred to ethanol since it produces a more stable biodiesel reaction over ethanol *(3).* As a result of transesterification with methyl alcohol, the triglycerides would yield methyl ester of fatty acids which is then referred to as biodiesel. During the reaction, each triglyceride molecule is transesterified into three methyl esters after they are being cleaved off from a glycerin molecule. Hence, during this process, a triglyceride molecule would offer three fatty acid methyl esters.

Sodium hydroxide (NaOH) is required for the transesterification process and act as a catalyst to shift the reaction forward. During this process, the alcohol acts as a nucleophile and undergoes an addition-elimination reaction, thus resulting in the methyl ester of fatty acids. Once the reaction is complete, the catalyst will remain dissolved in the aqueous layer along with glycerol at the bottom of the mixture.

2.2 Instrumentation Background

GC-MS is a common instrument used in the quantitative and qualitative analyses of liquid and gaseous molecules. GC-MS is a hyphenated analytical technique that comprises two instruments combined in series to output robust analytical results. In this instrument, gas chromatography separates the components in a sample and the results are displayed in the form of a chromatogram, where the band intensity informs about the quantity of the analytes and the number of bands represents different molecules in the mixture. Mass spectrometry, on the other hand, gives out the discrete number in the form of peaks that either represents the mass of the molecule or its fragments, which is characteristic to individual components in the mixture. Combining these techniques one can quantitatively and qualitatively analyze the compounds in a given mixture.

The separation of the compounds in a mixture is carried out by the GC portion of the instrument. The sample can be introduced to the GC manually or through an autosampler. The injected sample enters the instrument through a port called the inlet.

Inlets are kept at a temperature of 200°C to ensure that the sample is quickly vaporized as a gaseous phase. The mobile phase, such as helium, carries the sample through the column, where the individual compounds are separated (shown in Figure 2) because of different strength of intermolecular interactions with the stationary phase. The column is held in an oven that can ramp the temperature gradually to aid successful compound separation. As the temperature remains constant or temperature increases, low boiling compounds elute first from the column and so on. Control of temperature and the type of stationary phase in a GC column are the two important conditions for efficient separations in GC.

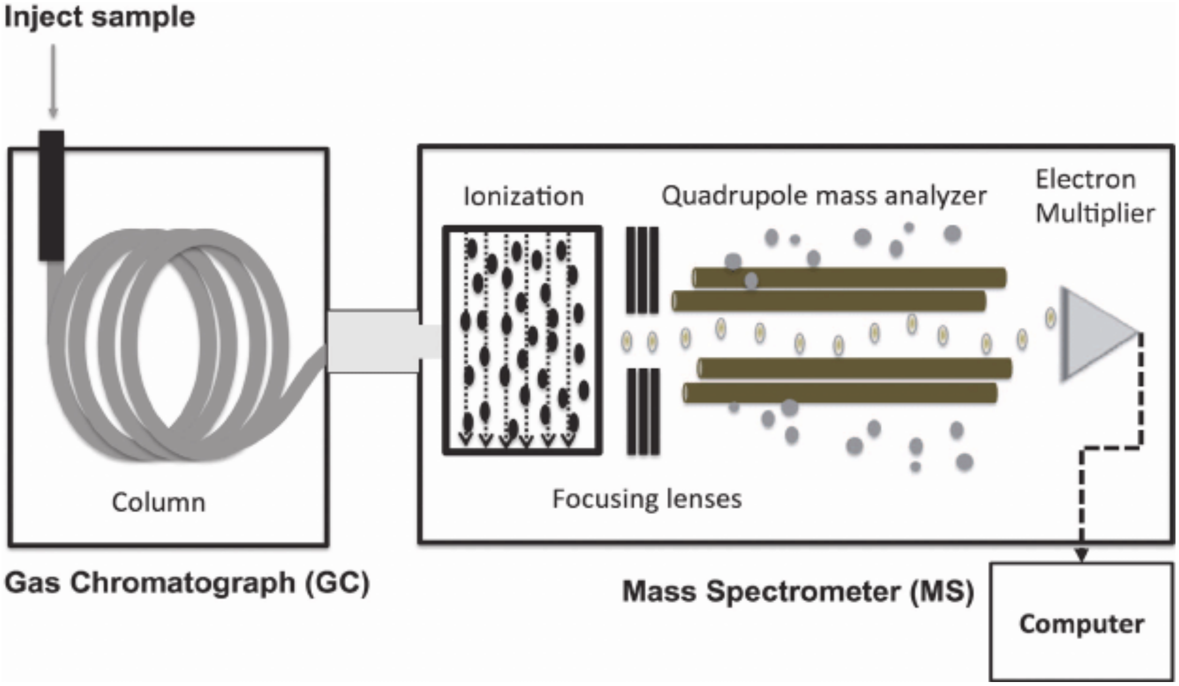


Figure 2: Diagram of the Gas Chromatograph coupled to a Mass Spectrometer *(4)*.

GC columns have a narrow diameter, usually, 0.1-0.5 mm and are made up of fused silica capillaries that have chemically bonded stationary phase inside the column *(4)*. The polarity of a stationary phase varies depending on its chemical composition. If the stationary phase is non-polar, it will tightly interact with non-polar analytes thus increasing the retention time (RT). A detector creates an electronic signal in response to the presence of a compound is. The greater the amount of the analyte, the bigger the signal.

As the individual solutes elute from the GC column, they enter the MS instrument. Here, the molecules are bombarded with a stream of electrons that have sufficient energy to break apart into fragments. The fragments are charged ions with a certain mass and the mass of the fragments divided by the charge is called the mass to charge ratio (m/z). Since most fragments have a charge of +1, the m/z usually represents the molecular weight of the fragment. The mass of a compound can be reproducibly obtained if the electron energy used to impact the molecule is the same. Therefore, the mass spectrum is essentially a fingerprint for the molecule, which can be used to identify a compound *(4).*

The GC-MS inbuilt computer has a library of spectra that can be used to help identify compounds within a mixture. A software searches the library of mass spectra of standards to find the closest mass spectrum of an unknown and reports a list of likely identifications along with the statistical probability of identification.

2.3 Fatty Acid Methyl Esters

Fatty acid methyl esters are typically produced by an alkali-catalyzed reaction between fats and methanol in the presence of a base such as sodium hydroxide. Structurally, all the FAMEs share similar chemical moieties - methyl and ester groups, as illustrated in Figure 3.

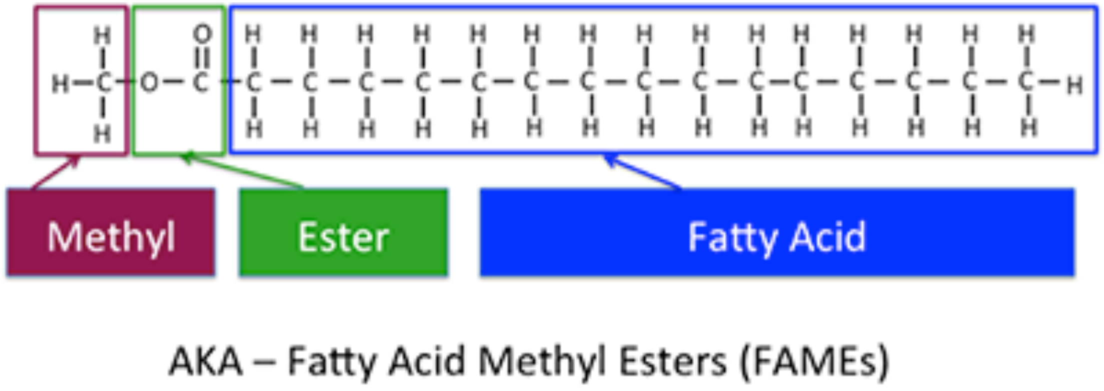


Figure 3: A Chemical Structure of the FAME Showing Different Moieties [19].

Omega-3 and Omega-6 fatty acids are important nutritional fatty acids commonly found in food sources such as fish, meat, nuts and oils. For example, salmon oil is an excellent source of polyunsaturated Omega-3 fatty acids, such as EPA and DHA. Proper identification and quantification of these FAMEs by GC is important for nutritional labeling and quality control tests *(8).* This is one of the reasons why we used the three most common fatty acids, DPA, DHA and EPA, found in fish oils to validate our GC-MS method (all shown respectively in Figures 4, 5 and 6).

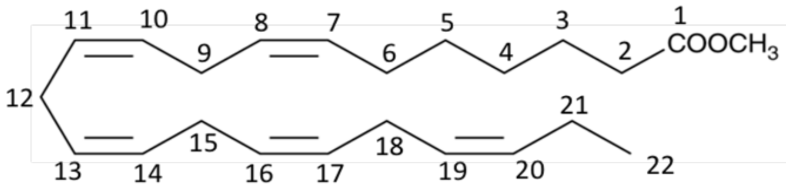


Figure 4: Structure of Methyl Ester 7, 10, 13, 16, 19 – Docosapentaenoic Acid (DPA)

DPA is an Omega-3 fatty acid, which is an intermediate of *in vivo* synthesis of DHA and EPA.

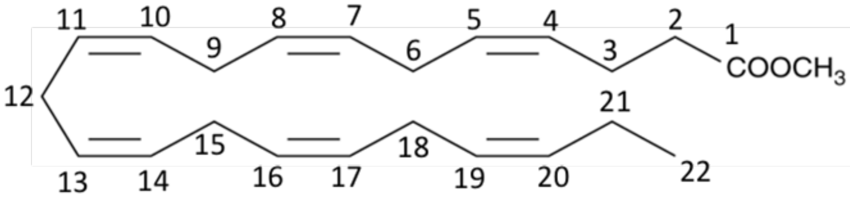


Figure 5: Structure of Methyl Ester 4, 7, 10, 13, 16, 19 – Docosahexaenoic Acid (DHA)

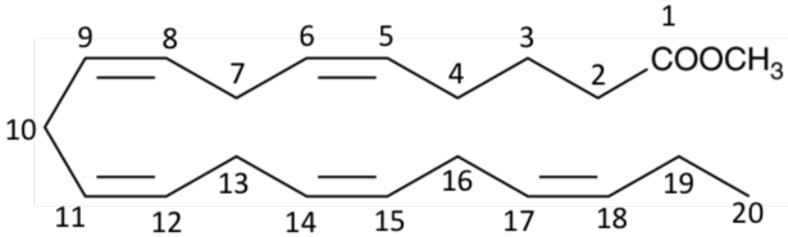


Figure 6: Structure of Methyl Ester 5, 8, 11, 14, 17 – Eicosapentaenoic Acid (EPA)

FAMEs are a type of fatty acid esters that are derived by the transesterification of fats with methanol as explained in section 2.1. The physical characteristics of FAMEs are closer to those of fossil diesel fuels than pure vegetable oils, but the properties depend on the type of vegetable oil. A mixture of different FAMEs is referred to as biodiesel. For our purpose, we used soybean and sunflower oils. From our qualitative GC-MS analysis, we found the following five fatty acids as the major biodiesel components obtained from soybean and sunflower oils:

1. methyl ester linoleic acid,
2. methyl ester oleic acid,
3. methyl ester linolenic acid,
4. methyl ester palmitic acid and
5. methyl ester stearic acid.

We therefore decided to pursue the quantitative analysis of these five FAMEs using the GC-MS instrument.

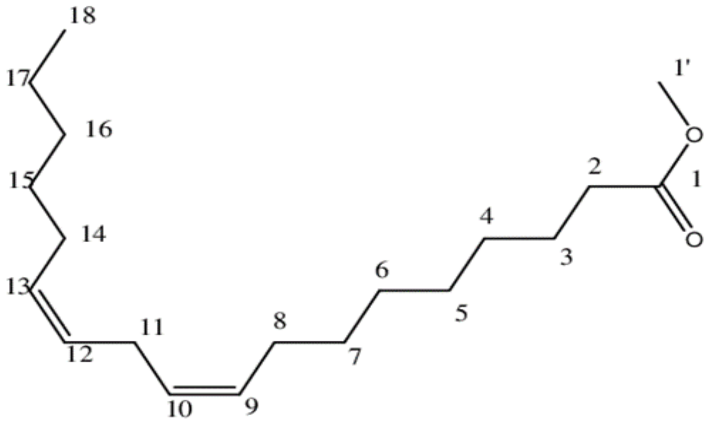


Figure 7: Structure of Methyl Ester 9, 12 – Octadecadienoic Acid (Methyl Ester Linoleic Acid)

Methyl ester linoleic acid is a polyunsaturated Omega-6 fatty acid. It is one of the two essential fatty acids from humans, and is found in nuts, seeds and their derived vegetable oils.

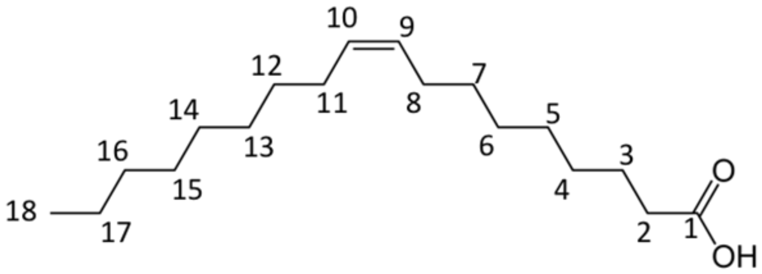


Figure 8: Structure of Methyl Ester 9 – Octadecenoic Acid (Methyl Ester Oleic Acid)

Methyl ester oleic acid is a monounsaturated Omega-9 fatty acid that is the most common fatty acid found in nature. It is in many animal and vegetable fats and oils as well.

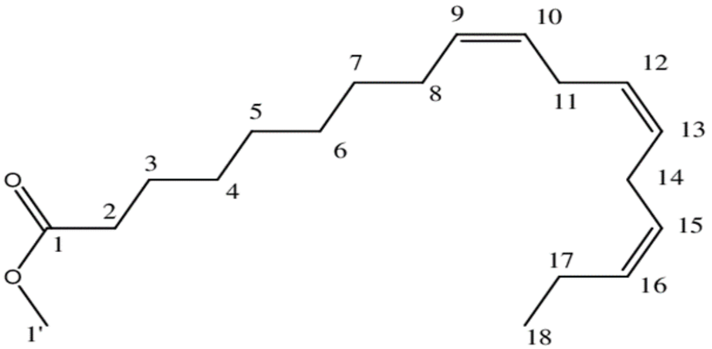


Figure 9: Structure of Methyl Ester 9, 12, 15 – Octadecatrienoic Acid (Methyl Ester Linolenic Acid)

Methyl ester linolenic acid is a polyunsaturated Omega-3 fatty acid, that is found in seed and vegetable oils.

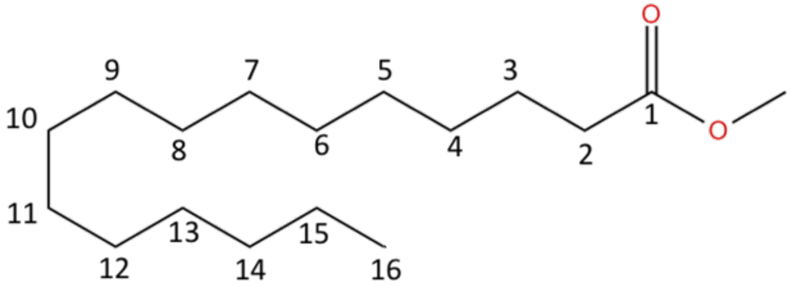


Figure 10: Structure of Methyl Ester Hexadecanoic Acid (Methyl Ester Palmitic Acid)

Methyl ester palmitic acid is the most common saturated fatty acid found in animals, plants, and microorganisms. It is also widely present in the meats, cheese, butter and other dairy products, as well as cocoa butter, soybean, and sunflower oil.

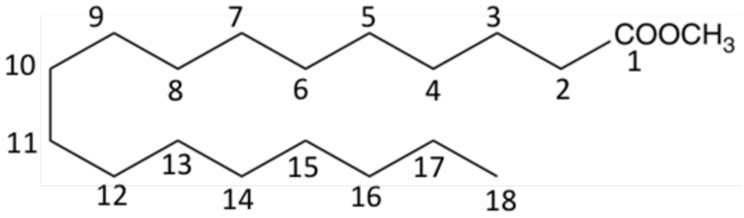


Figure 11: Structure of Methyl Ester Octadecanoic Acid (Methyl Ester Stearic Acid)

Methyl ester stearic acid is also a saturated fatty acid. It is the second most common saturated fatty acid in nature, after methyl ester palmitic acid. Fats and oils rich in stearic acid are more abundant in animal fat than in vegetable fat.

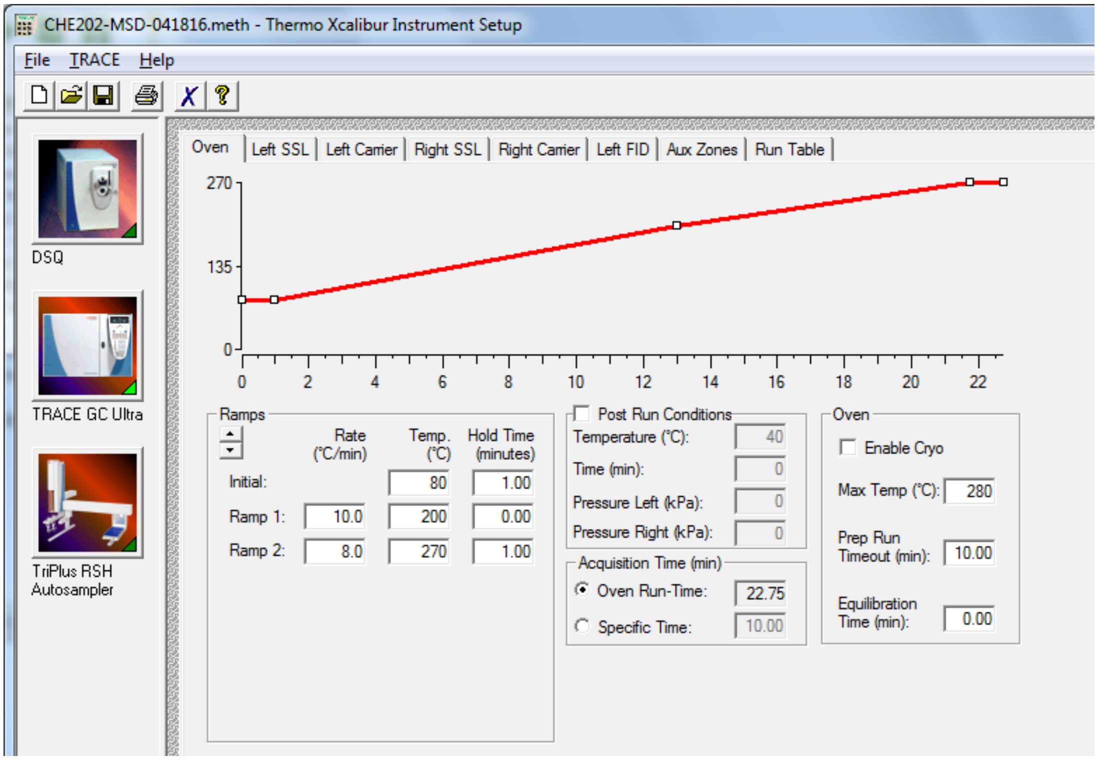
2.4 Goals of the Study

The objective of this project is to synthesize biodiesel from used vegetable oils and to develop an analytical method for the quantitative and qualitative analyses of the esters of fatty acids. For this, we planned to develop a GC-MS method and validate the protocol using fatty acid esters derived from fish oil. The GC-MS analysis of fatty acid esters from fish oils are well-established and widely published in the literature. Further, this would ease us to modify the existing method towards the analysis of biodiesel-derived esters of fatty acids. After the successful method validation, the same GC-MS method was used to measure the composition of biodiesels that are synthesized from cooked and uncooked soybean and sunflower oils. Further, these data were used for analyzing the composition of biodiesel and petroleum diesel fuel in a mixture. Finally, the physical properties were studied of the biodiesel fuels derived from different sources and confirmed that the data are in the literature accepted ranges.

III. Materials and Methods

3.1 Instrumentation

FAMEs were analyzed by a GC-MS instrument: Thermo-Finnigan Trace DSQ, that contains a Hewlett-Packard-5 (crosslinked 5%-phenyl-methyl-polysiloxane) column with dimensions of 30 m x 0.32 mm and a 0.25 μm film thickness. The auto-injector was operated in the splitless mode at 220°C, helium was used as a carrier gas at a flow rate of 1.5 mL/min. 1 μL of sample were injected using a Thermo Scientific TriPlis RSH autoinjector, the oven temperature was held 80°C held for 1 min and then it was increased to 200°C, at a rate of 10°C/min. Followed by an increase to 270°C at 8°C/min and held at that temperature for 1 min.



Ramps

	Rate (°C/min)	Temp. (°C)	Hold Time (minutes)
Initial:		80	1.00
Ramp 1:	10.0	200	0.00
Ramp 2:	8.0	270	1.00

☐ Post Run Conditions
Temperature (°C): 40
Time (min): 0
Pressure Left (kPa): 0
Pressure Right (kPa): 0

Acquisition Time (min)
● Oven Run-Time: 22.75
○ Specific Time: 10.00

Oven
☐ Enable Cryo
Max Temp (°C): 280
Prep Run Timeout (min): 10.00
Equilibration Time (min): 0.00

Figure 12: GC-MS Method Showing Ramp Temperature and Hold Time

3.2 Analytical Reference Standards

At first, the commercially available fatty acids esters derived from fish oils were analyzed: 99% methyl ester EPA, 99% methyl ester DHA, 99% methyl ester DPA. These samples were purchased from Sigma-Aldrich (St. Louis, Missouri USA). For each fatty acid ester six different concentrations were chosen to analyze. A serial dilution was used to create 3130, 1565, 782.5, 391.3, 195.6, and 97.8, ng/mL fatty acid ester concentrations from the Sigma-Aldrich standards in methanol.

The FAMEs that were analyzed: 99% methyl ester palmitic acid, 99% methyl ester linoleic acid, 99% methyl ester oleic acid, 98% methyl ester linolenic acid, and 99% methyl ester stearic acid as standard references were also purchased from Sigma-Aldrich (St. Louis, Missouri USA). A serial dilution was used to create nine different concentrations. Those concentrations for the FAMEs were 3130, 1565, 782.5, 391.3, 195.6, 97.8 48.9, 24.5 and 12.2 ng/mL fatty acid methyl ester concentrations from the Sigma-Aldrich standards in hexane.

3.3 Biodiesel Preparation

To synthesize the biodiesel, both cooked and uncooked vegetable oils were obtained from different locations. Table 2 shows the types of vegetable oils obtained from the restaurant and the locations.

Table 2: Types of Vegetable Oils Used, Restaurant Name & Location

Type of Oil	Restaurant Name	Location
Cooked Soybean Oil #1	Flip Burger	Niagara Falls, NY
Cooked Soybean Oil #2	Hilleview Cafe	Lewiston, NY
Uncooked Soybean Oil #2	Hilleview Cafe	Lewiston, NY
Cooked Sunflower Oil	Chipotle	Niagara Falls, NY
Uncooked Sunflower Oil	Chipotle	Niagara Falls, NY

The first soybean oil sample was cooked oil from Flip Burger Restaurant located in Niagara Falls, NY. The second soybean oil samples, both cooked and uncooked samples were from Hilleview Café in Lewiston, NY. The last vegetable oil sample was sunflower oil (cooked and uncooked) that was obtained from Chipotle, located in Niagara Falls, NY.

The transesterification of all cooked and uncooked vegetable oils was carried out the same way. First, 20 mL methanol was mixed with 0.35 g NaOH to obtain a sodium methoxide, which is a powerful corrosive base and harmful for human skin. Proper safety precautions were taken to avoid skin contact while handling it. Once NaOH was fully dissolved in methanol, we added 100 mL of the corresponding vegetable oil to the solution and vigorously mixed them for 15 min. The mixture was then transferred to a separatory funnel as shown in Figure 13A and a bilayer was formed in 2 hrs before gravity separation.

Figure 13 A Figure 13 B Figure 13 C Figure 13 D

Figure 13: Biodiesel Synthesis

The top layer is the organic layer, or biodiesel layer. The bottom layer is the unreacted components and the glycerin byproduct. The bottom layer was discarded, leaving the top biodiesel layer. The biodiesel was further washed with distilled water several times. In each wash, approximately 100 mL of distilled water was added to the organic layer and vigorously mixed until it looked milky, as shown in Figure 13C. Finally, the mixture was allowed to stand for several hours until clear biodiesel top-layer was formed, as illustrated in Figure 13C.

IV. Results

4.1 Qualitative Analysis of Fatty Acid Esters of Fish Oils

While using the GC-MS two important pieces of information should always be considered, retention time (RT) and peak area (PA). Often the PA could vary between each run, due to human and instrumental error. However, RT should remain consistent, due to intrinsic physical characteristic of the sample, as long as the analytical method remains the same. Before GC-MS analysis of a sample, it is also important to understand the elution order of the compounds in the mixture. For this, the GC-MS analytical method described in Section 3.1 was used in our purpose. Elution order also ensures that the method is ramped high enough for the compounds to elute off the column and show an accurate retention time. Upon analyzing a mixture of fatty acid esters in GC-MS, three peaks were observed with retention time 17.53, 19.38 and 19.50 minutes, as illustrated in

Figure 14 (Table 3).

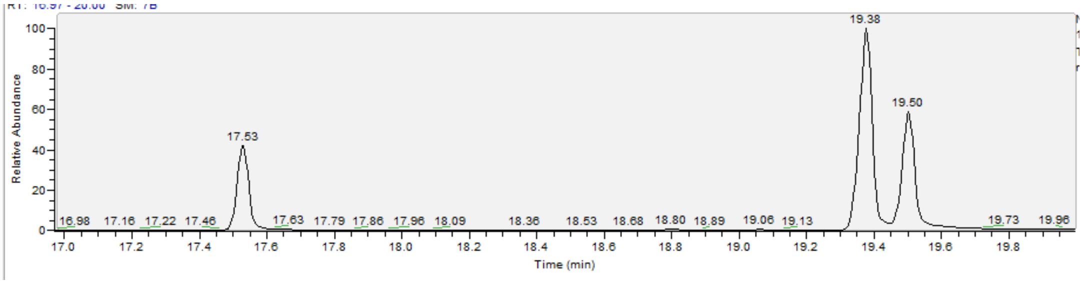


Figure 14: Elution order of fatty acid esters on GC-MS: EPA (17.53 min.), DHA (19.38 min) and DPA (19.50 min).

Since the GC-MS was run in TIC mode, chromatograms and MS were collected at the same time, the observed retention time along with the fragmentation pattern of each analyte can be compared. In the event when there is no match within the database, the relative ions and their relative abundances can be verified using online databases for the compound identification. Table 3 shows the RT of the different esters of fatty acids derived from fish oil and their corresponding molecular masses.

Table 3: RT & MW of Fatty Acid Esters

	RT (min)	MW (g/mol)
Methyl Ester EPA	17.53	316.5
Methyl Ester DHA	19.38	342.5
Methyl Ester DPA	19.50	344.5

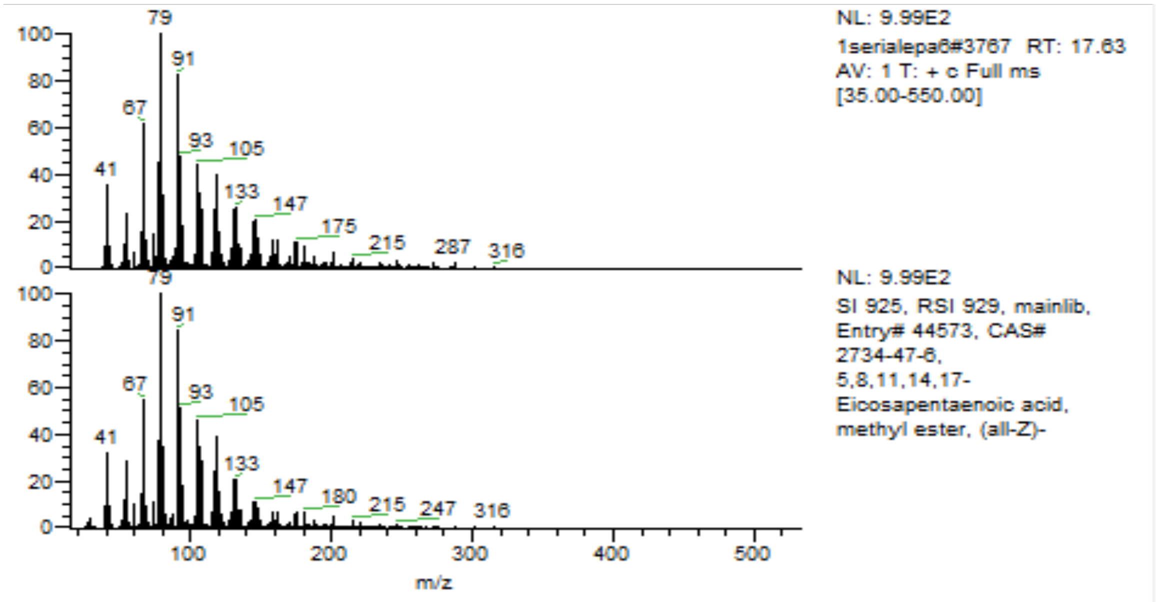


Figure 15: Database Comparison of Mass Spectra

Using the library search function on the inbuilt computer database, all three fatty acid esters were positively matched. Figure 15 shows a comparative mass spectral fragmentation pattern of methyl ester EPA of an unknown compound with the inbuilt database on the computer. Analyzing both mass spectrums of methyl ester EPA, the parent peak was at 316 m/z and the base peak was observed at 79 m/z. This process was used for all three fatty acid esters and were all positively identified. The parent peak of methyl ester DHA is 342 m/z and the base peak was observed at 79 m/z. Lastly, the parent peak for methyl ester DPA is 344 m/z and the base peak was also observed at 79 m/z. From this study, the elution order of the ester of fatty acids confirmed to be in the order of methyl ester EPA, methyl ester DHA and methyl ester DPA.

From the above experiment we validated that our GC-MS method is compatible with the esters of fatty acid derived from fish oil. Encouraged with these results, we further attempted to explore the chemical composition of the biodiesel using the same GC-MS method. We hypothesized that the above-mentioned esters have relatively lower molecular masses, and lesser hydrophobicity than most of the esters from vegetable oil. We therefore expected to see an increase in retention time in majority with the similar fragmentation pattern.

4.2 Qualitative Analysis of FAMEs of Biodiesel

Qualitative analysis of FAMEs of the biodiesel samples was initiated using the above-mentioned GC-MS analytical method as described in section 3.1. The gas chromatogram of the FAMEs showed that the mixture is composed of five different esters of fatty acids having retention time 14.00, 15.77, 15.83, 15.85 and 16.08 minutes. Comparing the MS-data of the sample with the inbuilt database in the computer, the esters were identified as methyl esters of palmitic acid, linoleic acid, oleic acid, linolenic acid, and stearic acid, as summarized in the Table 4.

Table 4: RT & MW of FAMEs

	RT (min)	**MW (g/mol)**
Methyl Ester Palmitic Acid	14.00	270.5
Methyl Ester Linoleic Acid	15.77	294.5
Methyl Ester Oleic Acid	15.83	296.5
Methyl Ester Linolenic Acid	15.85	292.5
Methyl Ester Stearic Acid	16.08	298.5

The mass spectra of FAMEs were analyzed to identify each compound using the computer inbuilt database the same way the fatty acid esters of fish oils were done. Each FAME was positively identified using the database. Methyl ester palmitic acid has a MW of 270.5 g/mol its parent peak was observed at 270 m/z and its base peak was observed at 74 m/z. The base peak observed at 74 m/z is caused by the McLafferty rearrangement ion, which is a site-specific sterically favored arrangement that occurs when a hydrogen atom at position 4 of the aliphatic chain migrates to the carbo-methoxy group through a six-membered transition state. This is more evident in the mass spectra of saturated fatty acids. There are also a series of related ions of the general formula $[(CH_2)_nCOOCH_3]^+$ at 87 m/z, followed by ions at 101, 115, 129, 143, etc. m/z. These are formed by losses of aliphatic radical cations from the terminal part of the molecule *(18).*

Likewise, the other methyl esters were identified. In brief, methyl ester linoleic acid has a MW of 294.6 g/mol, its parent peak was observed at 296 m/z and its base peak observed at 67 m/z. Methyl ester oleic acid has a MW of 296.5 g/mol, its parent peak was observed 296 m/z and its base peak was observed at 55 m/z. Methyl ester linolenic acid has a MW of 292.5 g/mol, with its parent peak observed at 292 m/z and its base peak observed at 79 m/z. Lastly, methyl ester stearic acid has a MW of 298.5 g/mol and its parent peak was observed at 299 m/z and its base peak was observed at 74 m/z. The elution order for the FAMEs were studied as follows: methyl ester palmitic acid, methyl ester linoleic acid, methyl ester oleic acid, methyl ester linolenic acid and methyl ester stearic acid.

4.3 Quantitative Analysis of Fatty Acids Esters of Fish Oils

After a successful qualitative analysis of the esters, we proceed onto quantitative analysis. A serial dilution was prepared of individual esters of fish oils, purchased commercially. The diluted samples of the individual ester were used to construct a calibration curve that can be used to find the concentration of the unknown sample. A serial dilution was created from 3130, 1565, 782.5, 391.3, 195.6, and 97.8 ng/mL of each fatty acid ester standard purchased from Sigma-Aldrich. The graphs were created by plotting peak areas against the concentrations for methyl esters of EPA, DHA and DPA. Subsequently, the calibration curves that are generated from the serial dilution, were used toward finding the concentration of individual esters of fatty acids from the unknown samples.

Table 5: The Squares of Correlation Coefficients (R^2) and Linear Regression Equations of Fatty Acid Esters

	RT (min)	**R^2**	**Equation of Line**
Methyl Ester EPA	17.53	0.9779	$Y=3 \times 10^9 X+8 \times 10^8$
Methyl Ester DHA	19.37	0.9784	$Y=4 \times 10^9 X+8 \times 10^8$
Methyl Ester DPA	19.49	0.9876	$Y=3 \times 10^9 X+6 \times 10^8$

From the calibration curve, R^2 values for esters of EPA, DHA and DPA were found to be 0.9779, 0.9784, 0.9876 as shown in Table 5.

The R^2 values for the esters of EPA, DHA and DPA are not up to par. One major reason behind our R^2 values is the number of samples analyzed, there were six concentrations used for each compound's concentration curve. Using more concentrations could have greatly improved the R^2 values to lie further within the accepted range. Therefore, for the quantitative analysis of FAMEs of biodiesel, there were nine concentrations that were analyzed.

4.4 Quantitative Analysis of FAMEs of Biodiesel

After the successful preliminary study on the ester of fatty acids from fish oils, the quantitative analysis of FAMEs from biodiesel was carried out. For each standard FAMEs purchased from Sigma-Aldrich, nine different serial dilutions were created - 3130, 1565, 782.5, 391.3, 195.6, 97.8, 48.9, 24.5 and 12.2 ng/mL. A graph was plotted between the peak areas against the concentrations for each methyl ester palmitic acid, linoleic acid, oleic acid, linolenic acid and stearic acid to come up with a calibration curve for each FAME with the data from the GC-MS (Figures 16-20: Table 6).

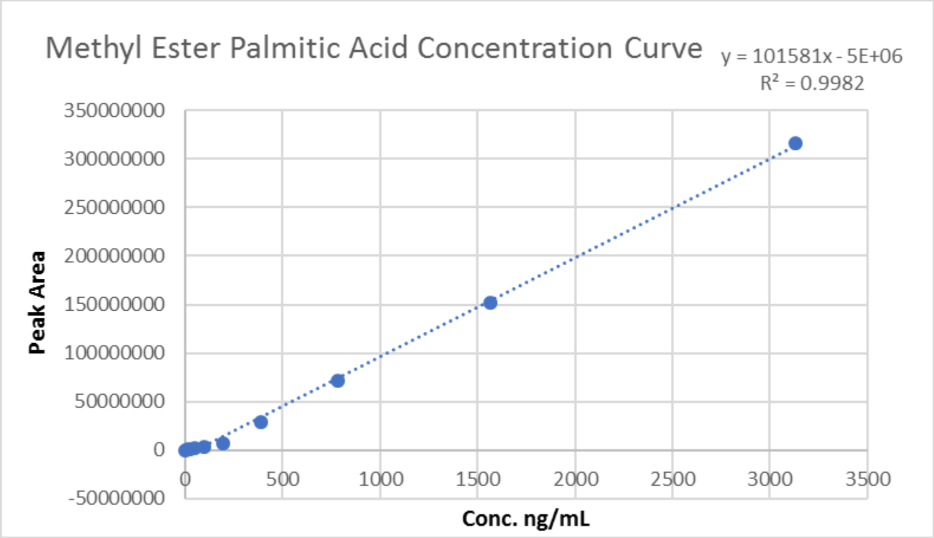


Figure 16: Calibration Curve for Methyl Ester Palmitic Acid

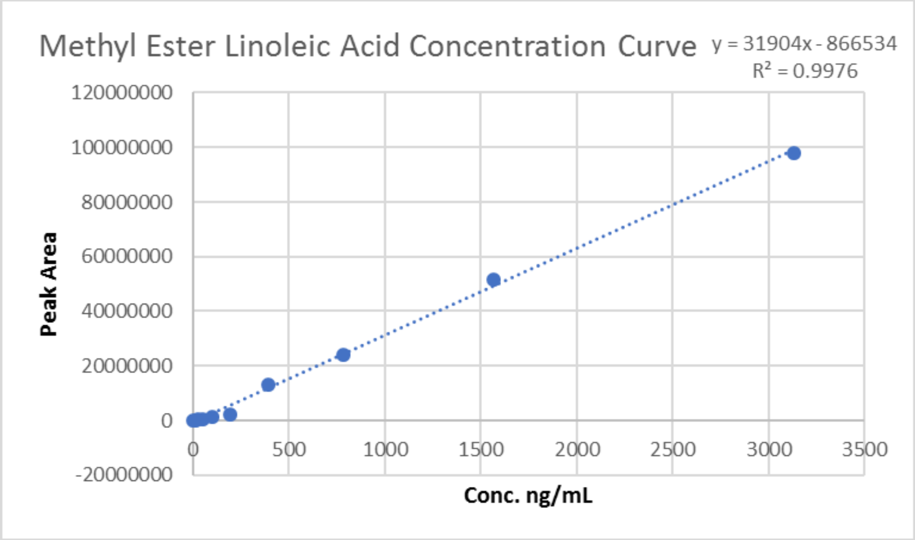


Figure 17: Calibration Curve for Methyl Ester Linoleic Acid

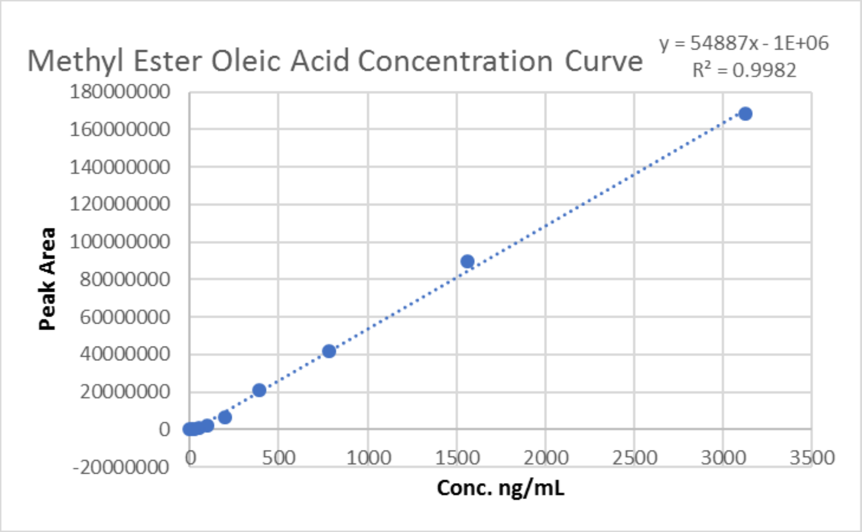


Figure 18: Calibration Curve for Methyl Ester Oleic Acid

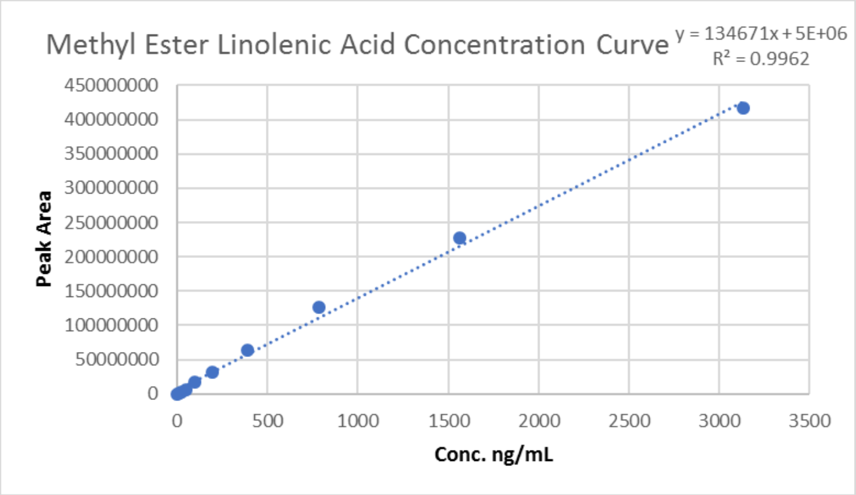


Figure 19: Calibration Curve for Methyl Ester Linolenic Acid

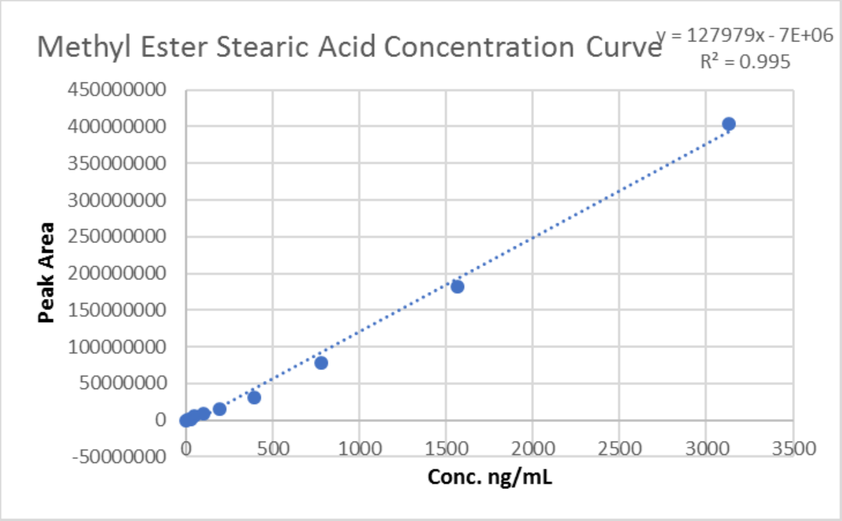


Figure 20: Calibration Curve for Methyl Ester Stearic Acid

Table 6: The Squares of Correlation Coefficients (R^2) and the Linear Regression Equations of Five Standard FAMEs

	RT (min)	**R^2**	**Equation of Line**
Methyl Ester Palmitic Acid	14.00	0.9982	$Y=101581\ X - 5 \times 10^6$
Methyl Ester Linoleic Acid	15.77	0.9976	$Y=31904\ X - 866534$
Methyl Ester Oleic Acid	15.83	0.9982	$Y=54887\ X + 1 \times 10^6$
Methyl Ester Linolenic Acid	15.85	0.9962	$Y=134671\ X + 5 \times 10^6$
Methyl Ester Stearic Acid	16.08	0.9950	$Y= 127979\ X - 7 \times 10^6$

The linearity (R^2) of the concentration curves for each fatty acid methyl ester greatly improved from our previous concentration curves from the fatty acid esters of fish oils. From the calibration curve, R^2 values for the methyl esters of palmitic acid, linoleic acid, oleic acid, linolenic acid and stearic acid were found to be 0.9982, 0.9976, 0.9982, 0.9962 and 0.9950 as shown in Table 6. Also, each FAME corresponding equation from the concentration curve can be found in Table 6 where y equals the peak area and x equals the concentration.

4.5 Quantitative Analysis of Biodiesel

For the quantitative analysis of contents of biodiesel, the samples synthesized using five different types of vegetable oils were explored, as shown in Table 2. Prior to quantitation, individual samples of biodiesel were diluted to the extent such that the peak areas of ester fall within the linearity of concentration curves. After the several runs of the diluted samples, we confirmed that approximately 100,000x dilution required for the satisfactory result. The first sample of biodiesel that was synthesized and analyzed shown in Figure 21 was a cooked soybean oil from Flip Burger restaurant.

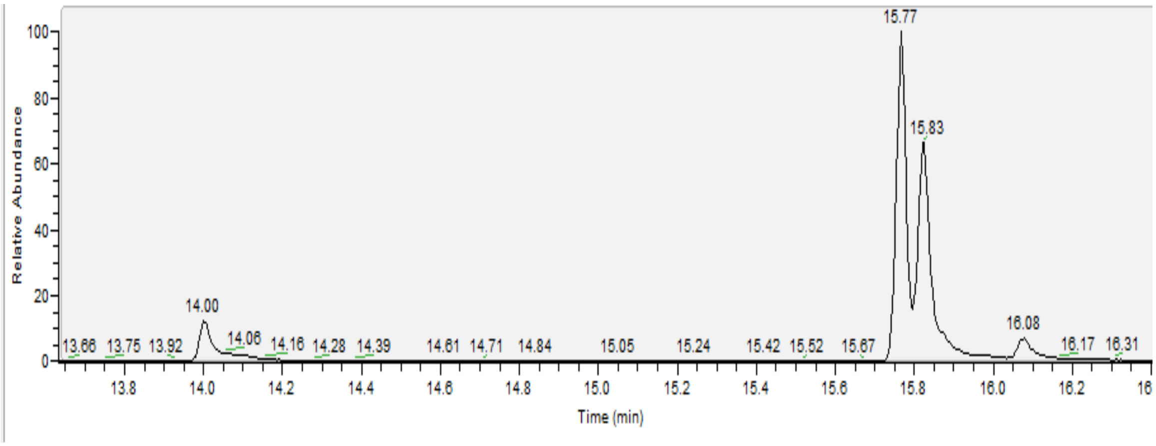


Figure 21: TIC Biodiesel Made from Cooked Soybean Oil #1 (Flip Burger)

The GC from the first biodiesel sample showed four different esters of fatty acids. Using the inbuilt database, all four compounds were successfully identified and are shown in Figure 21 (Table 7). The compound that has a retention time of 14.00 minutes was identified as methyl ester palmitic acid. With the aid of the calibration equation found in Table 6, the sample concentration was found to be 90.7 ng/mL. The second compound with a RT of 15.77 minutes was identified as methyl ester linoleic acid with its concentration of 437.7 ng/mL based on the calibration curve. Likewise, the third compound was identified as methyl ester oleic acid with a RT of 15.83 minutes and calculated concentration was 221.9 ng/mL based on the calibration curve. Lastly, the methyl ester stearic acid was observed at its RT of 16.08 and its calculated concentration was 77.3 ng/mL. Considering the calculated concentrations of all four compounds, the percentage composition of methyl ester linoleic acid was found to be the most abundant, which was 52.9%. Similarly, the percentage composition of other methyl esters of fatty acids were found to be 26.8%, 11.0% and 9.30% representing methyl esters of oleic acid, palmitic acid and stearic acid, respectively.

Table 7: Compounds Detected from Biodiesel from Cooked Soybean Oil #1

Compound	**RT (min)**	**Peak Area**	**Calculated Conc. (ng/mL)**	**Relative Percentage**
1. Methyl Ester Palmitic Acid	14.00	4213952	90.7	11.0 %
2. Methyl Ester Linoleic acid	15.77	13096361	437.7	52.9%
3. Methyl Ester Oleic Acid	15.83	11181287	221.9	26.8%
4. Methyl Ester Stearic Acid	16.08	2895592	77.3	9.30%

The second sample of biodiesel that was synthesized and analyzed was from another cooked sample of soybean oil from Hilleview Café, shown in Figure 22.

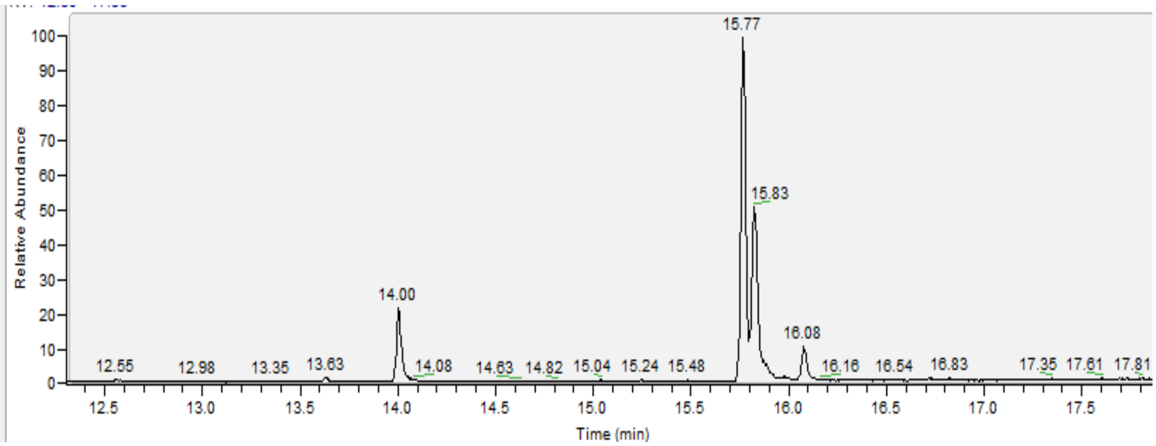


Figure 22: TIC Biodiesel Made from Cooked Soybean Oil #2 (Hilleview Cafe)

The GC showed the second biodiesel also comprises four different esters of fatty acids, same as the previous sample. With the aid of an inbuilt database, the identified compounds were methyl esters of palmitic acid, linoleic acid, oleic acid, and stearic acid as shown in the Table 8. As evident from the GC, the RT for these esters were 14.00 min, 15.77, 15.83 and 16.05 corresponds to the methyl esters as explained in our previous sample (Figure 21). Using the calibration equation from Table 6 the concentration of methyl ester palmitic acid was calculated to be 98.5 ng/mL, 660.4 ng/mL was found to be to concentration of methyl ester linoleic acid. Likewise, the concentration of methyl ester oleic acid was calculated to be 200.7 ng/mL, lastly the concentration of methyl ester stearic acid was 74.3 ng/mL. Considering the calculated concentrations of all four compounds, the percentage composition of methyl ester linoleic acid was found to be the most abundant, which was 63.9%. Similarly, the percentage composition of other esters of fatty acids were found to be 19.4%, 9.51% and 7.19% representing methyl esters of oleic acid, palmitic acid and stearic acid, respectively. Comparing the biodiesels synthesized from the soybean oils obtained from two different origins, the trend of the composition of methyl esters looks similar however the percentage composition of methyl esters was found to be different.

Table 8: Compounds Detected from Biodiesel from Cooked Soybean Oil #2

Compound	RT (min)	Peak Area	Calculated Conc. (ng/mL)	Relative Percentage
1. Methyl Ester Palmitic Acid	14.00	5002604	98.5	9.51%
2. Methyl Ester Linoleic acid	15.77	20203813	660.4	63.9%
3. Methyl Ester Oleic Acid	15.83	10013733	200.7	19.4%
4. Methyl Ester Stearic Acid	16.08	2508465	74.3	7.19%

The third biodiesel sample was synthesized from an uncooked sample of soybean oil from Hilleview Café, shown in Figure 23.

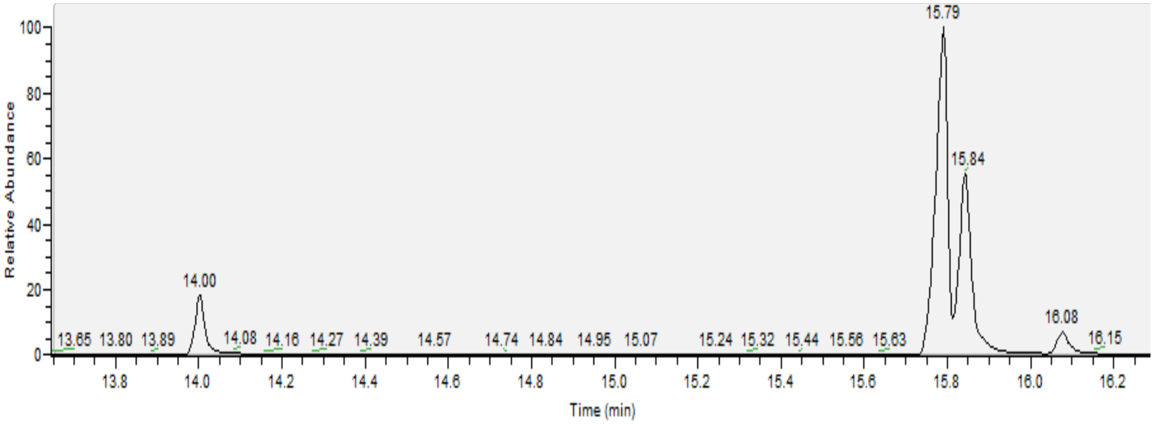


Figure 23: TIC Biodiesel Made from Uncooked Soybean Oil #2 (Hilleview Cafe)

The GC from the third biodiesel sample similarly showed four different esters of fatty acids, as seen in the previous two samples. Using the inbuild database, all four compounds were successfully identified and are shown in Figure 23 (Table 9). The compound that has a retention time of 14.00 minutes was identified as methyl ester palmitic acid. With the aid of the calibration equation found in Table 6, the sample concentration was found to be 58.2 ng/mL. The second compound with a RT of 15.79 minutes was identified as methyl ester linoleic acid with its concentration of 246.8 ng/mL based on the concentration curve. Likewise, the third compound was identified as methyl ester oleic acid with a RT of 15.84

minutes and calculated concentration was 108.0 ng/mL based on concentration curve. Lastly, the methyl ester stearic acid was observed at its RT of 16.08 and its calculated concentration was 62.3 ng/mL. Considering the calculated concentrations of all four compounds, the percentage composition of methyl ester linoleic acid was found to be the most abundant, which was 51.9%. Similarly, the percentage composition of other esters of fatty acids were found to be 22.7%, 13.2% and 12.2% representing methyl esters of oleic acid, stearic acid and palmitic acid, respectively. Comparing the biodiesels synthesized from the cooked soybean oils to the uncooked soybean oil, the trend of the composition of methyl esters looks similar however the percentage composition of methyl esters was found to be different.

Table 9: Compounds Detected from Biodiesel from Uncooked Soybean Oil #2

Compound	**RT (min)**	**Peak Area**	**Calculated Conc. (ng/mL)**	**Relative Percentage**
1. Methyl Ester Palmitic Acid	14.00	912297	58.2	12.2%
2. Methyl Ester Linoleic acid	15.79	7006209	246.8	51.9%
3. Methyl Ester Oleic Acid	15.84	4925463	108.0	22.7%
4. Methyl Ester Stearic Acid	16.08	970708	62.3	13.2%

After the soybean oil samples were synthesized and analyzed. biodiesel was then synthesized using sunflower oil. The sunflower oil samples were gathered from Chipotle. Figure 24 represents the chromatogram of the biodiesel made from the cooked sample of sunflower oil.

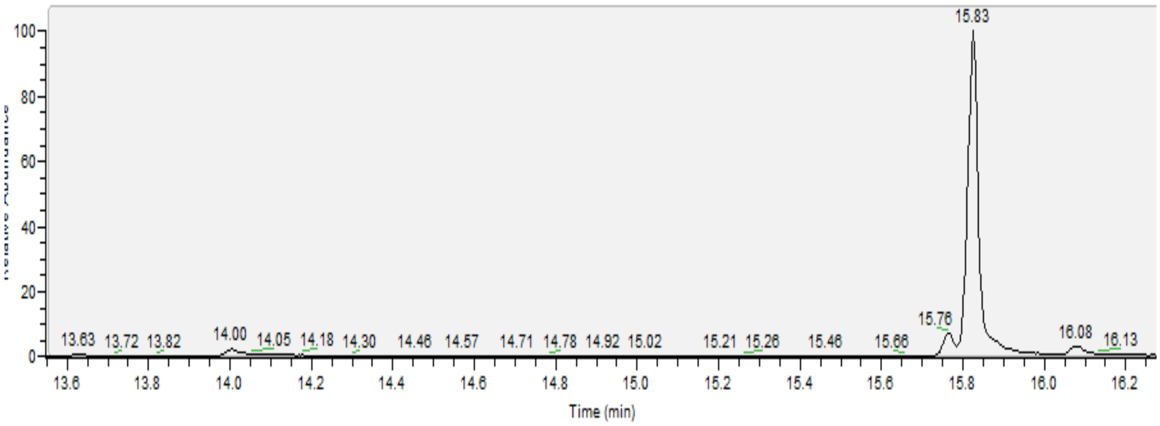


Figure 24: TIC Biodiesel Made from Cooked Sunflower Oil (Chipotle)

The GC showed the fourth biodiesel also comprises the same four different esters of fatty acids as the previous samples. Methyl esters of palmitic acid, linoleic acid, oleic acid, and stearic acid were identified as shown in the Table 10. Using the RT and mass spectrum library, the RT for these esters were 14.00 min, 15.76, 15.83 and 16.08 which corresponds to methyl ester palmitic acid, linoleic acid, oleic acid and stearic acid, respectively. Using the calibration equation from Table 6 the concentration of methyl ester palmitic acid was calculated to be 51.6 ng/mL, 41.2 ng/mL was found to be to concentration of methyl ester linoleic acid. Likewise, the concentration of methyl ester oleic acid was calculated to 2,464 ng/mL, lastly the concentration of methyl ester stearic acid was 75.4 ng/mL. Considering the calculated concentrations of all four compounds, the percentage composition of methyl ester oleic acid was found to be the most abundant, which was 93.5%. Similarly, the percentage composition of other esters of fatty acids were found to be 2.87%, 2.11% and 1.50% representing methyl esters of stearic acid, palmitic and linoleic acid, respectively. Comparing the biodiesels synthesized from the soybean oils obtained to the sunflower oil sample the trend of the composition of methyl esters are different, along with their percentages.

Table 10: Compounds Detected from Biodiesel from Cooked Sunflower Oil

Compound	**RT (min)**	**Peak Area**	**Calculated Conc. (ng/mL)**	**Relative Percentage**
1. Methyl Ester Palmitic Acid	14.00	650319	55.6	2.11%
2. Methyl Ester Linoleic acid	15.76	447867	41.2	1.50%
3. Methyl Ester Oleic Acid	15.83	134281734	2,464	93.5%
4. Methyl Ester Stearic Acid	16.08	2655013	75.4	2.87%

Lastly, an uncooked sample of sunflower oil from Chipotle was used to synthesize the last sample of biodiesel, shown in Figure 25.

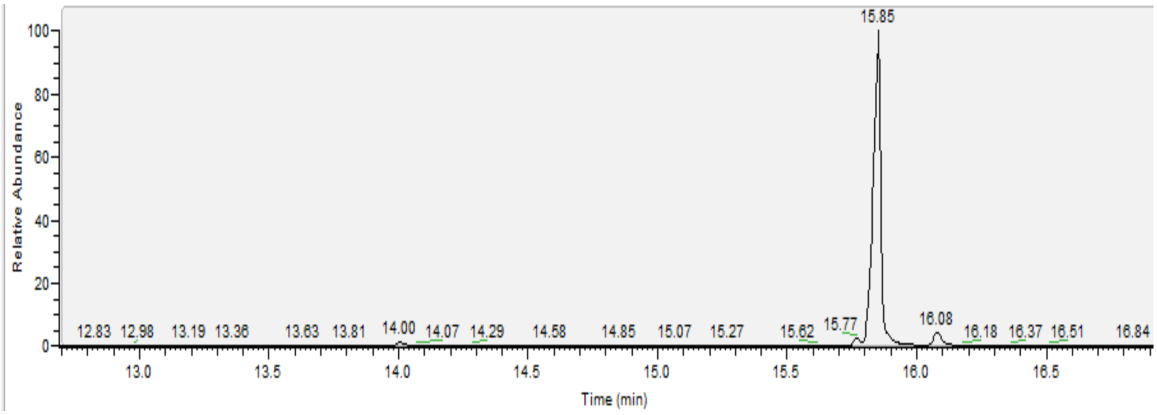


Figure 25: TIC Biodiesel Made from Uncooked Sunflower Oil (Chipotle)

The GC showed the fifth biodiesel similarly comprises four different esters of fatty acids, as the previous samples. With the aid of an inbuilt database, the identified compounds were similarly, methyl esters of palmitic acid, linoleic acid, oleic acid, and stearic acid as shown in the Table 11. As evident from the GC, the RT for these esters were 14.00 min, 15.77, 15.85 and 16.08 which corresponds to methyl ester palmitic acid, linoleic acid, oleic acid and stearic acid, respectively. Using the calibration equation from Table 6 the concentration of methyl ester palmitic acid was calculated to be 50.3 ng/mL, 39.2 ng/mL was found to be to concentration of methyl ester linoleic acid. Likewise, the concentration of methyl ester oleic acid was calculated to be 1,290 ng/mL, lastly the concentration of methyl ester stearic acid was 81.6 ng/mL. Considering the calculated concentrations of all four compounds, the percentage composition of methyl ester oleic acid was found to be the most abundant, which was 88.0%. Similarly, the percentage composition of other esters of fatty acids were found to be 5.58%, 3.44% and 2.68% representing methyl esters of stearic acid, palmitic acid and linoleic acid, respectively. Comparing the biodiesels synthesized from the soybean oils obtained to the sunflower oils obtained the trend of the composition of methyl esters are different among the different vegetable oils. There are also slight differences between the cooked and uncooked sunflower oil biodiesel.

Table 11: Compounds Detected from Biodiesel from Uncooked Sunflower Oil

Compound	**RT (min)**	**Peak Area**	**Calculated Conc. (ng/mL)**	**Relative Percentage**
1. Methyl Ester Palmitic Acid	14.00	108604	50.3	3.44%
2. Methyl Ester Linoleic acid	15.77	384463	39.2	2.68%
3. Methyl Ester Oleic Acid	15.85	69822782	1,290	88.3%
4. Methyl Ester Stearic Acid	16.08	3443731	81.6	5.58%

4.6 Quantitative Analysis of Diesel Standards

A DG #2 sample was obtained from Tops in Lewiston, NY and was labeled as an ultra-low sulfur highway diesel fuel, having 15 ppm of sulfur at maximum. The TIC chromatogram of the sample is shown in Figure 26.

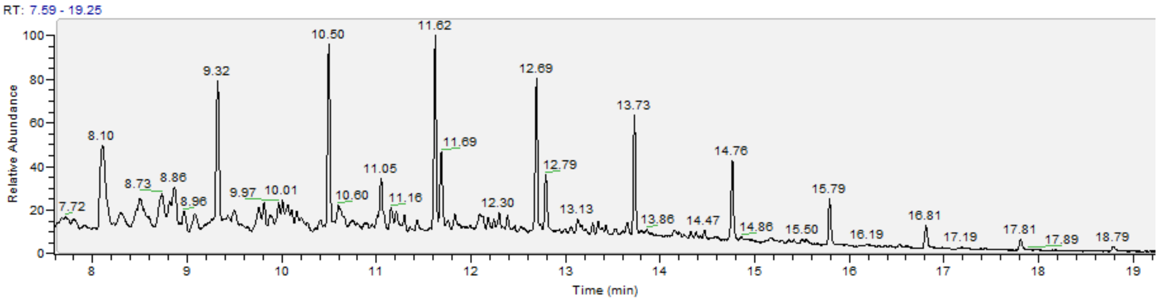


Figure 26: TIC Diesel Fuel Standard

There were eleven differentiated peaks within this chromatogram. Seen in Table 12, the first compound tetadecane, has a RT of 8.10 minutes; pentadecane with a RT at 9.32 minutes; hexadecane has a RT 10.50 minutes; heptadecane with a RT of 11.62 minutes; octadecane with a RT of 12.69 minutes; nonadecane has a RT of 13.73 minutes. The last five compounds that were found are as follows: eicosane with a RT at 14.76 minutes; heneicosane with its RT at 15.79 minutes; docosane with a RT at 16.81 minutes; tricosane with a RT at 17.81; and lastly tetracosane with a RT at 18.79 minutes.

Table 12: Compounds Detected from TIC Diesel Fuel Standard

Compound	**RT (min)**	**Peak Area**
1. Tetradecane	8.10	119497634
2. Pentadecane	9.32	91661836
3. Hexadecane	10.50	102788101
4. Heptadecane	11.62	95213897
5. Octadecane	12.69	77703632
6. Nonadecane	13.73	65348204
7. Eicosane	14.76	48643166
8. Heneicosane	15.79	27264371
9. Docosane	16.81	14527118
10. Tricosane	17.81	6033907
11. Tetracosane	18.79	3266392

The second sample of diesel fuel that was obtained and analyzed, seen in Figure 27 was a biodiesel blend with petroleum diesel fuel. This sample was obtained from Milleville Farms located in Sanborn, NY.

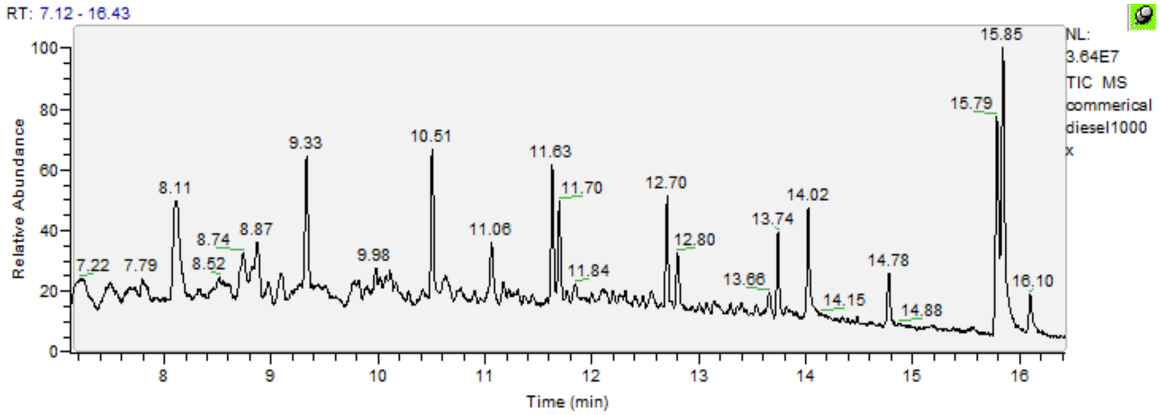


Figure 27: TIC Biodiesel Blended with Petroleum Diesel Fuel

In this biodiesel blend, there were also eleven differentiated peaks within this chromatogram. The first compound was tetradecane, with a RT of 8.11 minutes; pentadecane with a RT of 9.33 minutes; hexadecane with a RT of 10.51; heptadecane with a RT of 11.63; octadecane that has a RT 12.70 minutes; nonadecane which has a RT of 13.74. The next compound found in the blend was methyl ester palmitic acid, with a RT of 14.02 minutes, then eicosane was the next peak with a RT of 14.78 minutes. Then methyl ester linoleic acid with a RT of 15.79 minutes, methyl ester oleic acid with a RT of 15.85 minutes, and lastly methyl ester stearic acid with a RT of 16.10 minutes.

Table 13: Compounds Detected from Biodiesel Blend

Compound	RT (min)	Peak Area
1. Tetradecane	8.11	50769033
2. Pentadecane	9.33	31187555
3. Hexadecane	10.51	27779635
4. Heptadecane	11.63	20185252
5. Octadecane	12.70	19409861
6. Nonadecane	13.74	12812745
7. Methyl Ester Palmitic Acid	14.02	23680323
8. Eicosane	14.78	26739503
9. Methyl Ester Linoleic Acid	15.79	21417992
10. Methyl Ester Oleic Acid	15.85	28818823
11. Methyl Ester Stearic Acid	16.10	10362690

The composition of the diesel fuel sample is very different from that of synthesized biodiesel. As shown in Tables 12 and 13, the diesel fuel sample mainly consists of hydrocarbons, but the biodiesel sample consists of FAMEs. In the biodiesel blended with petroleum diesel fuel, there are a mixture of both hydrocarbons and FAMEs. After observing that these samples are diversely composed of compounds, the physical properties of each fuel can put into perspective how biodiesel can replace diesel fuel in a diesel engine.

4.7 Physical Properties

4.7.1 Specific gravity:

Specific gravity is a dimensionless unit, also known as relative density. It is the ratio of the density of a substance to the density of a reference substance, or the ratio of the mass of a substance to the mass of a reference substance for the same given volume. The reference substance for liquids is water at 15 °C at 1 atm *(13).* The specific gravity was measured for water, all soybean and sunflower oil samples, biodiesel samples and diesel fuel standards which can be seen in Table 14. Each sample was measured three times and the average specific gravity was calculated, along with the standard deviation.

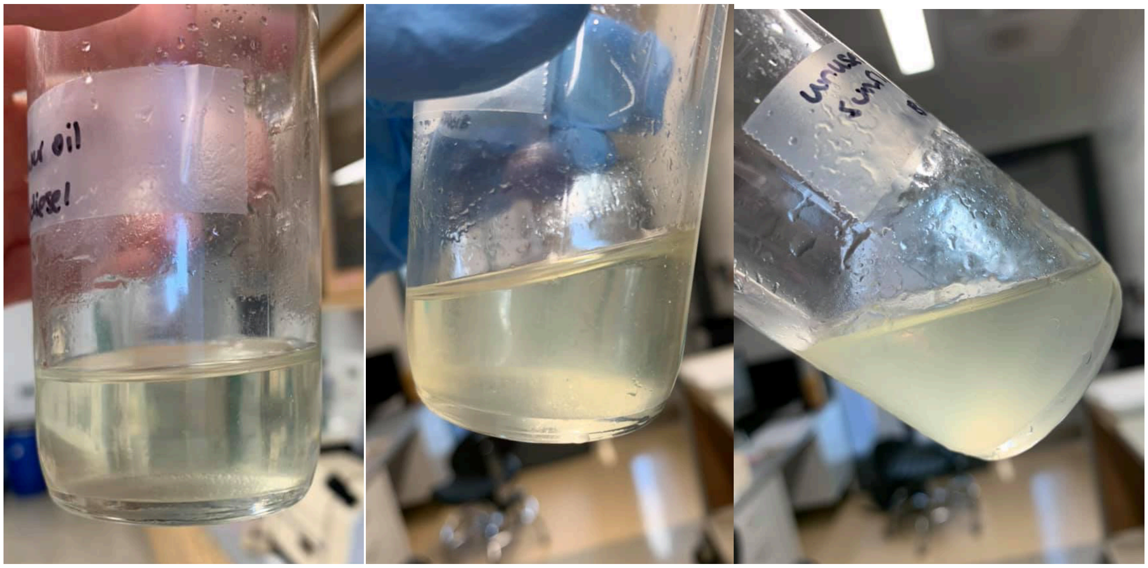
Table 14: Specific Gravity

Specific Gravity (15 °C)				
Water	0.9996	0.9997	0.9996	**0.9996 ± 0.0001**
Cooked Soybean Oil #1	0.9153	0.9158	0.9152	**0.9154 ± 0.0003**
Cooked Soybean Oil #2	0.9152	0.9151	0.9152	**0.9151 ± 0.0001**
Uncooked Soybean Oil #2	0.9079	0.9077	0.908	**0.9079 ± 0.0002**
Cooked Sunflower Oil	0.9235	0.9239	0.9241	**0.9238 ± 0.0003**
Uncooked Sunflower Oil	0.9229	0.9227	0.9225	**0.9227 ± 0.0002**
Cooked Soybean Oil Biodiesel #1	0.8702	0.8705	0.8703	**0.8703 ± 0.0002**
Cooked Soybean Oil Biodiesel #2	0.8705	0.8707	0.8708	**0.8707 ± 0.0002**
Uncooked Soybean Oil Biodiesel #2	0.8709	0.8708	0.8712	**0.8710 ± 0.0002**
Cooked Sunflower Oil Biodiesel	0.8696	0.8698	0.8699	**0.8698 ± 0.0002**
Uncooked Sunflower Oil Biodiesel	0.8692	0.8695	0.8696	**0.8694± 0.0002**
Biodiesel Blend	0.8435	0.8439	0.844	**0. 8438 ± 0.0002**
Commercial Diesel	0.8343	0.8342	0.8344	**0.8343 ± 0.0001**

4.7.2 Cloud point and pour point:

In the petroleum industry, the cloud and the pour points, or the cold flow properties are extremely important. The cold flow properties of all vegetable oils samples, biodiesel sample and diesel fuel standards were measured. The cloud point refers to the temperature below which wax in diesel or biodiesel forms a cloud appearance. As you can see in Figure 28A, this is a sample at room temperature. In Figure 28B you can see the cloudy appearance as compared to room temperature. The presence of solidified waxes thickens the oil and clogs fuel filters and injectors in engines. The wax also accumulates on cold surfaces and forms an emulsion with water. Therefore, cloud point indicates the tendency of the oil to plug filters at cold operating temperatures *(14).*

The pour point is the temperature below which a liquid loses its flow characteristics. It is defined as the minimum temperature in which the oil has the ability to pour down from a beaker *(15).* As shown in Figure 28C, there is a gel-like consistency in crude oil. A high pour point is associated with a high paraffin content.



A: Room Temp (20.6 °C) **B:** Cloud Point **C:** Pour Point

Figure 28: Biodiesel Sample at Varying Fluidity

The cloud point and pour point varied between the vegetable oils and the biodiesel, as seen in Table 15.

Table 15: Cloud Point & Pour Point

	Cloud Point (°C)	**Pour Point (°C)**
Cooked Soybean Oil #1	10.1	2.4
Cooked Soybean Oil #2	10.3	2.5
Uncooked Soybean Oil #2	9.8	2.2
Cooked Sunflower Oil	12.1	4.9
Uncooked Sunflower Oil	12.6	4.6
Cooked Soybean Oil Biodiesel #1	6.3	-1.3
Cooked Soybean Oil Biodiesel #2	5.6	-1.8
Uncooked Soybean Oil Biodiesel #2	6.0	-1.0
Cooked Sunflower Oil Biodiesel	9.8	2.0
Uncooked Sunflower Oil Biodiesel	10.0	2.3
Biodiesel Blend	Little at -2.2	N/A
Commercial Diesel	Little at -2.6	N/A

4.7.3. Viscosity:

Viscosity is an important fluid property when analyzing liquid behavior and fluid motion. The viscosity of a fluid is a measure of its resistance to gradual deformation by shear stress or tensile stress. The shear resistance in a fluid is caused by inter-molecular friction exerted when layers of fluid attempt to slide by one another *(16).* Kinematic viscosity is the measure of a fluid’s inherent resistance to flow with no external force, except gravity acting on it. Kinematic viscosity is measured by measuring the time it takes a fluid to flow through a capillary tube, the time is then converted directly into kinematic viscosity using a calibration constant provided for the specific tube used, using this formula: $\nu = \mu / \rho$ (v: kinematic viscosity, μ: measured viscosity, ρ: density). Kinematic viscosity is measured in measured in centistrokes (cSt) or mm^2/s. Only liquids that are characterized as Newtonian liquids, or liquids that do not change viscosity with changes in applied force can be measured using this method *(17)*.

There were two cannon-fenske viscometers that was used for this experiment, two different size viscometers were used, size 75 and size 150, shown in Figure 29. Size 75 has a range of 1.6 to 8 cSt, which was used for the biodiesel and diesel fuel samples, size 150 has a range of 7 to 40 cSt, which was used for the vegetable oil samples. The sample is introduced into the viscometer by inverting it and immersing tube "A" into the liquid and applying suction to "I", this causes the sample to rise to etched line "E". Once there is enough liquid, the viscometer can be returned to normal position and wiped clean. The viscometer then is inserted into a temperature bath, where it sits for 10 minutes for the viscometer to reach equilibrium. Suction is then applied to tube "A" to bring the sample into bulb "B", a short distance above etched "C". The efflux time is measured by the sample flowing freely through to etched "C" and measuring the time for the meniscus to pass from "C" to "E" *(20)*. Each sample was tested three times, and the average kinematic viscosity was calculated, along with its standard deviation, as shown in Table 16.

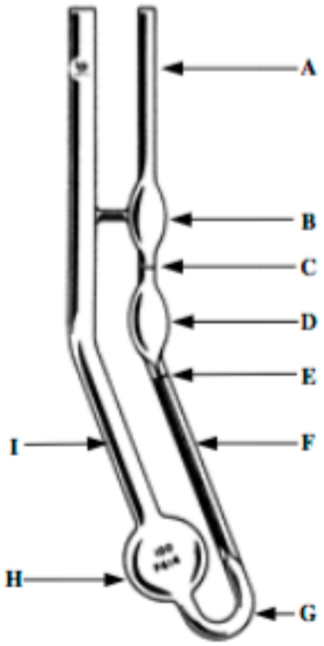


Figure 29: Cannon-Fenske Viscometer [20].

Table 16: Kinematic Viscosity

Kinematic Viscosity (mm^2/s) at 40°C				
Cooked Soybean Oil #1	36	37	36	**36.3 ± 0.6**
Cooked Soybean Oil #2	35	36	36	**36.7 ± 0.6**
Uncooked Soybean Oil #2	33	34	33	**33.3 ± 0.6**
Cooked Sunflower Oil	37	37	36	**36.7 ± 0.6**
Uncooked Sunflower Oil	37	36	38	**37.0 ± 1**
Cooked Soybean Oil Biodiesel #1	4.8	5.1	4.9	**4.9 ± 0.2**
Cooked Soybean Oil Biodiesel #2	5.6	5.9	4.8	**5.4 ± 0.6**
Uncooked Soybean Oil Biodiesel #2	4.8	5.1	4.9	**4.9 ± 0.2**
Cooked Sunflower Oil Biodiesel	4.9	5.2	5.4	**5.2 ± 0.3**
Uncooked Sunflower Oil Biodiesel	4.9	4.8	4.9	**4.9 ± 0.1**
Biodiesel Blend	4.2	4.4	4.3	**4.3 ± 0.1**
Commercial Diesel	3.9	3.8	3.8	**3.8 ± 0.1**

In the literature, there were accepted ranges for the physical properties that were measured in our experiment, for biodiesel, diesel fuel, soybean oil and sunflower oil. The accepted ranges are shown below for the four samples in Table 17 *(3)*.

Table 17: Accepted Ranges of Physical Properties *(3)*.

Properties	**Biodiesel Std.**	**Diesel Std.**	**Soybean Oil**	**Sunflower Oil**
Specific Gravity (15°C)	0.86 to 0.90	~0.85	0.91 to 0.93	0.9 to 0.93
Cloud Point (°C)	-3 to 12	-15 to 5	1 to 15	2 to 18
Pour Point (°C)	-15 to 10	-35 to -15	-8 to 5	-6 to 8
Kinematic Viscosity (mm^2/s) at 40°C	1.9 to 6.0	1.3 to 4.1	30 to 36	32 to 38

All the physical properties that were analyzed for diesel fuel, soybean oil and sunflower oil fell within the accepted ranges. Most importantly, the biodiesel that was synthesized from all five samples of soybean oil and sunflower oil fell within the accepted ranges from biodiesel standards. This indicated that the biodiesel was successfully synthesized and is able to be used in a diesel engine.

V. Discussion

5.1 R^2 Value in Fatty Acid Ester Analysis

The linearity of the concentration curves of the fatty acid esters is significantly lower compared to the R^2 value of the FAMEs. Only six concentrations were used for the fatty acid esters of fish oils which could contribute to the lower R^2 value. These values are shown in Table 5 - R^2 values for methyl esters of EPA, DHA, and DPA were found to be 0.9779, 0.9784, and 0.9876, respectively. For the FAME concentration curves, nine concentrations were used, as a result we observed significant improvement in R^2 for all five samples, as shown in Table 6 - R^2 values of the FAMEs were found to be 0.9982, 0.9976, 0.9982, 0.9962, and 0.9950 corresponding to methyl esters of palmitic acid, linoleic acid, oleic acid, linolenic acid, and stearic acid, respectively.

5.2 Cooked vs. Uncooked Oil Biodiesel

From the quantitative analysis of the biodiesels (cooked and uncooked) derived from soybean oil and the sunflower oil, a difference in calculated concentration of methyl ester palmitic acid was observed. As shown in Table 8 and Table 9, the methyl ester of palmitic acid derived from the cooked soybean oil found to contain 98.5 ng/ml of the molecule in comparison to that obtained from uncooked biodiesel had 58.2 ng/ml. Similarly, in the sunflower oil biodiesel (shown in Table 10 and 11), the calculated concentration of methyl ester palmitic acid was slightly higher in the cooked oil (55.6 ng/ml) in comparison to that from uncooked oil (50.3 ng/ml). To our understanding, palmitic acid is the most common saturated fatty acid present in the meats, cheese, and butter that are commonly used in restaurants, the observed spike in the ester of palmitic acid can be attributed from those foods.

5.3 Soybean Oil Biodiesel vs Sunflower Oil Biodiesel

The biodiesel samples were prepared via the transesterification method to investigate the composition and physical properties. In the soybean oil biodiesel, methyl ester linoleic acid was the most abundant compound. While in the sunflower oil biodiesel, methyl ester oleic acid was the most abundant compound.

Soybean oil is mainly composed of polyunsaturated fats, followed by monounsaturated fat, then saturated fat. The major unsaturated FAMEs in soybean oil are methyl ester linoleic acid, and the monounsaturated FAME, methyl ester oleic acid. It also contains the saturated fatty acids, methyl ester stearic acid and methyl ester palmitic acid.

Sunflower oils have a various composition, because they are produced through plant breeding and industrial processing. There are four types of sunflower oils depending on its composition: such as high-linoleic, high-oleic, mid-oleic and high-stearic combined with high-oleic. In our case, the sample was derived from oil having a high-oleic composition. Therefore, the sunflower oil sample under investigation was mainly composed of monounsaturated fat, polyunsaturated fat, and then saturated fats.

Each type of fat affects biodiesel differently. The lower the saturated fat content, the lower the gel point of the biodiesel that is synthesized from it. Unsaturated fats tend to spoil faster than saturated fats. When an oil contains too high concentration of unsaturated ester of fatty acids, the oil is considered a drying oil, which is not a good oil for synthesizing biodiesel. This is because they break down quicker. These are the types of oils that are ideal in paints and varnish to dry quickly. However, the cooking oils contain a single or double bond per fatty acid along with a lower concentration of saturated fatty acid, which makes such oil suitable for synthesizing biodiesel. The properties of the oil also depend upon the frequency of filtration and the temperature at which the oil is heated. If a restaurant filters their oil often, the oil lasts much longer and is free of contaminants, while if it is cooked at a temperature that is too high, the oil breaks down quickly and would not result in high-grade biodiesel.

Between the oils that were used for this study, soybean oil is the better of the two types of vegetable oils that were synthesized into biodiesel, although they both were successfully made and fell within the accepted ranges for the physical properties. Soybean oil is more readily available within the restaurant industry since it is cheaper based upon its demand. Also, the cloud point and pour point of the vegetable oil biodiesels were significantly lower than that of the sunflower oil biodiesels (Table 15). The cloud point for the sunflower oil biodiesels ranged from 9.8 °C to 10.0 °C, while the range for the soybean oil biodiesels ranged from 5.6 °C to 6.3 °C. Similarly, the pour point for the sunflower oil biodiesels ranged from 2.0 °C to 2.3°C, the range for the soybean oil biodiesels ranged from -1.8 °C to -1.0 °C. As stated in section 4.7.2, the cloud and pour points are important physical characteristics of biodiesel fuel. A fuel able to withstand lower temperatures, the better it is to use in a diesel engine.

VI. Summary and Future Work

In conclusion, we successfully developed a GC-MS method used for qualitative and quantitative analysis of the biodiesel. From these studies, we found that biodiesel derived from the soybean and sunflower oil contains four different types of esters of fatty acids. Using the calibration curve derived from the standard FAMEs we are also successful in quantifying the individual esters present in the synthesized biodiesel. Importantly, we are also successful in identifying biodiesel blended with petroleum diesel fuel using our GC-MS method and quantify the FAMEs composition in the biodiesel blend. Further, we studied the quality of biodiesel by comparing their physical characteristics, such as cloud point, pour point, specific gravity, and kinematic viscosity. From these studies, we conclude that the biodiesel derived from the soybean could be performing better as it showed more ideal physical properties. We are confident that this method can be further extended toward the analysis of biodiesel blended with other fuels as well, such as kerosene. Further studies could be done using the GC-FID compared to the GC-MS to calculate the concentrations of the FAMEs found in biodiesel.

Scientists at the National Renewable Energy Laboratory (NREL) have cultivated and cataloged 300 strains of algae which eat carbon dioxide and produce oil. The quantity of oil that can be produced by algae is astonishing. Since algae reproduce by cellular division, they divide continuously until they fill whatever space they are in, or exhaust their nutrients. The southwestern region of the United States has the best climate condition for diatom algae to grow. They need very high temperatures and dry climates. The oil is extracted by physical extraction, using a filter or chemical to separate the algae from the water in which they grow. The filter or chemical forces the algae to clump together, resulting in algae paste. The paste is then spun in a large three-phase centrifuge to extract more moisture. A solvent is then used to extract the oil from the protein and carbohydrate parts of the algae.

At a testing facility by NREL diatom algae productivity reached 50 grams of algae per square meter per day. Each pond was 1,000 square meters in size, making a possible daily yield of 50,000 grams of algae per pond. It is estimated that algal biodiesel could be produced for approximately $1.65 per gallon *(3)*. With these developing researches on algae, we hypothesize biodiesel would be widely used in the vehicles and types of machinery in the days to come. We are confident that our GC-MS analytical method could play a significant role in a wide variety of biodiesels and the blend that will be produced from different industries. We also advocate that the abundance of cooked and uncooked vegetable oils should be wisely utilized towards the production of biodiesel thus mitigating the use of petroleum resources, which can improve the quality of the atmospheric air.

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