Analysis of Substrates for a Continuous Fermentation Reactor for Mead

A Major Qualifying Project Report
Submitted to the faculty of
WORCESTER POLYTECHNIC INSTITUTE
In partial fulfillment of the requirement for the
Degree of Bachelor of Science

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Abstract

In the preparation of iQhilika, a traditional mead of the Xhosa of South Africa, roots are used to immobilize yeast, allowing for the continuous fermentation of the must that passes through the packed column. Dr. Garth Cambray's continuous fermentation reactor design was inspired by this traditional practice. Our prototype reactor on campus is based on Dr. Cambray's design, and was originally designed using ginger root as the biomass substrate to immobilize the yeast. In this project, we set out to try several types of biomass substrates to work as a replacement for ginger; dried apple, lemon rinds, orange rinds, habanero peppers, and jalapeño peppers. We fermented small batches of mead using each of these substrates, and used a GC-MS to observe how the different biomasses affected the fermentation process, and whether they imparted their own flavors onto the resulting mead. Additionally, we tested the orange rinds as the substrate in our packed column fermentation reactor.

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Background

Mead, an alcoholic beverage made from the fermentation of honey, has been around for thousands of years. While its origins are uncertain, there is strong evidence of mead production in several ancient cultures. The earliest archeological evidence for the production mead dates to 7000 BC in the Neolithic village of Jiahu, in the Henan province of Northern China [1].

Mead is traditionally produced by mixing water, honey, and some type of yeast to allow it to ferment. Many different variations of mead can be produced by varying the type of honey and yeast used. Typically, the honey is diluted with water to form "must" and then yeast is added. It can also be flavored with fruit, spices, or vegetables that function as substrates. With many different options and variations, the possible flavors and methods are limited by the brewer's imagination.

During mead production several complications may occur at different steps of the process. The brewer has to be aware of several guidelines and conditions to follow in order to produce quality mead and avoid setbacks. One of the most common setbacks is the inability to reach the desired alcoholic content and flavor. Yeast refermentation or bacterial secondary fermentation are two common factors that prevent achieving the desired alcohol content and flavor. Yeast refermentation is common mostly in red wines but may occur in other alcoholic beverages. Bacterial secondary fermentation occurs after the first fermentation process in the beverage and can produce undesirable compounds associated with unpleasant flavors and aroma. Another possible reason for undesirable flavors is ineffective filtration of the remaining yeast after the first fermentation period is complete. The most common compounds associated with these off-flavors are ethyl acetate, octanoic acid, and hexanoic acid [2]. It is important that the brewer be aware of correct filtration methods and fermentation periods in order to achieve flavorful mead. Another parameter to consider during the production of mead is the temperature where the fermentation process takes place. For the yeast species *S. cerevisiae,* higher fermentation rates are achieved between 20 and 30 °C, and temperatures lower than 15 °C tend to decrease fermentative performance [2]. For this reason many brewers prefer to ferment their mead in a warm place that is approximately 25°C. It is also important to note that temperatures above 30°C may slow down the fermentation process of yeast.

Continuous Reactor Apparatus

The continuous reactor that was used for this experiment was constructed by former WPI students. Its construction was based on the research and advice of Dr. Garth Cambray, as well as his design for a small scale packed bed reactor made for mead fermentation. Cambray's design was inspired and informed by the production of iQhilika, a traditional mead of the Xhosa of South Africa. Cambray describes the reactor used for iQhilika production as a "non-homogenous anaerobic batch tank bioreactor"[3]. A crucial part of this continuous reactor model is the immobilization of yeast. In both traditional iQhilika production and Cambray's own design, plant roots are used to immobilize the yeast.

Substrates

Yeast immobilization is a common strategy used in continuous fermentation that involves suspending the yeast on a substrate in order to hold the yeast in place while conserving biological activity. Immobilization has many advantages over free yeast cell methods in continuous fermentation systems. Some specific advantages include an increased cell density, increased product yield, and better reproducibility [4]. Typically, inert substrates are chosen, such as silica beads or ginger root, as they provide the necessary structure without imparting any unwanted flavors [5]. Cells can also be immobilized with membrane systems and organic materials such as alginate [4].

Alcohol Content

The alcohol content of mead typically ranges from eight to eighteen percent [6]. The alcohol content results from ethanol produced during the fermentation process [7]. The amount of alcohol produced is contingent on the strain of yeast and the fermentation circumstances, largely defined by must makeup and temperature [7]. Must made with high ratios of honey to water, such as ratios of 2:1 or 1:1 (volume to volume basis) , can lead to premature termination of the fermentation process from too much osmotic pressure. Fruits and spices added to mead have been known to increase the fermentation process and alcohol production [2]. Typically, increasing temperature decreases ethanol production in most fermentation processes. Many factors, including honey variety and the fermentation environment, may alter the final product quality of the mead [8]. For this reason, consistency in the production process is very important, as variations in flavor and alcohol content can be drastic between batches for even small changes in brewing conditions.

Clarity and Color

The clarity and color of mead is largely dependent on the type of honey used, as well as any added ingredients. For the mason jar tests, we used the same must mixture for each sample, so the only factor affecting a difference in color was the substrates added. If we see major differences in color/clarity between different samples, we will have a greater visual indicator of how the substrates interacted with the mead differently. For further investigation, we may choose to use the lab's spectrophotometer to find the different absorbances of the samples.

Chemical Makeup

Mead is an alcoholic beverage so by definition it contains some percent ethyl alcohol. Mead additionally contains sugars, organic acids, vitamins, minerals, and polyphenols, which vary depending on the process and ingredients used to make the mead. As the main ingredient of mead is honey, it contributes most significantly to the aroma of mead. Other substrates used to brew the mead also contribute to the aroma, and these typically include floral, fruity or spicy substrates. This combines overall to give mead a sweet, wine-like aroma.

Flavor

The fruits, spices and other nutrient additives, the honey to water ratio, the honey variety, the conditions of fermentation, as well as the strain of yeast all have an impact on the flavor profile of mead. Fruits and spices add different aromas to the beverage by leaving behind traceable compounds. For example the compound 2-Phenylacetaldehyde may be responsible for a sweeter flavor, while the compound (R)-limonene may account for a more citrus-like flavor [9]. Higher ratios of honey to water in the must increase the sugar content in its final production yielding a sweeter flavor to the beverage [2]. Mineral makeup of the honey, which can have a significant impact on flavor, is affected by its sourced location and surroundings. For instance honeys from dark blossoms usually contain higher concentrations of minerals that affect flavor. In addition to this the acidity of honey in its production can be accredited to the plant species involved and the time of harvest [8]. The different strains of yeast, as well as the conditions of fermentation can sometimes yield unexpected and unpleasant flavors. This is due to the unforeseeable nature innate to the fermentation process [8].

Analysis

In order to detect specific compounds in the mead, a gas chromatography mass spectrometer (GC-MS) can be used. GC-MS works in two stages: the gas chromatography stage and the mass spectrometry stage. Mass spectrometry is a process that can determine the chemical composition of unknown compounds. It works by bombarding the unknown samples with an electrical field, which is essentially a beam of electrons [10]. After coming into contact with the field, the molecules in the sample break apart and become ionized. The ions are then directed through a magnetic field, causing them to curve due to the repulsion of the field [10]. The ions have different mass to charge ratios. The degree to which an ion curves is a result of the ions mass to charge ratio, so by measuring how much the ions are deflected by the magnetic field, the mass to charge ratio can be determined. A spectrum is created with different peaks along the spectrum where multiple ions hit the same location, indicating that there were multiple ions with the same mass to charge ratio. It was found that when conducting this process under the same conditions it will always produce the same spectrum when analyzed, this means that unknown compounds can be matched to known compounds by comparing their resulting spectrum of mass to charge ratios [10]. The intensity of the spectrum also indicates the relative abundance of a specific compound. This process works very well for a pure unknown substance, but when multiple unknown substances are present, a complicated mass spectrum with many peaks can result. In this case, gas chromatography is used. Gas chromatography is a process that “relies on different affinities of vapor components for surfaces” [10]. The sample is vaporized and an inert gas is run through it to carry it through a packed tube. The different compounds are separated from each other based on how much they interact with the surfaces in the tube and how fast they are able to travel through the tube. This allows the unknown compounds in the mixture to be separated out from each other so they can be run separately through the mass spectrometer [10].

For the purpose of this experiment this means that known quantities of compounds can be run through the GC-MS and the mass spectrum can be collected. Then a sample of mead can be run through the GC-MS and the resulting mass spectrums can be compared to the known ones already collected to see what compounds are present.

Methodology

Mason Jar Batch Tests

For this experiment, the team chose to use habanero peppers, ginger root, dried apple slices, lemon peels, orange peels, and silica gel crystals as substrates. The silica gel crystals were chosen to act as a control group.

Column Operation

After sanitization of both of the columns, we prepared the yeast for each column by introducing it to a mixture of warm water (95-98℉) and honey. We let the mixture stand for about ten minutes or until the yeast was visibility activated. For the left column, we then introduced the yeast to the previously sanitized orange peels in order to initiate growth on the surface of the substrate. This mixture of substrate and yeast was then poured into the left column. For the column on the right, the substrate was loaded into the column and the activated yeast was then poured into the column afterwards. Initially, we used small pieces of cheesecloth to plug the entrance of the tubing at the top of each column to keep yeast and substrate from the product collection bucket. This cheesecloth was later replaced by metal mesh screens because the cheesecloth was blocking the flow of must, causing leaks.

Approximately five gallons of must was poured into the feed buckets provided. These feed buckets were each placed into the fridges provided. Each fridge and feed bucket were previously drilled into to allow the feed tubing in. Both columns were run using the same pump and same size tubing. The pump was set to its lowest setting in order to maximize the residence time. Even at the lowest pump setting, the entirety of the must in the feed buckets ran through the column in approximately sixteen hours. In order to increase the contact between the must and the yeast, the contents of the product buckets were moved back into their respective feed buckets twice a day.

Substrate Preparation

Apples

1. Removed apple skin using a fruit peeler.
2. Cut apples into thin cross-sectional slices
3. Arranged slices on a baking sheet
4. Put in oven at 250℉ to dry out and sanitize apples
5. Removed apples from oven after 50 minutes

Ginger

1. Peeled ginger skin using a sanitized peeler
2. Added peeled ginger to boiling water for 10 minutes to sanitize
3. Remove ginger from boiling water to cool and for further use

Lemons

1. Peeled lemons by hand using a sanitized knife.

2. Put peels into boiling water for 10 minutes to sanitize
3. Remove lemon skin from boiling water to cool and for further use

Oranges

1. Peeled orange skin using a sanitized knife.
2. Placed peeled orange skin into boiling water for 10 minutes to sanitize.
3. Remove orange skin from boiling water to cool and for further use.

Peppers

1. Sliced peppers into small pieces
2. Removed seeds
3. Put pepper pieces into boiling water for 10 minutes to sanitize
4. Remove pepper from boiling water to cool and for further use

Silica

1. Removed silica beads from packing

Must Preparation

1. Heated 1 gallon of water on the stove.
2. Added 2 lbs of honey and mixed until combined. (4 parts water to 1 part honey)
3. Added about 0.75 grams of Fermax yeast nutrient.
4. Removed solution from heat until cooled to under 140℉.

Bottling

1. Rinsed out all mason jars lids and airlocks with a sanitizing mixture
2. Let mason jars and lids air dry before use
3. Added 10 oz of must into each of the six labeled mason jars.
4. Then added approximately 10 grams of the prepared substrates into each of their respective jars.
5. Once the must was cooled to the proper temperature (under 140℉), about 2 grams of yeast was added to each jar.
6. Lids and airlocks were put on the mason jars.
7. The mason jars were allowed to sit and ferment in a warm and dry environment

Sample Preparation for GC-MS

To prepare samples for gas chromatography-mass spectroscopy analysis, the organic components must be separated from the aqueous solution. In order to do that, the mead samples in mason jars were filtered until the sample was clear. Then 3 ml of the filtered mead sample was extracted and placed in a sample vial along with 6 ml of di-chloromethane (DCM). The samples were then shaken for 10 minutes in order to ensure separation of the organic components. After clear separation of components can be seen in the samples, the organic components were extracted using a pipette. The extracted organic components were then heated using a heating plate until their volume was reduced by approximately 50%. The samples were then sent for analysis.

Sanitization Steps:

This Procedure takes a total of 6 days.

1. Purified water was pumped through columns at 5 mL/min to test for leaks. The purified water was allowed to run for an hour.
2. In a bucket 76 g solid NaOH was prepared along with 2 gal of water in order to create NaOH sanitizing solution (1% w/v). The solution was shaken up in the bucket to clean it.
3. The NaOH solution was pumped through columns at 2.5 mL/min for 36 hours.
4. The solution was fed back into the feed bucket and then disposed of into a waste bucket.
5. The feed and product buckets were thoroughly rinsed.
6. 2 ½ gal of tap water was pumped through the reactor at 5mL/min for 24 hours.
7. 2 cups of "Sanidate" was combined with 2 ½ gal of tap water to create the "Sanidate solution".
8. The Sanidate solution was pumped through the reactor at 2.5 mL/min for 36 hours.
9. The tap water should be pumped at 5mL/min for 24 hours.
10. The purified water was then pumped through the system at 5mL/min for 24 hours.

Table 1. This is a table of all of the runs, their corresponding solutions, flow rates, and times allowed to run through the two columns.

Run	Solution	Flow rate (mL/min)	Time (hr)
1	Purified water	5	< 1
2	NaOH solution	2.5	36
3	Tap water	5	24
4	Sanidate	2.5	36
5	Tap water	5	24
6	Purified Water	5	24

Results

Gas Chromatography-Mass Spectrometry (GC-MS) is a powerful tool capable of breaking down and analyzing the chemical components in a solution. The principle behind it consists of separating the sample into its different components based on their physical and chemical properties by using gas chromatography. After the components have been separated, they are sent to a mass spectrometer where they are analyzed. The result is a mass spectra which provides a unique peak for each compound. The peaks are then matched to known compounds until the correct one is found.

Table 2. This chart indicates the presence of common compounds found in the batch tests. The presence of a compound for a given substrate is indicated with an X.

Chemical Name	Lemon	Orange	Apple	Jalapeño	Habanero	Silica	Ginger
Octadecenoic acid	X		X	X	X	X	X
Benzene 1,3-bis(1,1 dimethylethyl)	X	X	X	X	X	X	
Phenylethyl alcohol	X	X	X	X			X
Hexadecenoic acids	X	X	X	X	X	X	X
1- butanol, 3- methyl (isoamyl alcohol)			X	X			
2,4 dimethyl-1-heptene		X	X	X		X	
Methyl 11-eicosenoate					X	X	

Common compounds

Octadecanoic acid was found most abundant in the mead samples prepared with ginger, apple, habanero, jalapeño, and silica as the substrates. Octadecanoic acid, also known as stearic acid, is a fatty acid frequently found in plants and animals as a glycerol ester. This compound is also known for having a bitter flavor [11]. This fatty acid appears more in animal fats but is still present in plant fats [12]. Due to its association with numerous plant species, including *Senegalia pennata*, *Senegalia catechu*, *Anchieta pyrifolia*, and *Lepidium meyenii*, octadecenoic acid may have entered the honey through pollen [13]. In addition to this beeswax, octadecanoic acid happens to be the most common free fatty acid fraction [14]. Any traces of beeswax likely came from honey used to produce the must.

Despite not expecting the presence of octadecanoic acid in the mead samples, beeswax and plant pollens often contain this compound. Given that the compound is more commonly found in animal fats and that several samples of the mead contain the compound it is most likely derived from the beeswax in the honey rather than the substrates used.

The compound Benzene 1,3-bis(1,1 dimethyl ethyl) was found in the habanero, jalapeño, apple, orange, and silica samples. In the case of the apple, jalapeño, and orange, the compound was identified in both samples of the second batch test.

Benzene 1,3-bis(1,1 dimethyl ethyl) is described as having a "waxy" odor to it. No known association was found between the compound and any of the previously listed substrates. However, one study did find significant levels of the compound in green olives [15]. This study suggests that benzene 1,3-bis(1,1 dimethyl ethyl) could be present in other vegetables/fruit, perhaps in lower concentrations.

Because of the presence of this compound across the various samples, it is likely that this compound was a derivative of the honey rather than a flavor compound of one of the substrates. Its presence in the silica is especially indicated as the silica beads were assumed to be inert and unlikely to impart any flavor-producing compounds. Additionally, the "waxy" flavor could be a residual element of beeswax present in the honey.

Phenylethyl alcohol was found in relatively low to medium abundance in samples from five of the substrates: jalapeño, apple, lemon, ginger, and orange. Phenylethyl alcohol is a compound commonly found in mead and is associated with a sweet, floral aroma [16]. It is a known byproduct of fermentation and, more specifically, it is a known metabolite of the strain of yeast (*Saccharomyces cerevisiae*) we used throughout this project [16].

While we expected to find phenylethyl alcohol in our samples, its presence was not detected in the habanero or silica samples, or any other sample from our first batch tests except apple. We think that it is probable that phenylethyl alcohol was actually present in all samples but in such low relative abundance that it could not be detected by GC-MS. Because phenylethyl alcohol is only a byproduct, it should only be found in low concentrations in mead if fermentation occurs under optimal conditions. However, we do not believe that its presence in the GC-MS spectra is an indication of bad fermentation conditions. Since the GC-MS uses relative abundance and not overall concentration as a scale, the scale varies between samples. This means that a large peak on a spectrum may still correspond to a low concentration if the sample was diluted.

The compound hexadecanoic acid, also known as palmitic acid, appeared in at least one sample of each substrate. This acid did not appear on our list of expected compounds, but its presence was unsurprising nonetheless. Palmitic acid is a common saturated fatty acid commonly associated with plants [17]. It is described as having a "waxy" flavor. Taking these factors into consideration, coupled with the fact that each substrate yielded a detectable amount of the

compound, it is reasonable to conclude that the palmitic acid was present due to the must solution, rather than any added biomass substrate.

The same compound with the addition of a methyl ester group was also commonly seen in the results, appearing in at least one sample of each substrate, save for the silica sample. Methyl palmitate is yet another fatty acid methyl ester. It also has the role of a metabolite in humans and other organisms [18].

1-butanol, 3-methyl, commonly known as isoamyl alcohol, was found in low relative abundance in one of the apple samples and one of the jalapeño samples. Isoamyl alcohol is a known byproduct of fermentation and contributes to the overall alcoholic flavor of mead [16]. It is the most abundant secondary metabolite produced by yeast during fermentation and in beer making it is typically found in concentrations above the flavor threshold [19]. It is also known for its fruity, banana-like aroma [19]. In low concentrations, it can be considered beneficial to the overall flavor profile of alcoholic beverages [16]. However, high concentrations of isoamyl alcohol can cause an undesirable “heavy” flavor [19]. Isoamyl alcohol was most likely present in all of our samples but was below the detection limit.

2,4 dimethyl-1-heptene was found in four of the samples tested: silica, jalapeño, apple, and orange. This compound is an alkene with a couple of methyl groups attached to the 2nd and fourth carbon. Like most alkenes, it is colorless and odorless. This compound is also known as a common human and animal metabolite, so it likely was produced by the bees that made the honey [20].

Methyl 11-eicosenoate ($C_{21}H_{40}0_2$) was present in both the habanero and silica samples. These samples had a mildly sweet, and fruity odor. In comparison to the other components found in the samples, methyl 11-eicosenoate was not as abundant, but it is still present. It is formed from the reaction of an alcohol and a carboxylic acid, which makes it an ester. Methyl 11-eicosenoate is a colorless liquid with a sweet and slightly fruity odor, which supports the samples having a slightly sweet odor. It is a nonpolar compound which means it is not soluble in water, it is, however, soluble in alcohols. It can be found naturally in various plants and insects, among them honeybees. It has been found that methyl 11-eicosenoate can be found in the venom extracted from honeybees and the honey they produce [21]. This suggests that this compound originated from the must solution that was made from mixing water and honey. This compound is a relatively stable compound, and it is classified as a low-toxicity compound which means it is safe to use.

Uncommon Compounds

While the majority of the compounds identified appeared in several samples, there were some compounds that were unique to their samples. The GC-MS results for the silica sample showed a compound that was not found in other samples. Cyclohexasiloxane, a cyclic silicone

compound with the chemical formula $C_{12}H_{36}0_6Si_6$. This compound likely originated from the silica substrate that was used in the control sample.

Additionally, phenol 2,4 bis (1,1 dimethyl) was found exclusively in the second batch test for the jalapeno sample. Literature sources could not confirm its presence in jalapenos, however it is present in avocados [22], which suggest that it may also be found in similar organics.

Of the many compounds identified across the samples, only two were found to be unique to one specific substrate. The rest were seen across multiple samples. The most likely explanation for this is that any flavor compounds imparted unto the mead from the substrates existed in a far smaller abundance than the actual honey and fermentation byproducts. Since the scales of the spectra produced by GC-MS analysis are so dependent on the abundance of each chemical identified, it would be extremely difficult to identify any compound that did not appear in a high enough abundance without some sort of control method to isolate these compounds from the rest. Another factor in the lack of flavor compounds identified by the GC-MS could have been the fact that the samples were evaporated before they were entered into the GC-MS. This was done by simply placing the vials of extracted samples in a beaker of hot water. By warming the samples, we evaporated off some of the dichloromethane to produce more concentrated samples for testing, but we also likely lost some of the more volatile compounds present, including compounds that contribute to aroma.

Despite the lack of conclusive data from the GC-MS showing the presence of flavor compounds from the substrates, olfactory analysis was all we needed to be certain that there were flavors from the substrates present in the samples. Each of the substrates produced samples that distinctly smelled of the biomass that was in the sample. Specifically, the more pungent smells, like the two different types of peppers used, were easily identifiable.

Continuous Reactor Results

Table 3. This chart indicates the presence of common compounds found in the continuous tests. The presence of a compound for a given column is indicated with an X. Right Column "Mid" indicates the sample taken during the process as opposed to the "End" which was taken after the process was complete.

Chemical Name	**Right Column End**	**Left Column End**	**Right Column Mid**
Ethylidenecyclobutane	X	X	X
2,4 Dimethyl-1-heptene		X	
Benzene, 1-4-bis[1,1-dimethylethyl]	X	X	X
Hexadecanoic acid, methyl ester	X		
Hexadecanoic acid	X	X	X
9-octadecenoic acid, methyl ester	X	X	X
Octadecanoic acid		X	X

We collected three samples from our continuous fermentation reactor setup. One from each of the two columns at the end of the process, and one from the right column from the middle of the time frame. The GC-MS results from these samples were all quite similar to one another, and nearly all of the chemicals identified were previously seen as "common compounds" in our batch samples. The only unique compound we saw appeared in all of the samples: ethylidenecyclobutane. Very little data on this compound and its characteristics exist, so it was difficult to conclude why it would appear in all of our column samples but none of the batch samples. The only conclusion we could come to is that, since the honey we used for the batch samples was too expensive, we used a different brand of honey for the must in our continuous reactor.

Unfortunately, we were unable to obtain much data on the alcohol content of the mead produced from both the continuous fermentation reactor and batch processes. This was largely due to a lack of a reliable method to do so. We initially tried using hydrometer readings to determine the alcohol content through the change in specific gravity of the samples over time. However, we found the specific gravity readings to be inadequate in determining alcohol percentage as the readings were always close to 1.00, the specific gravity of water. These

inaccurate specific gravity readings were likely due to the presence of other compounds, besides ethanol and water, in the samples tested. Due to time and material constraints, we were unable to use GC-MS techniques to determine alcohol percent. This unfortunately meant we had only qualitative measurements to gauge an estimate of the alcohol percent achieved by continuous fermentation. Based on smell and literature expectations, we can estimate a broad alcohol percentage of 6 to 15 percent [23].

Conclusion

This project aimed to explore the use of different biomass substrates in mead to impart different flavors. Specifically, the substrate would serve to immobilize yeast in a continuous fermentation process. We first conducted batch tests for various substrates to evaluate the differences between them and identify one substrate to use for the continuous fermentation process. Ultimately, the orange rind was chosen as it immobilized the yeast and imparted its flavor into the mead most effectively. This continuous fermentation process was modeled using a pilot-scale reactor. Using orange rind as the immobilized substrate, we were able to produce orange-flavored mead. Gas Chromatography-Mass Spectrometry testing was used alongside qualitative measurements to analyze the chemical profile of the mead. The results from the GC-MS showed that the compounds identified originated from the must solution rather than from the substrates. However, the qualitative analysis did support that the flavor profiles from the substrate were imparted on the mead. The results from the project can potentially be used to develop more effective and efficient fermentation processes.

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Appendix A

Scanned GC-MS Results

Appendix of GC-MS Spectra

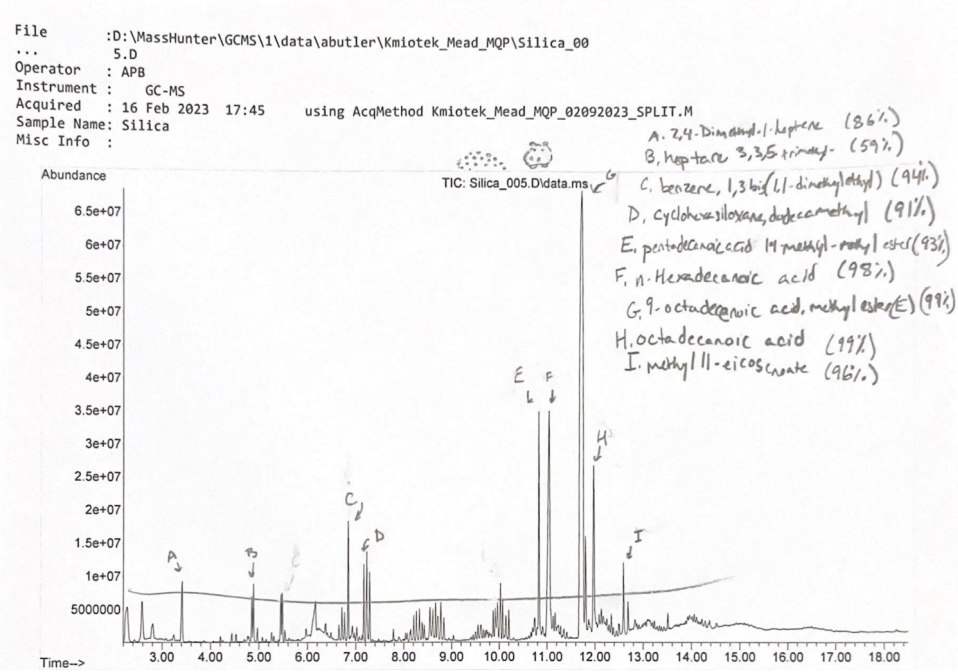

Figure 1. Labeled GC-MS spectra of the silica sample from the first batch (SIL B1S1).

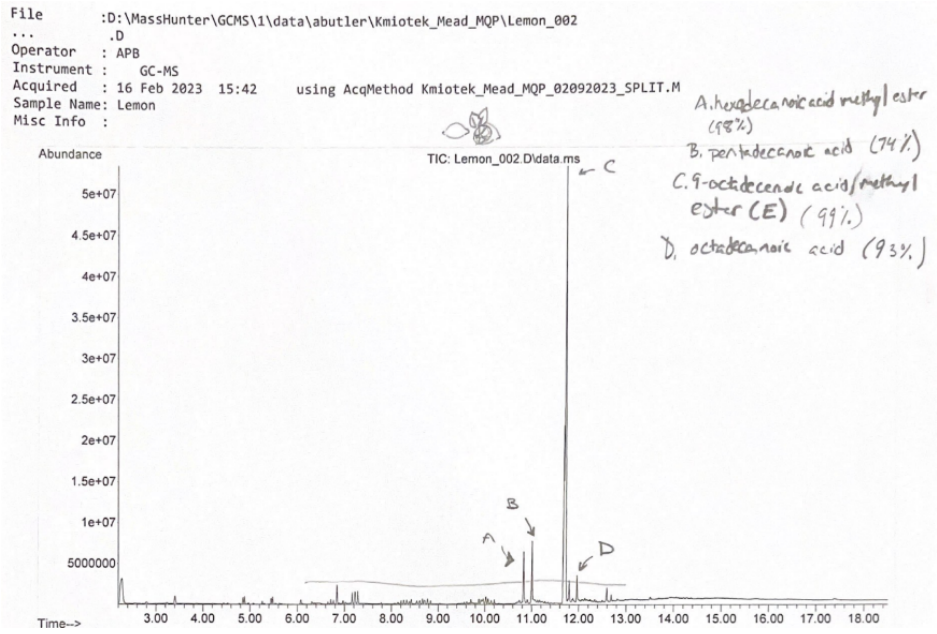

Figure 2. Labeled GC-MS spectra of LEM B1S1.

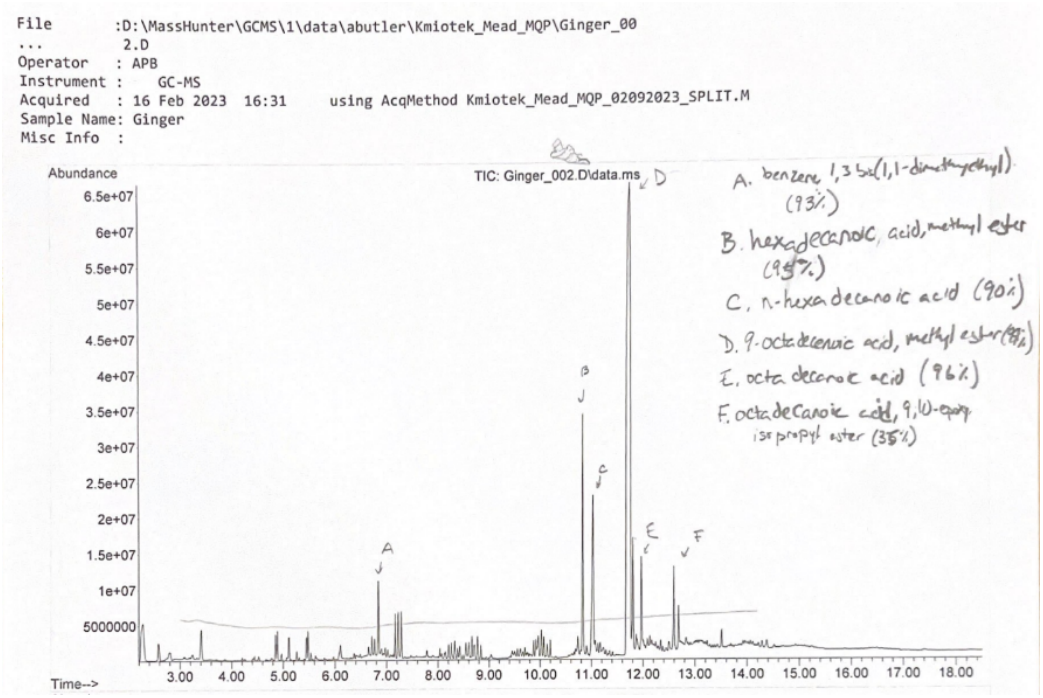

Figure 3. Labeled GC-MS spectra of GIN B1S1.

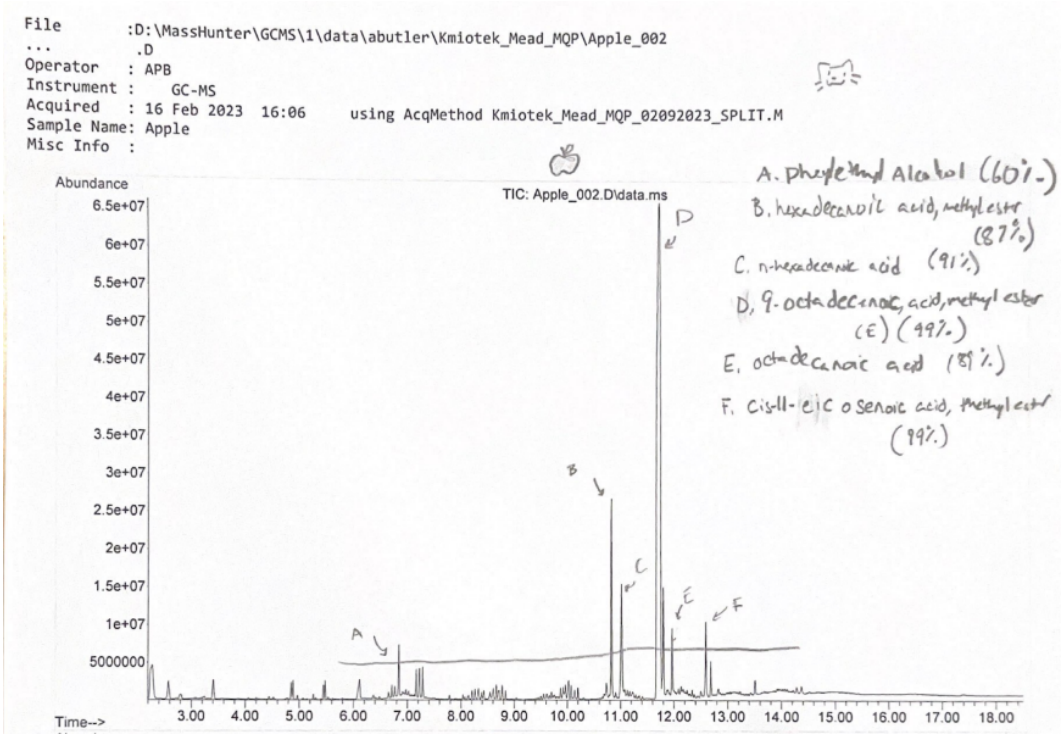

Figure 4. Labeled GC-MS spectra of APL B1S1.

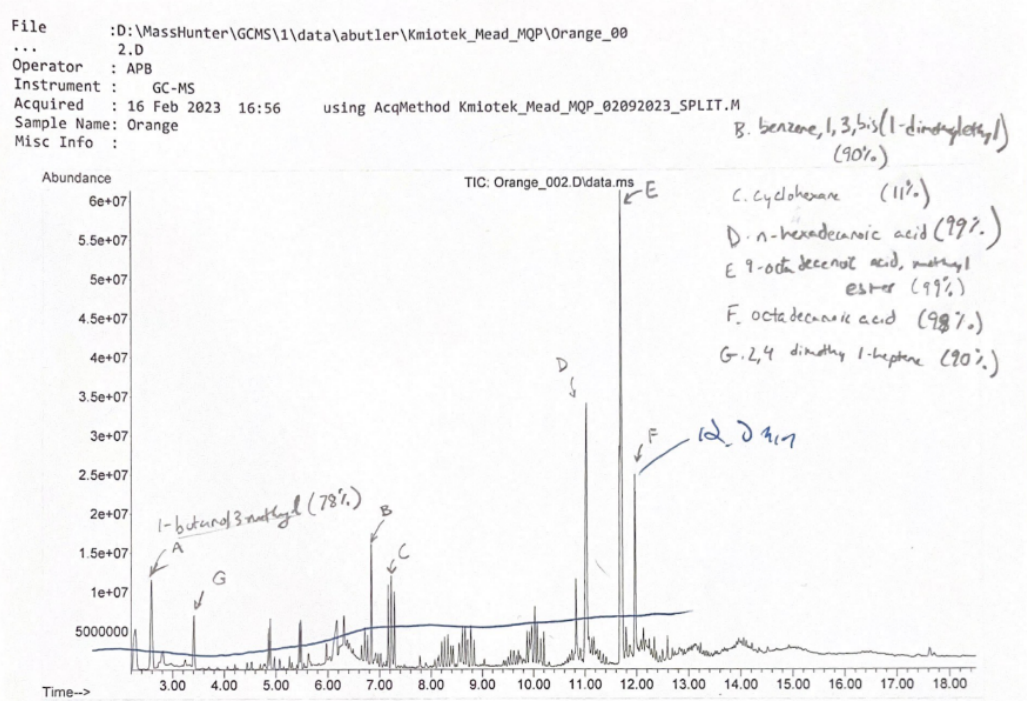

Figure 5. Labeled GC-MS spectra of ORG B1S1.

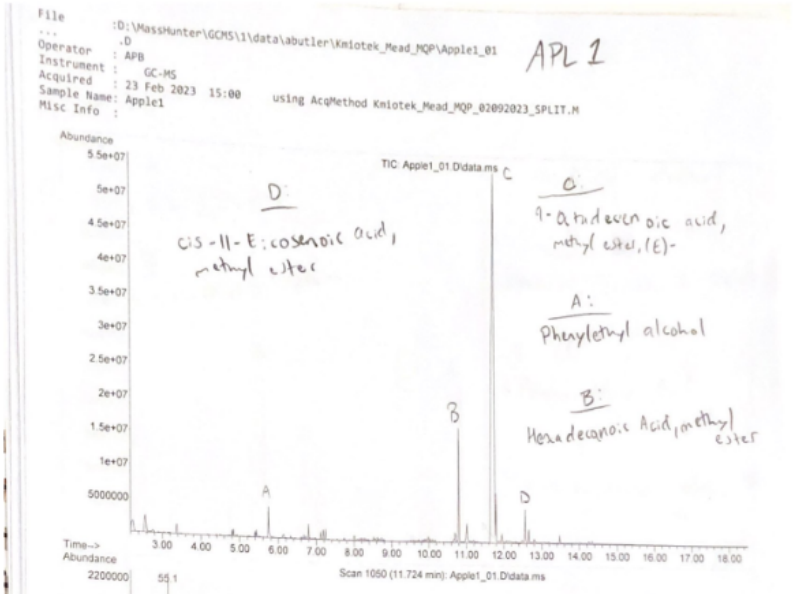

Figure 6. Labeled GC-MS spectra of APL B2S1.

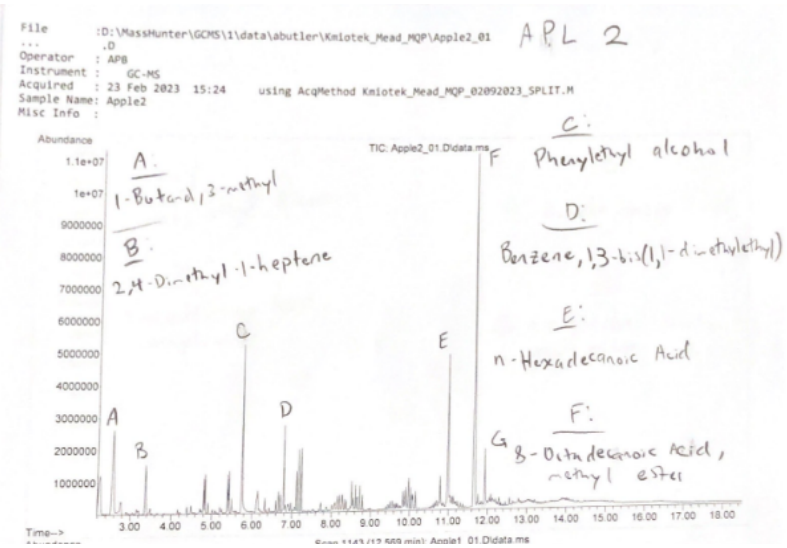

Figure 7. Labeled GC-MS spectra of APL B2S2.

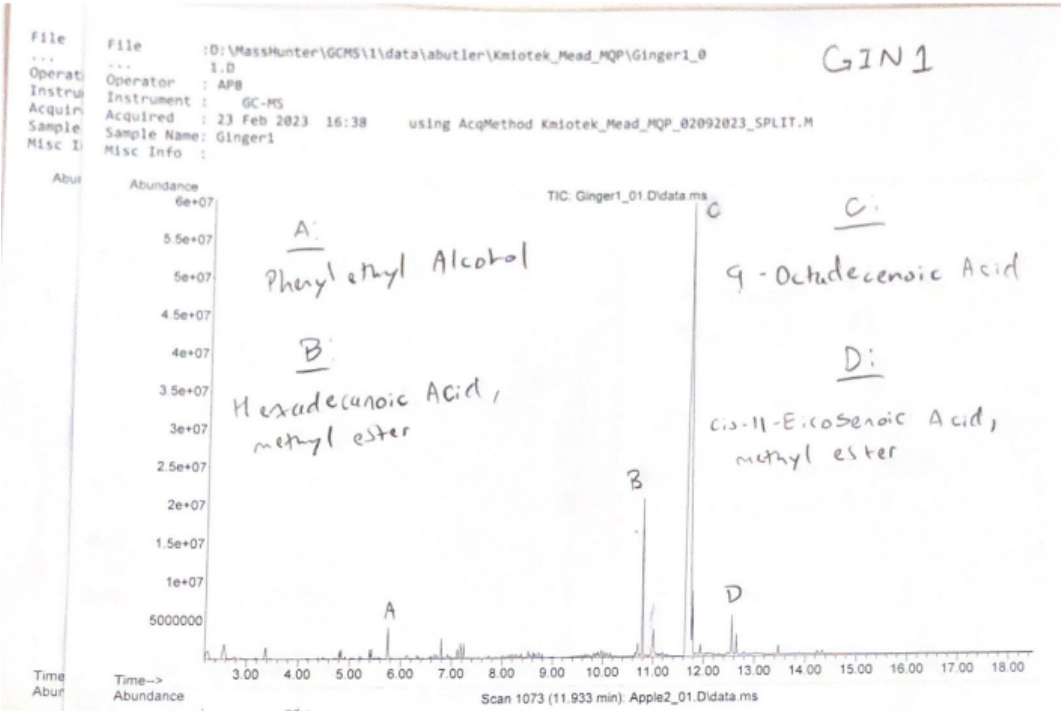

Figure 8. Labeled GC-MS spectra of GIN B2S1.

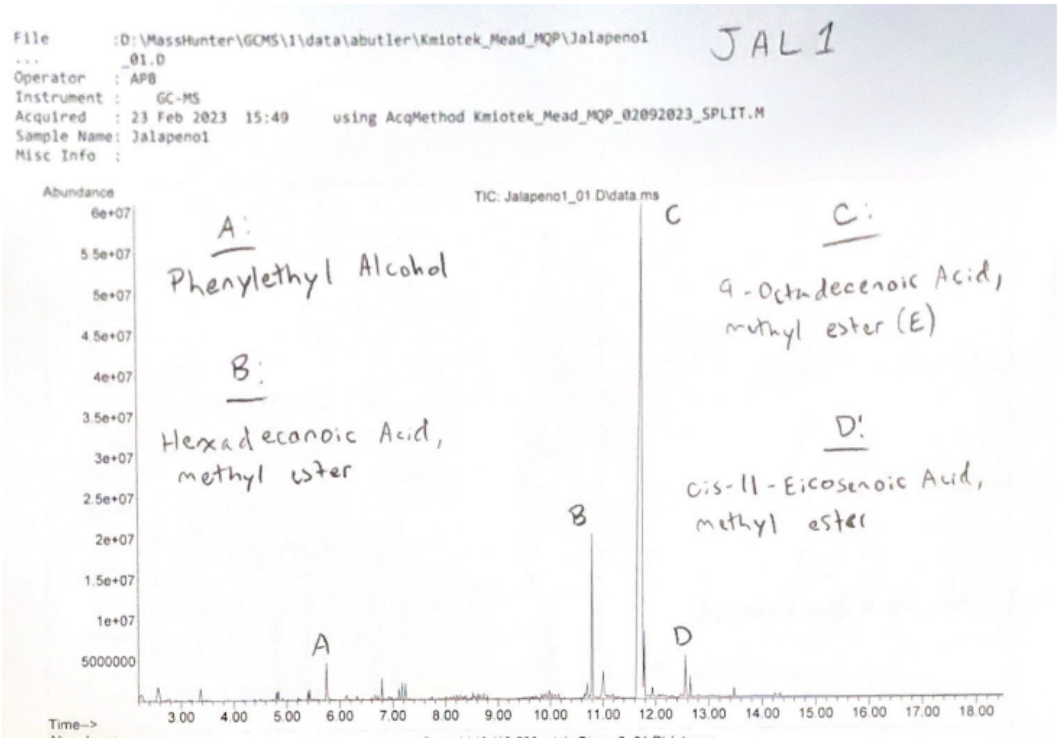

Figure 9. Labeled GC-MS spectra of JAL B2S1.

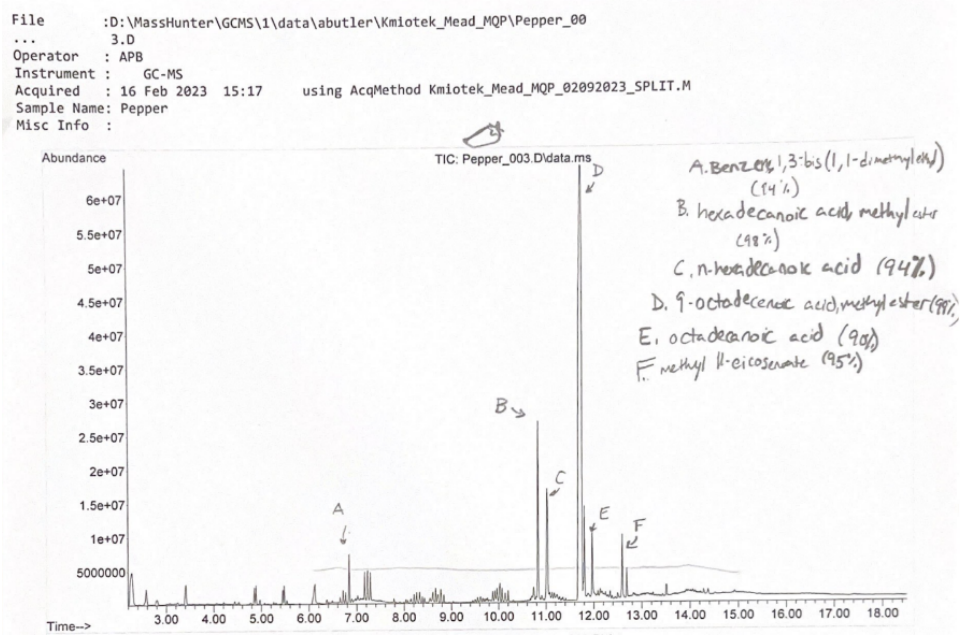

Figure 10. Labeled GC-MS spectra of PEP B1S1.

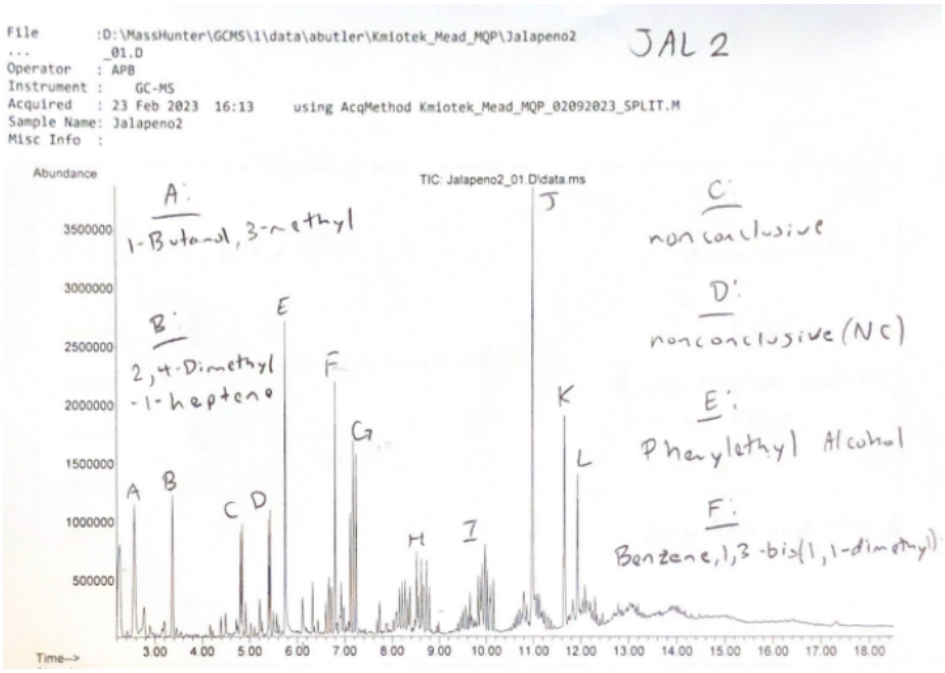

Figure 11. Labeled GC-MS spectra of JAL B2S2

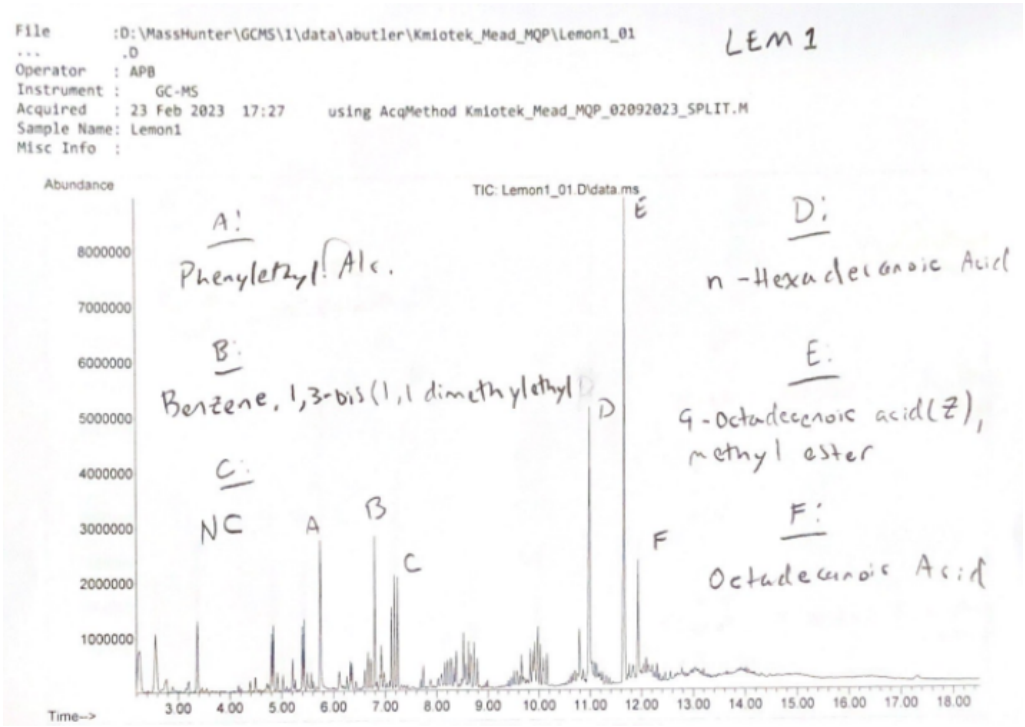

Figure 12. Labeled GC-MS spectra of LEM B2S1.

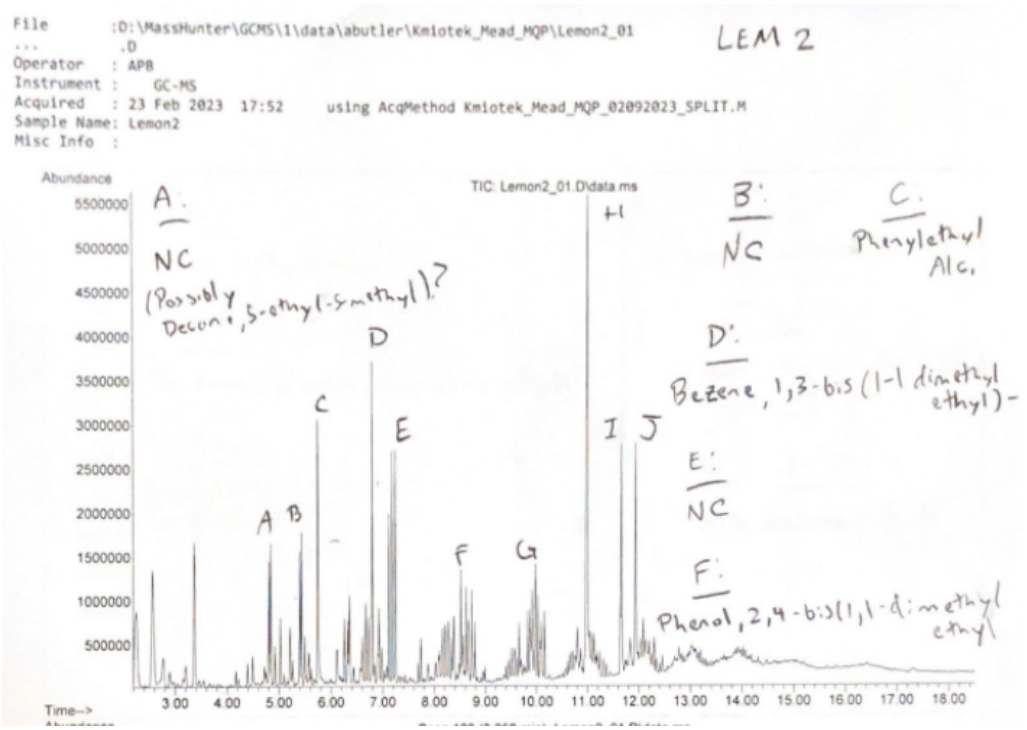

Figure 13. Labeled GC-MS spectra of LEM B2S2.

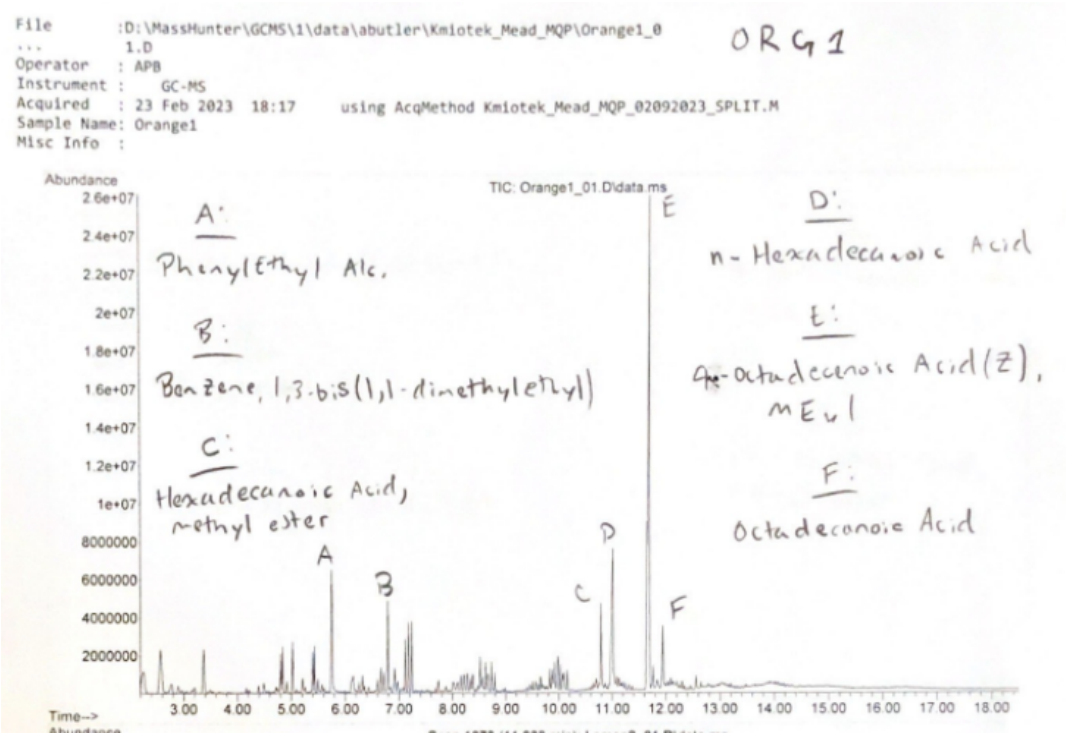

Figure 14. Labeled GC-MS spectra of ORG B2S1.

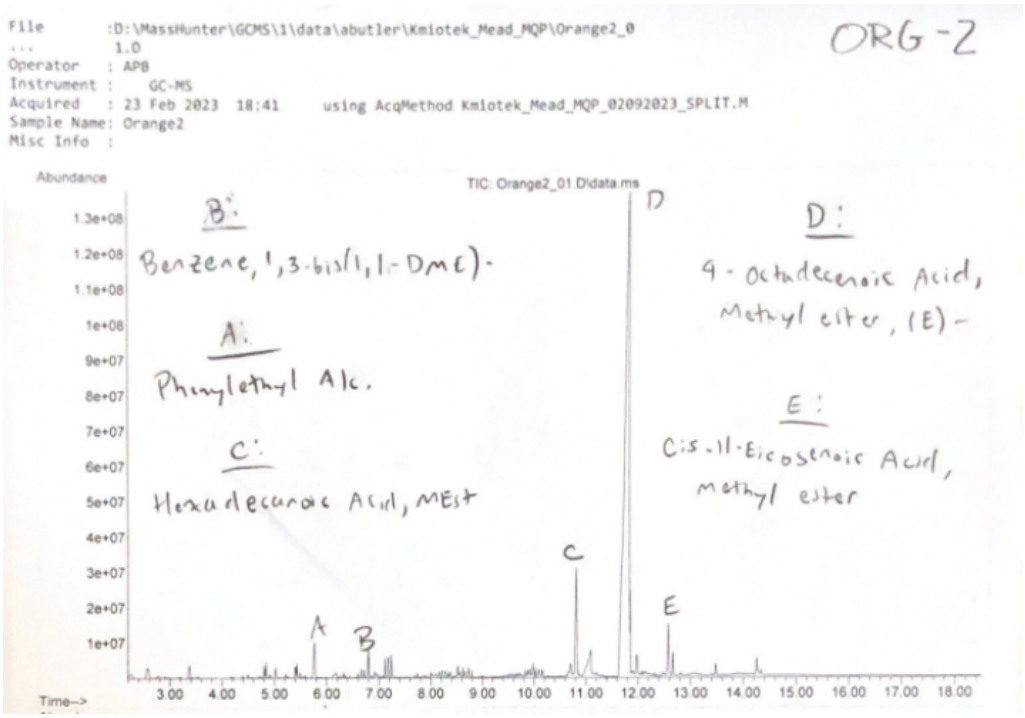

Figure 15. Labeled GC-MS spectra of ORG B2S2.

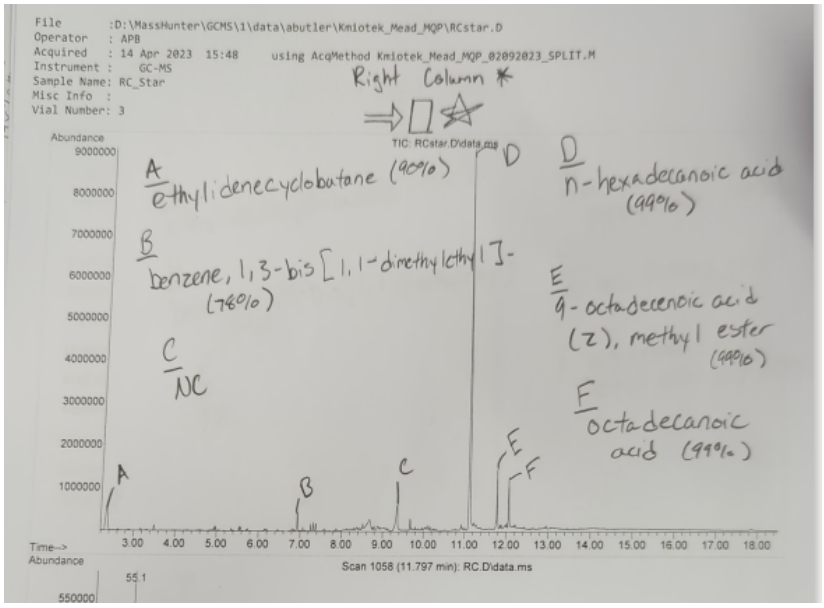

Figure 16. Labeled GC-MS spectra of the right column sample taken in the middle of our time frame.

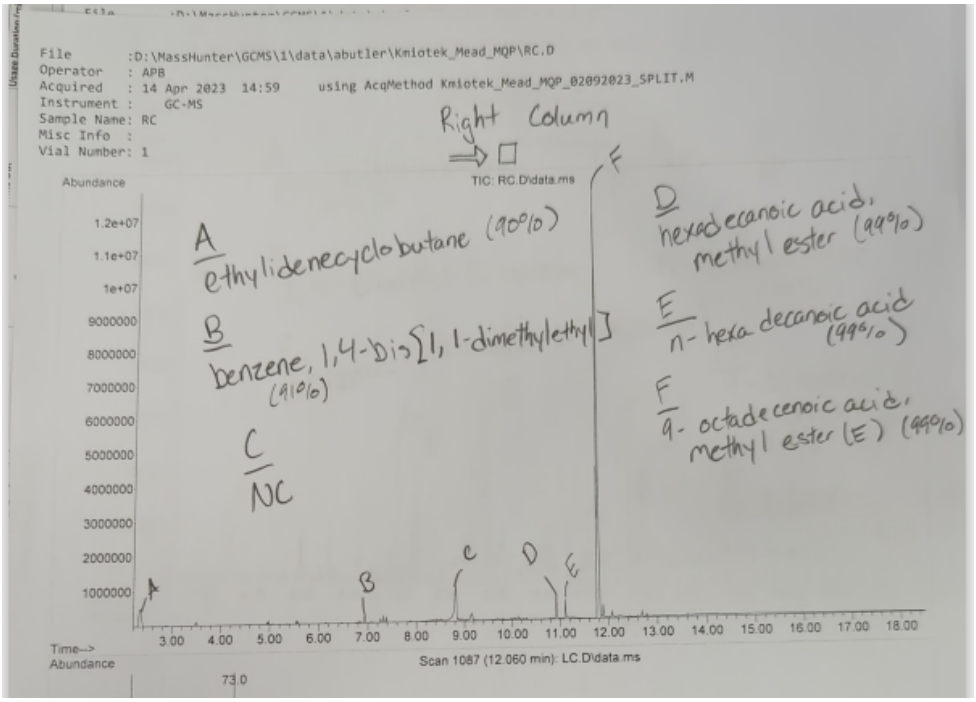

Figure 17. Labeled GC-MS spectra of right column sample taken at the end of the process.

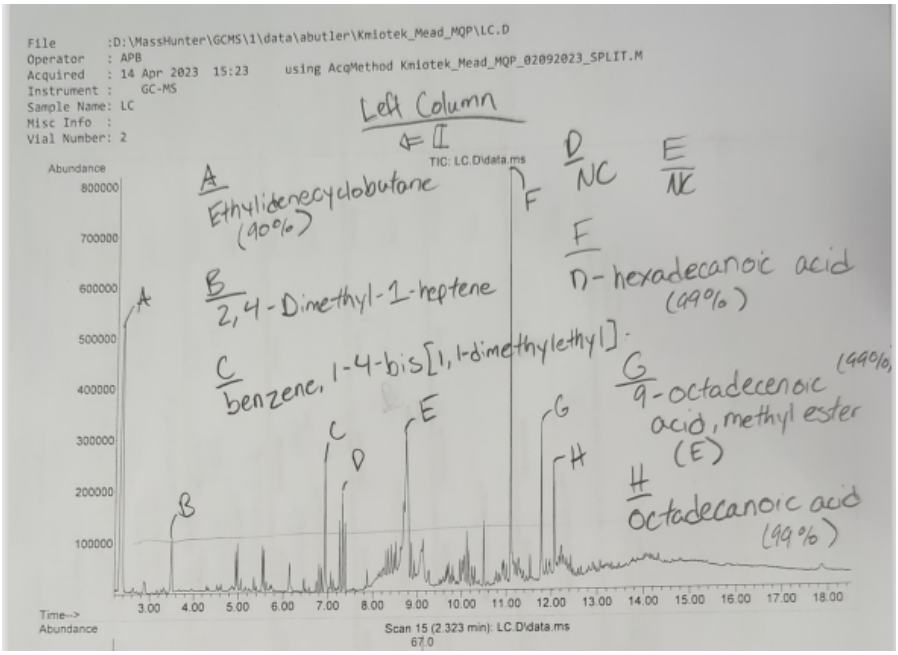

Figure 18. Labeled GC-MS spectra of left column sample taken at the end of the process.