Zoll Cellars Winery

A Major Qualifying Project
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Sponsored by:
Zoll Cellars
110 Old Mill Rd
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Abstract

The MQP team worked with Zoll Cellars, in Shrewsbury, Massachusetts, to create a more consistent and better quality product. This was accomplished with research, experimentation, and the analysis of the results. Chardonnay and Riesling were the two wines investigated. The sugar content, Fermaid K, and yeast were varied in 44 different samples. After various obstacles were overcome, the samples were extracted and analyzed. The results were compared to a generic brand of Chardonnay and Riesling. One wine did not stand out from the rest, but some were more similar to the generic brand than others. Relationships between the Fermaid K additions and the amount of certain compounds were discovered, however none were found for sugar content or yeast.

Authorship

This report was completed with the combined efforts of both MQP partners. Each member contributed equally to the project as well as the report.

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Table of Contents

1.0 Introduction

Zoll Cellars is a winery located in Shrewsbury, Massachusetts. Frank Zoll owns and operates the winery out of his home. Zoll Cellars makes their own wine, and they offer the opportunity for wine enthusiasts to learn how to make their own wine as well. Zoll Cellars grow some of their own grapes on their property, but they also purchase grapes from other areas such as Massachusetts, Connecticut, Rhode Island, and New York. The grapes are received or harvested at Zoll's home. The grapes are then crushed, pressed, fermented, clarified, and finally bottled accordingly. The entire process takes place at his home. Zoll's wines are then sold at local stores and famer's markets. The current wines offered by Zoll Cellars are: Chardonnay, Hard Cider, Wildflower Mead, Harvest Pumpkin Mead, Late Harvest Vidal Blanc, Dry Riesling, Cabernet Franc, Sand Castle Blend, Lighthouse Blend, and Pinot Noir.

The team met with Frank Zoll to discuss the goal of the project as well as learn about his process of winemaking. The team also had an opportunity to spend a day at Zoll Cellars to learn Zoll's process of fermentation. Frank Zoll is a Pastry Chef and Vintner and is well versed with the art of food and beverages. Zoll was looking to gain knowledge on the scientific side of winemaking to help improve product quality and consistency in his Chardonnays and Rieslings. After researching, the team created a plan and presented it to Zoll. He agreed with the plans and provided fresh juice, yeast, and additional nutrients. He also offered his knowledge and assistance throughout the duration of the project.

2.0 Background

2.1 History

Wine making is thousands of years old, and there is even mention of it in the bible. The use of the microorganism yeast is one of the oldest uses of microorganisms by humans. Wine was first produced 6,000 to 8,000 years ago ("Wine making," 2007). Since it first began it has spread throughout the Middle East, the Nile Valley, and the Mediterranean Basin. The Egyptians first recorded the making of wine around 2000 B.C. Egyptians would crush fruit and keep it in a warm place to produce a liquid that produced feelings of elation ("Wine making," 2007). In Egypt, wine played a significant role in many religious ceremonies, and it still does today in some religions. Besides the religious ceremonies, Pharaohs and the elite also used wine. The spread of wine to the Mediterranean Basin, Greece and Rome was due to the Phoenicians' trading ("Wine making," 2007). Once wine was introduced to the Greek and Romans, it started to become an important role in daily life. The potential for medicinal properties was revealed along with the effects of over consumption leading to inebriation. Wine was extremely important to both ancient civilizations and was tied to the religious myths of Dionysus and Bacchus McGrew & Wagner, 1977). Romans had a significant impact on wine, including production methods, storage, classification and production throughout Europe, such as modern day France, Italy, and Spain. During the dark ages after the fall of the Roman Empire, religion and wine were again intertwined. Since wine was so important in the Christian faith, the monks in the monasteries continued to produce wine. In Saint Benedict's rules, Chapter 40 was all about wine and even required monks to consume wine in moderation daily. Today, wine is still significant in many religions (McGrew & Wagner, 1977; Trevisan, 2011).

Throughout history, it was a common tradition for many families to make their own wine at home worldwide. Presently, most wine is produced industrially, although it is still commonplace in some areas. The wine market has grown significantly, and it now has significant economic impact both locally and globally through exports. Approximately one quarter of wine purchased is outside of the country of production (McGrew & Wagner, 1977; Trevisan, 2011). Wine production is very concentrated. For example, there are countries such as Italy, France, and Spain that contribute a large percent: 21%, 20.4% and 11.7% respectively, of globally produced wine. (McGrew & Wagner, 1977; Trevisan, 2011).

2.2 General

Grapes are one of the largest fruit crops in the world. Out of this large amount, 60 million metric tons of grapes are used for dry wine alone. The grapes are the main ingredient of wine and as a result have a large impact on the quality of the wine. Grapes have one of the highest sugar contents of all fruits and that is why they are the most common fruit for winemaking. A good wine starts with a good grape. There are hundreds of different varieties of grapes from all over the world (McGrew & Wagner, 1977).

Wine making at its core is very simple and straightforward. Wine is produced from the fermentation of grape juice, or must. The grapes are crushed to produce the must. Must is freshly pressed grape juice that includes skins, seeds, and stems. Red wine ferments the must, whereas white wine ferments just juice (McGrew & Wagner, 1977). For white wine, the must is directly pressed after being crushed. Pressing is a form of separation that removes the skins, stems, and seeds from the juice. When this juice is left in an appropriate vessel for an extended period of time, it will begin the fermentation process. Fermentation will normally begin within a few days. This process occurs because of the yeast that naturally exists on the grapes. Once the grapes are crushed the yeast begins to multiply. In the basic reaction, the yeast consumes the sugar in the juice and produces half alcohol and half carbon dioxide. Fermentation can last up to a few weeks, or longer depending on the temperature. Fermentation stops when all the sugar is consumed. Once fermentation is complete, the liquid is then drained and clarified. The product is wine (McGrew & Wagner, 1977).

Wine quality can vary greatly. A large portion of this can be contributed to the constituents in the grapes. Everything needs to be in balance for a high quality wine. Even though the process of making wine is simple and there is one main reaction, there is a lot happening in conjunction with the main process. There are many complex biochemical reactions that happen throughout fermentation. Many of these reactions are not fully understood ("Wine making," 2007). It is because of these reactions that wine making can be as simple or as complicated as one makes it. The better these reactions and the variables that affect these reactions are understood, the better the wine can be (McGrew & Wagner, 1977).

2.3 Red Wine

The juice should be placed in a fermentation vessel after all necessary additives are mixed in. The juice is now ready to ferment, and it should be covered. Red wines are fermented with the skins of the grapes, which is where they get their color ("Winemaking," 2007). Once fermentation begins for red wines, a layer of solid mass will form on top of the juice. This is called a cap. Twice a day this should be pushed down and mixed in with the rest of the must. Once fermentation has ended, red wine will need to be pressed. This involves separating the wine from the solid mass of skins, seeds, and stems. A small basket press is recommended although there are a variety of presses available. Once the red wine is pressed, it should be put into 5-gallon glass jars. The jars should be filled to the top and an air trap or bubbler should be used as a cover. The bubbler allows the carbon dioxide out but does not allow air in. Once the fermentation stops, which is noted by a stop in the formation of bubbles, the air trap should be removed and replaced with a rubber stopper (McGrew & Wagner, 1977).

2.4 White Wine

White wine is pressed immediately after being crushed. As a result, it is only juice and does not have skins or stems. It is recommended that white wine be fermented in 5-gallon glass jars. They should be filled only two-thirds of the way full to prevent

overflow which can happen due to the formation of too many bubbles. Once fermentation has completed, the jars can be consolidated and filled. The jars should be topped with an air trap or bubbler similar to the process of the red wine (McGrew & Wagner, 1977).

2.5 Sugar

Two important variables to look at when making wine are the sugar content and the tartness, or total "acidity" of the juice. The sugar content, also known as the brix, is important because the amount of sugar determines the amount of alcohol that the wine will have. Approximately two mass percent of sugar in the juice contributes one percent alcohol to the final wine ("Wine making," 2007). For a common table wine, the normal alcohol content ranges from 10 percent to 12.5 percent. This means that appropriate sugar content would range from 20 percent to 25 percent. Normally grapes from California have high enough sugar content, however grapes grown elsewhere can be deficient. Adding the correct amount of granulated sugar can compensate for this deficiency. The ordinary granulated sugar is converted to grape sugar once it contacts the juice. It is recommended if non-California grapes are used, that the sugar content of the juice should be tested. The sugar content of the juice can be tested using a saccharometer. The saccharometer is floated in a sample of the juice. The buoyancy of the saccharometer is dependent on the sugar content of the water. The height of the juice aligns with the sugar content scale of the saccharometer (McGrew & Wagner, 1977). The average level of brix varies depending on the type of wine. For example, a white wine has an average of 22-24 brix. A red wine has a slightly higher average of 22-25 brix. The higher the brix, the higher the alcohol content. This means that red wines are typically sweeter with higher alcohol contents. The lower the brix level, the more acidity you will taste in the wine. This means most white wines have a more acid like taste ("Wine making," 2007; McGrew & Wagner, 1977).

2.6 Acidity

The acidity or tartness also has a large effect on the flavor of the wine. If it is not corrected the resulting wine will not be of high quality and may be too tart. Acidity can be measured, but most home winemakers rarely do the chemical test required to check for total acidity. Instead, if the juice tastes too acidic most winemakers use a rule of thumb. This requires making a sugar solution by adding two pounds of sugar to one gallon of water. One gallon of the sugar solution should be added to four gallons of juice. Acidity protects the juice from spoiling. Therefore grapes with low acidity, such as those from California are susceptible to spoilage from bacteria during fermentation. To prevent spoilage in grapes with low acidity, potassium metabisulfite can be added. One-quarter ounce of potassium metabisulfite should be added to 100 pounds of crushed grapes (McGrew & Wagner, 1977). The average acidity of a red wine is around .6-.9 grams/liter (Winemaker's Academy). The average acidity for a white wine is typically higher than a red wine and is around .7-.9 grams/liter (Winemaker's Academy). Red wine has a pH between 3.2-3.6, and a white wine has a pH of 3.2-3.5 (Winemaker's Academy).

2.7 Yeast

As previously stated, yeast is the main component that goes into winemaking. Yeast is a single-celled fungus and because of this, it is able to reproduce by fission or budding. Yeast belongs to the phylum Ascomycota. There are many different kinds of yeast available. The yeasts that are suitable for making wine are the seven species of yeast that are in the genus Saccharomyces. The most common species used for wine making is Saccharomyces cerevisiae. Many different strains of this species can be used to make wine. Wine making is both a science and an art. Part of the art of winemaking is choosing the yeast. The yeast should pair well with the grape species and the fermentation conditions to make the most ideal and highest quality wine. There is natural yeast that is already present in most grapes. This is the natural strain of yeast that populates and dominates the grape vineyard. Some of the newer, less mature vineyards have to rely on inoculation of, or the addition of, yeast to the crushed grapes ("Wine making," 2007).

Yeast is the agent for fermentation, or the production of carbon dioxide and alcohol from glucose sugar. Fermentation is possible because of certain enzymes the yeast possess. More than two-dozen yeast enzymes contribute to the degradation of glucose. This degradation of glucose is a pathway, meaning that one reaction is dependent on the occurrence of the reaction prior to it and subsequent reactions are dependent on the occurrence of that one reaction. In total, there are approximately 30 chemical reactions that are involved in the process of fermentation ("Wine making," 2007).

It is recommended that the juice be inoculated with yeast, also known as a yeast starter. Different kinds of yeast can be bought as dehydrated yeast. Dehydrated yeast is a powder that is a form of yeast that is dormant ("Wine making," 2007). Most come in packets and should come with directions on the package. Some directions will recommend the yeast just be pitched directly into the juice. However, this is not the most ideal way to add the yeast to the juice. Directly pitching the yeast into the juice is said to kill a large portion of the variable yeast cells. This leaves the remaining surviving yeast cells to do extra work and leaves them stressed. Stressed yeast can lead to unwanted flavors and aromas in the resulting wine. This is why it is best to rehydrate the yeast instead of directly pitching the yeast into the juice (American Homebrewers Association, 2015). To rehydrate the yeast add the yeast to a small cup. Add a few ounces of warm water to the yeast. Let the yeast absorb the water and once all of the water has been absorbed, add more water to bring the consistency to that of cream. Let the yeast stand for a total of at least 20 minutes, but some recommend waiting an hour (American Homebrewers Association, 2015; McGrew & Wagner, 1977; Palmer, 1999). It is recommended to try to bring the yeast's temperature to within 15 degrees of the juice temperature (American Homebrewers Association, 2015). Then the yeast can be added to the grapes. You can also add a small amount of warm juice or sugar water to the yeast mixture to make sure the yeast is still active (American Homebrewers Association, 2015). Approximately 30 minutes after the juice is added, the yeast should begin bubbling

and foaming. If bubbling, churning, or foaming is not seen then the yeast is probably dead or too old. This can be a common problem with dehydrated yeast that is not name brand. Yeast should be added in a ratio of 20 to 30 grams per hectoliter of juice (Palmer, 1999; "Zymaflore X16 Product Data Sheet").

Zoll gave two different types of yeast that were to be used during the experiment, D47 and X16. Both were dehydrated yeast. Zymaflore X16 is recommended for chardonnay, chenin, ugni blanc, and colombard grape varieties, but it can also be used for any white or rose wines. It is for aromatic wine with a strong fermentation aroma profile, such as white peach, white flower, and yellow fruit. It has a very high fermentation capacity and high aromatic production. The profile is delicate and clean. It is a tough yeast as it has a high alcohol tolerance, and tolerance of low temperature and low turbidity ("Zymaflore X16 Product Data Sheet,"). ICV D47 Yeast is recommended for white and rose wines. It adds citrus and floral notes to the wine. It produces a high amount of polysaccharides, which contributes to the fruity characteristics, volume, and complexity of the wine. It has a slightly small alcohol tolerance than X16, 16% versus 14%. It is also sensitive to low temperatures in clarified juices ("ICV D47 Product Data Sheet,").

2.8 Fermaid K

In order for the yeast to ferment, nitrogen is required. The minimum amount of fermentable nitrogen necessary for fermentation is 140 mg/L. If there is no or little nitrogen present in the fermentation, the yeast cells become stressed and produce excess H2S. This produces an off-odor therefore making it noticeable. This is one of the most common problems home winemakers experience (Palmer). Therefore, it is important to monitor the YAN, which stands for Yeast Available Nitrogen. It is a measure of the amount of nitrogen available to the yeast in grape juice (Church). YAN is measured in parts per million or milligrams per liter. The YAN come in two different forms within the grapes. The first is the assimilable nitrogen from the alpha amino acids in the grapes (a-amino nitrogen). The second is Free Ammonia Nitrogen (FAN). Both are important, but the a-amino nitrogen has a bigger role at the beginning of fermentation. The FAN supplies the nitrogen required during the later stages of fermentation (Church). That natural YAN varies greatly in grapes depending on the variety and the season (Church). In today's harvests, it is typical to have a higher brix which means a low YAN level. Because of this, it is imperative to add Fermaid K or DAP to raise the YAN levels (Church). However, there is no set amount of Fermaid K or DAP to add to raise the YAN levels. YAN required based on brix is as follows: 21 brix 200-250 YAN range, 22 brix 225-275 YAN range, 23 brix 250-300 YAN range, 24 brix 275-325 YAN range, and 25 brix 300-350 YAN range (Church). The best way to add Fermaid K or DAP is to periodically check on your wine. You should add a smaller amount in the very beginning of fermentation, and then throughout the fermentation process continue to watch the wine. If it begins to smell like H2S, the yeast is becoming stressed from a lack of nitrogen and you should add more Fermaid K or DAP (Church; Palmer).

2.9 Fermentation Temperature

Fermentation can be negatively affected by the poor growth of the yeast. This can happen if the amount of light or temperature is unfavorable. This in turn, can then cause unfavorable organisms in the wine to negatively impact the wine flavor and aroma. These organisms can then also outgrow the yeast and compete with the yeast for nutrients (Winemaker's Academy). Maintaining the correct fermentation temperature can prevent all of this. Maintaining the correct fermentation temperature can also facilitate proper yeast growth, extract flavors from the skins for red wine, increase the production of desirable byproducts, and prevent a rise in temperature that could kill the yeast. The best temperature for the growth of wine yeast is approximately 77°F (Winemaker's Academy). However, this is not the ideal temperature to start fermentation because then it is hard to prevent the fermentation temperature from rising to 86°F. This is because fermentation also produces heat, along with carbon dioxide and alcohol. The production of heat can cause the temperature of the fermentation vessel to rise to potentially unacceptable temperatures ("Wine for Beginners," ; "Wine making," 2007; "Winemaking Temperatures," 2015).

For white wines, a cooler fermentation temperature from 50 to 60°F is preferred. Some even cited temperatures as low as 45°F. This cooler fermentation temperature results in better production and retention of desirable by-products. It also helps to preserve the volatile aromatic compounds, keeps volatile acidity low, and also contributes to a full mouth feel. However, when fermented at cooler temperatures fermentation takes longer to complete. Instead of one to four weeks, it take six to ten weeks at a warmer temperature. Fermenting at a cooler temperature can also result in fermentation stopping with some residual sugar still remaining in the wine. In practice most white wines are fermented at 68°F (Winemaker's Academy). For red wines a warmer fermentation temperature, from 70 to 85°F, is preferable (Winemaker's Academy). The increased temperature allows for better color and tannin extraction from the grape skins. It also limits the fruitiness in the wine, which is undesirable for red wines ("Wine for Beginners," ; "Winemaking Temperatures," 2015).

2.10 Storage

After the juice has fermented and been pressed, the wine should be stored for a few weeks at 60 degrees Fahrenheit. This is for two reasons: some of the suspended material will settle out of the wine, and some beneficial reactions continue to take place at this temperature. After a few weeks, the wine should be siphoned from the glass jar and put into clean containers. While completing this, more potassium metabisulfite should be added to the wine. It should be added at a ratio of ¼ teaspoon per 5 gallons of wine. As stated before, potassium metabisulfite prevents unwanted aromas and spoilage of the wine (McGrew & Wagner, 1977).

2.11 Clarification

After storage, the containers with the wine should be chilled. The drop in temperature will help some of the unwanted suspended solids to settle and fall out

of the wine. It should be chilled for approximately 4 or 5 months until it has “fallen bright” and is stable. Placing a lit match on the opposite side of the container can check the clarity of the wine. If the wine is brilliantly clear it can be siphoned into wine bottles and corked (McGrew & Wagner, 1977).

If the wine is not brilliantly clear after the first 4 or 5 months of chilling, the wine should be fined. Fining is a process that helps remove impurities and suspended solids from the wine. The process involves dissolving a small amount of normal gelatin in a small amount of hot water. Gelatin should be added at a rate of 2 teaspoons for 5 gallons of wine. This mixture should be added to the wine while the wine is being siphoned into new containers. The wine will turn milky when the mixture is added. As the gelatin mixture settles it will take with it impurities and suspended solids. This should take two weeks to a month (McGrew & Wagner, 1977).

After fining the wine, it should be siphoned again into a container and more potassium metabisulfite should be added at the same ratio of ¼ teaspoon for every 5 gallons of wine. This is because if the wine is not completely clear there are still some reactions that need to take place and if the wine was bottled before it was ready the pressure inside the bottle could build and potentially shatter the bottle or pop the cork. Once siphoned into new containers and the potassium metabisulfite is added, the containers should be left for approximately five more months. Then it can be siphoned for the final time, the last addition of potassium metabisulfite should be added and the wine can be bottled (McGrew & Wagner, 1977).

3.0 Methodology

3.1 Observations

The goal of the project was to help Zoll Cellars improve their overall process with Chardonnay and Riesling. These are both white wines. So we first observed his current processes. After observing Zoll's procedure we concluded that there were some variables that were nearly impossible for him to control. One variable that he challenged us with was trying to maximize incoming juice with lower brix as most of the grapes he receives have a brix at the lower end of the scale. He said most juices have a sugar content around 21 brix. Sometimes he adds sugar and sometimes he does not. Another variable that was considered was the fermentation temperature. However, the fermentation currently takes place outside, so it is not a variable that could be controlled. He has also been experimenting with different yeasts that result in slightly different flavor profiles. Additionally, the amount of Fermaid K is another variable to explore. At the end of the fermenting process, Zoll sometimes adds further additions without precise measurement. After observing his process and the environment it takes place in, we narrowed down our project to three separate variables. We decided to further our experiment with different Fermaid K additions, different sugar contents, and different yeasts.

3.2 Obstacles

After receiving the fresh juice from Zoll cellars, the juice was refrigerated. The refrigeration slowed down the fermentation, however it did not prevent the juice from fermenting. Nothing was added to the juice before it entered the refrigerator. The juice fermented on its own with natural yeast that was present in the juice. When the juice was needed to do the experiment, it was taken out of the refrigerator and the brix was tested. The brix was expected to be around 21. Various juices were tested at random and had a brix ranging from 1 to 10. A low brix indicated a low sugar content of the juice. Low sugar content indicated that fermentation had already taken place. Some bottles of the Riesling juice were rebottled after one of the bottles shattered due to the pressure of the carbon dioxide build up that is produced from fermentation. When the bottles were rebottled, they were put into mason jars that had an air trap in the cap, however the air trap was never filled with water. This resulted in the Riesling juice being exposed to air, causing severe oxidation. It was agreed that this juice was no longer suitable to use for the experiments. Regarding the Chardonnay, after contacting our sponsor, it was decided that we would add sugar to the already fermented juice to bring the brix back up to where it should have been and continue the experiment as normal.

3.3 Zoll Cellar Juice

3.3.1 Yeast

Two different types of yeast were used, X-16 and D-47. The yeast that was used as a constant throughout this experiment was X-16. This was chosen as the constant as it is the yeast that Zoll commonly uses for white wines. For this set of experiments we also had a variable in which no additional yeast was added, letting the natural yeast

in the juice do the fermentation. D-47 yeast was added to two jars of juice. No yeast was added to two jars of juice. X-16 yeast was added to all other jars of juice. Yeast was added in a ratio of 5 grams to 20 liters. Each small jar had 8 ounces of juice, so 0.04 grams of the yeast were added.

The yeast was dehydrated yeast so it had to be re-hydrated for it to become active. This was done by first adding the necessary amount of yeast to a pint mason jar. Then 5 pipets of hot water were added to each mason jar. After 15 minutes, two pipets of juice were added to the rehydrated yeast to see if it was active. Once the yeast was confirmed active, indicated by foaming or churning, 8 ounces of juice were added to the mason jar.

3.3.2 Sugar

Sugar had to be added to all the juice for this experiment. Different bottles were at different points in the fermentation, and many had different brix. Each bottle was shaken and some of the juice was poured into a 100 mL graduated cylinder. The triple scale hydrometer was then inserted into the graduated cylinder and allowed to settle to measure the brix. Once the brix was measured the juice was returned to the bottle. The juice in the bottle was massed using a mason jar. An approximation of the amount of sugar left in the juice was calculated. With a desired brix the amount of sugar added to the juice could then be calculated.

First the necessary amount of sugar was weighed and put into a small mason jar. Then juice was added to the small mason jar so it was full. It was then microwaved for 30 seconds and then shaken. This was repeated until the sugar was completely dissolved. Once it was dissolved the concentrated sugary mixture was poured back into the juice. The juice was then shaken and the brix was measured to confirm the brix was correct.

For this experiment three different brix were used: 20, 21, and 22. These brix were chosen because they were common brix of juice that Zoll received for white wines. A brix of 21 was chosen as the constant because that was the brix the juice had originally. The varied brix were chosen because they were common brix for white wine juice and Zoll was interested in lower brix juices. Two small jars of juice were brought to a brix of 20 and two small jars of juice were brought to a brix of 22. All other juice was brought to a brix of 21.

3.3.3 Fermaid K

Three different amounts of Fermaid K were added to the juice. Fermaid K was recommended to be added once at the beginning and once when fermentation was one third to one half complete. The total amount of Fermaid K that is supposed to be added is 5 grams per 20 liters of juice, or 0.25 g/L. Once converted to ounces, it adds up to 0.0074 g/oz. Each jar had 8 ounces of wine, so this number was multiplied by 8 to get 0.06 grams for each jar. However, because the total amount of Fermaid K is split between two different additions, this number would be divided by two. This would result in each of the two additions to be 0.03 grams of Fermaid K. Since our

scale only went to the hundredths place it was decided to also do 0.02 grams and 0.04 grams to vary the additions. This totaled to adding 0.04, 0.06 and 0.08 grams of Fermaid K to different jars.

3.4 Fresh Juice

Since it was not known how re-fermenting the juice would affect the experiment, it was agreed that the experiment should also be done using fresh juice. Frozen wine juice was ordered. The Chardonnay was from California and the Riesling was from Lanza Vineyards in the Suisun Valley in California. Once it arrived it was still frozen. It was thawed overnight and used the next day.

3.4.1 Yeast

The same yeasts were used for the fresh juice, both X-16 and D-47. D-47 yeast was used for two small jars of juice. There was not a variable without yeast for this experiment. X-16 yeast was used for the rest of the juice. The same amount of yeast, 0.04 grams, was added to each small jar. The yeast was re-hydrated and added as stated above.

3.4.2 Sugar

For this experiment three different brix were used: 21, 22, and 23. The brix for this experiment were larger than that of the other experiment. The brix of both frozen juices was 22 brix. For this reason it was decided that the standard brix would be 22 for the experiment with frozen juice. For both juices, the juice was put into a 100 mL graduated cylinder and the brix was checked with the triple scale hydrometer. To get a brix of 21 the juice was massed on the scale and the amount of water to be added was calculated. The water was then massed and added to the juice. To get a brix of 23 the juice was massed and the amount of sugar to be added was calculated. The correct amount of sugar was then massed out and some of the juice was added to it. The mixture was then microwaved until all the sugar dissolved. This mixture was then added back into the juice, as stated above.

3.4.3 Fermaid K

Fermaid K was added the same as above. Two small jars had two Fermaid K additions of 0.03 grams each and two small jars had two Fermaid K additions of 0.05 grams each. The other small jars had two additions of 0.04 grams each, which was the constant.

3.5 Analysis

There is a validated method that can determine major and minor volatile compounds. This method is accepted across the wine industry. In order to analyze the components of the wine, the wine is diluted with water and then salt and dichloromethane are added. Using this method, the volatile compounds in the wine are extracted into the dichloromethane phase. The dichloromethane is denser than water and therefore it sinks to the bottom of the tube. The dichloromethane phase can be extracted and run through gas chromatography mass spectrometry. This will separate the different compounds in the sample by running it through a long coil in the GC mass spec. The different compounds take different amounts of time to make

it through the coil. The larger compounds take longer and the smaller compounds take less time, and this is how the compounds are identified and differentiated. A previous MQP project was also used as a reference for this methodology.

There were forty-four samples of different experimental wines with varying yeast, sugar content, and Fermaid K additions. For each of these samples 5 ml of wine, 5 ml of water, 2.25 grams salt (NaCl), and 1.0 ml of dichloromethane were added in a centrifuge tube. Each mixture was hand shaken for 10 minutes and then put into a centrifuge for 10 minutes at approximately 3000 RPM. After it was shaken, 0.2 ml of the dichloromethane phase was extracted and put into a GC mass spec vial. It was later concluded that the analyte should be filtered because the extraction was not clean enough and there was concern about the salt in the GC. One of the samples was filtered using a 0.45 microliter filter. The sample of analyte was not large enough to be filtered and have enough analyte left over to run through the GC. The filter absorbed the entire sample. This set back lead to having to re-do the analysis methodology. After researching about the salt, it was determined that the salt was used in the aqueous solution to decrease the solubility of the volatile compounds, so that they are more soluble in the dichloromethane phase, making a better extraction. This ruled out the potential hazard of the salt in the GC, and now the focus was to get a clean, clear analyte that only included the dichloromethane phase.

To get a larger sample size that could be used for a cleaner extraction the amounts of the analysis were doubled. Since the extraction was time consuming and due to the time frame of the project, the decision was made to cut down the amount of samples. The forty-four samples were simplified to twenty-one samples that still represented our variables. The list of these samples can be seen below.

Sample No.	Juice Type	Yeast	Brix	Fermaid K (g)
1	OC	D47	21	0.06
2	OC	X16	21	0.06
3	OC	X16	23	0.06
4	OC	X16	22	0.06
5	OC	X16	21	0.08
6	OC	X16	21	0.04
7	OC	none	21	0.06
8	NC	X16	22	0.08
9	NC	X16	22	0.04
10	NC	X16	22	0.06
11	NC	D47	22	0.06
12	NC	X16	21	0.06
13	NC	X16	23	0.06
14	NR	X16	21	0.06
15	NR	X16	22	0.06
16	NR	X16	23	0.06
17	NR	D47	22	0.06
18	NR	X16	22	0.04
19	NR	X16	22	0.08
20	Store C	n/a	n/a	n/a
21	Store R	n/a	n/a	n/a

Table 1: Second Extraction Wine Samples

In a centrifuge tube, 10 ml of wine, 10 ml of water, 4.5 grams salt, and 2.0 ml of dichloromethane were added. This was hand shaken for 20 minutes and then spun in the centrifuge at 3000 RPM for 20 minutes. After the extraction was completed a pipet was used to extract the dichloromethane phase. There was no set amount of analyte that was extracted. The largest amount of the dichloromethane phase was extracted without extracting any other phase. To assure that there was enough analyte and the GC sampler would be able to break the surface tension, an extra 0.2 ml of pure dichloromethane was added to each analyte sample. The samples were then run through the GC.

3.5.1 Gas Chromatography Mass Spectrometry

The GC mass spectrometry injection was run the same way as the previous MQP project. It was run using the AOC-20i auto sampler injector in splitless mode. This injected 0.5 µl of analyte into the injection port at 230°C. The carrier gas had a constant pressure of 80 kPa. Column oven had a temperature profile that began with a hold at 50°C for 2 minutes. It then ramped for 20 minutes from 10°C/min to 250°C, where it was held for 3 minutes. The mass spectrometer interface temperature was

set at 230 °C and the ion source was set at 200 °C. The detection window started at 3 minutes and ended at 25 minutes. The GC had a computer program along with it that was able to specify the most probable compound associated with each peak. Using this program we were also able to get details of the peaks for each run. A search was run looking at the details of sharp peaks with a slope of five thousand or greater. The retention time, start time, end time, area, area percent, height, height percent, and the name of the compound were specified.

4.0 Results

4.1 First Extraction

The results of the first extraction were good, but the sample was too small. The analyte was clear and the separation of all samples was relatively good. The large issue for this extraction was the amount of analyte, or the dichloromethane phase. There was not enough analyte to be extracted and transferred to the vial. This resulted in a difficult extraction causing some of the other phases, including the gel phase to be pipetted into the vial along with the dichloromethane phase. This could not be run through the GC because it would be hazardous to the coil. The attempt to filter the analyte resulted in failure and the samples could not be used.

4.2 Second Extraction

The second extraction had better results. However, only eight out of the twenty-one samples were usable. The extraction of these eight samples was clean, clear and large enough to pipette. Six of the eight samples were the frozen chardonnay juice. These samples were numbered eight to thirteen. As previously stated and as seen in Table 1, they represent different yeasts, sugar contents, and Fermaid K additions. The other two samples of the successful eight were from the store bought wine which was used as a comparison. These were sample numbers twenty and twenty-one, as seen in Table 1 above. The extraction of the other ten samples out of the eighteen were unsuccessful and could not be used. These thirteen samples did not have a distinguishable dichloromethane phase and instead seemed as though it had an increase in the amount of gel phase. Due to the formation of the gel phase and the lack of the liquid dichloromethane phase, these samples could not be used and were not run through the GC.

4.3 GC Mass Spec

After running the samples through the GC and getting the results, the area percent of the six largest peaks were compared to that of the constants. Vials 8-13 had different variables, but were all wine from the frozen chardonnay. The results from these vials were compared to the results of vial 20, which was the sample of chardonnay from the store bought wine. The six peaks that had the largest area percent were compared across the different GC run results. As seen below in the GC run result from vial 11, there are six large peaks at approximately 4, 9.5, 20, 21.5, 21.75, and 22.

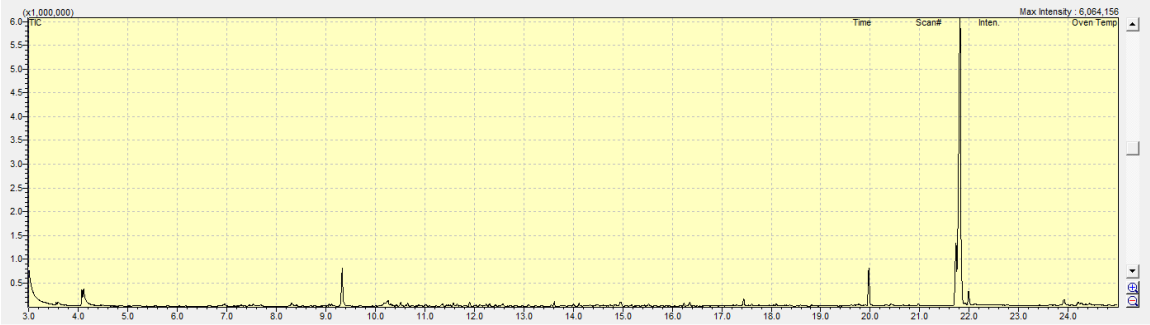

Figure 1: Vial 11 GC Run Result

After getting the details of the run and looking at the start time along with the most probable compound, the compounds and the start time associated with these peaks were determined. This can be seen in Table 2.

Compound	Start Time
Propanoic Acid	4.05, 4.09
Phenylethyl Alcohol	9.3
Hexadecanoic acid	19.93
9,12-Octadecadienoic acid	21.68
9-Octadecenoic acid (Z)-	21.75
Methyl stearate	21.99

Table 2: Six Largest Peaks with Compound Name and Start Time

It was discovered that for propanoic acid there were two distinct peaks very close together. The first start time is approximately 4.05 and the second is at 4.09. For comparison purposes the area percent of the two propanoic peaks were added together to form a total propanoic peak area percent. The comparison can be seen below in Table 3.

Vial No.	Propanoic Acid	Phenylethyl Alcohol	Hexadecanoic acid	9,12-Octadecadienoic acid	9-Octadecenoic acid (Z)-	Methyl stearate
8	9.8	16.72	6.29	3.74	59.98	n/a
9	4.63	6.84	11.84	7.24	61.99	3.81
10	7.11	10.05	3.56	2.63	44.24	1.58
11	5.11	6.13	4.86	10.63	68.44	2.12
12	5.34	7.44	5.09	4.32	64.26	2.55
13	6.24	7.27	6.33	4.12	60.03	2.6
20	n/a	18.96	4.3	6.37	56.24	2.11
21	n/a	4.97	6.36	9.42	69.53	2.82

Table 3: Comparison of the Six Largest Peaks

After looking at the results, it was noted that only vials 8-13 have peaks around 4.05 and 4.09 for propanoic acid. The constants, vials 20 and 21, do not have peaks at that time with a large enough slope. For the comparison of propanoic acid, it was thought that because the store bought wine did not have a peak with a slope as large as that of the experimental wine, the smallest area percent would be considered more like that of the store bought wine. After comparing the area percent of the different wines, vial 9 has the smallest area percent, which is most like vial 20. From the results, it can be seen that the addition of more Fermaid K resulted in a larger area percent of propanoic acid. This can be seen when comparing vials 8, 9, and 10. Vial 10 has the largest addition of Fermaid K and vial 9 has the smallest Fermaid K addition. No correlation is seen for sugar content.

All vials have a peak around 9.3 for phenylethyl alcohol. The area percent for phenylethyl alcohol varies greatly between the two constants, the store bought Chardonnay and the store bought Riesling. Vial 20 (Chardonnay) has an area percent of 18.96 and vial 21 (Riesling) has an area percent of 4.97. Vial 8, with the largest amount of Fermaid K, and vial 10, the normal sample, have a large area percent at 16.7 and 10.0 accordingly. Vials 9, 11, 12, and 13 have a smaller area percent of 6.8, 6.13, 7.44, and 7.27. For phenylethyl alcohol, vial 8 was closest to vial 20 and therefore is considered most like the store bought wine for this peak. Based on the results, it seems that there is a correlation for all the samples between the Fermaid K amount and the area percent of phenylethyl alcohol except for vial 11, which has a different yeast. The more Fermaid K, the higher area percent of phenylethyl alcohol. This can be seen specifically in vials 8, 9, and 10. There is no correlation seen for sugar content.

A peak for hexadecanoic acid is seen in all the samples at approximately 19.93. The area percent for most of the samples are relatively similar except for vial 9, which is much larger than all of the other samples. The area percent for vial 9 is 11.84 and the next largest area percent is vial 21 with an area percent of 6.36. It may be worth noting that vial 9 has double the amount of hexadecanoic acid than the next largest amount. This may be because vial 9 had the smallest amount of Fermaid K. However, vial 8, which has the largest amount of Fermaid K, also has a relatively large amount of hexadecanoic acid with an area percent of 6.29. Therefore, it cannot be stated that Fermaid K directly affects hexadecanoic acid. There is no correlation between the area percent and sugar content for hexadecanoic acid. Vial 11, with yeast D47, has the area percent closest to vial 20, and is considered the most like store bought wine for this peak.

All samples have a peak at approximately 21.68 for 9,12-Octadecadienoic acid. Out of the variable samples, vial 11 with yeast D47, has a much larger area percent. There is no trend seen for Fermaid K additions or sugar content for 9,12-Octadecadienoic acid. Vial 9, with the smallest Fermaid K addition has the area percent closest to that of vial 20. It is worth noting that there were no other samples that came close to the area percent of vial 20.

There is another peak very close to the 9,12-Octadecadienoic acid peak around. The second peak is at approximately 21.75 and most likely 9-Octadecenoic acid (Z). After looking at the area percent for vials 8, 9, and 10 it can be seen that there is no relationship between the area percent of 9-Octadecenoic acid (Z) and the amount of Fermaid K added. Sugar content does not show a trend for this peak either. After comparing the percent area of all peaks, it was seen that vial 8, which had the largest addition of Fermaid K, had the area percent most similar to that of vial 20.

The last of the six peaks was seen in all samples except for vial 8. The peak was approximately at time 21.99 and the most probable compound is methyl stearate. After looking at vial 8, 9, and 10 there is a trend that with increasing Fermaid K additions, the area percent of methyl stearate decreases. No relationship was seen with sugar content. Vial 11, with D47 yeast, was closest to vial 20 and therefore is the most like the store bought wine for this peak.

5.0 Conclusions

In conclusion, one wine sample does not stand out against the rest. Vials 8, 9 and 11 appear to be more like the store bought Chardonnay than the other samples. Although there is not one conclusive sample that is better than the rest, there are various relationships between the samples and the peaks from the analysis that are worth noting. For example as seen in the results section, it can be noted that the addition of more Fermaid K resulted in a larger area percent of propanoic acid. In addition, the more Fermaid K added, the higher area percent of phenylethyl alcohol. There is also a trend in the analysis results that the more Fermaid K added, the area percent of methyl stearate decreased. The best results we were able to observe and make note of involved Chardonnay and different Fermaid K additions. Since vial 11 contained high amounts of 9,12-Octadecadienoic and 9-Octadecenoic acids, and acids result in dryness, it can be concluded that vial 11 will be a dry wine. This could be a result of the different yeast, D-47, which was used in vial 11. It could also be concluded that since Fermaid K had an effect on the amount of Propanoic Acid, it could be manipulated to alter the dryness of the wine. Likewise, alcohols are thought to contribute to the sweetness of wine. Since vials 8 and 10 had a high amount of Phenylethyl Alcohol, these would be sweeter wines. Therefore it could be concluded that since Fermaid K had an effect on Phenylethyl Alcohol, it again could be manipulated to alter the sweetness of the wine. In addition, all samples had very low ester concentrations which is important to note because this results in a bitter wine. We were unable to determine anything conclusive from the Riesling juice because not enough of the dichloromethane phase was able to be extracted to run through the GC for analysis. In addition, no affirmative results or conclusions could be made for the sugar content in the Chardonnay juice. Further research, comparisons, and possible taste testing should be done to obtain more conclusive results.

6.0 Recommendations

After completing this project, the team has a few recommendations for what could be done differently and considerations for future projects. The team encountered several obstacles throughout the duration of this project, and had to adjust accordingly. The first obstacle encountered was that of the juice provided from Zoll Cellars to test had already begun to ferment before the team began to adjust the components for testing. The team came up with two solutions to this problem. Sugar was added to the already fermented juice to bring the brix back up to 21. Additionally, fresh juice from another vender was ordered to do further experiments with. In the future, we would suggest freezing the initial juice given from Zoll Cellars to prevent initial fermenting before experimentation can get started. We also recommend having more juice on hand in case of errors such as this.

Another obstacle the team encountered was regarding the extraction done prior to the analysis. When the samples were done fermenting, they were ready to be extracted and analyzed. To analyze the samples, the team used the Gas Chromatography Mass Spectrometry analysis method. As stated previously, the sample, water, salt, and dichloromethane were all added into a tube, which was then shaken. The sample was also run through the centrifuge to further shake and separate the tube into phases. After completing all of these steps, there was supposed to be a separation between the dichloromethane phase and a solid phase. The dichloromethane phase was then to be extracted and put into a new vial, which was to be run through the GC and analyzed. However, a strange gel formed on top of most of the samples. The team was unable to determine what this gel was and why it formed. There also wasn't enough of the dichloromethane phase to extract to run through the GC, so the team decided to repeat this part of the project only doubling the amount of the wine sample, salt, water, and dichloromethane. The intent of this was to get more pure sample to extract without any solid. However, we ran into the same issues as before. In many cases, there wasn't enough of the dichloromethane phase to extract. After the first extraction failed with 44 samples, the team narrowed the second extraction down to 21 samples. Out of 21 total samples, the team was only able to extract and analyze 8 samples. For these reasons, the team would suggest finding an alternate way to analyze the samples since we encountered so many obstacles with this specific part of the project. A more effective analysis method would make the project run more smoothly, and also produce better results. However, if the GC is still used we would suggest using or obtaining a new centrifuge machine since the one in the lab is outdated and there was trouble running it and getting the samples in and out of it. We would also suggest finding a way to determine what the gel is that formed on the top of the samples as well as find a way to eliminate it. In addition, make sure the samples are pure enough for extraction so they can in turn be analyzed. A way to do this could be more precise measuring of the components that go into the wine samples, as well as use juice that has not already been fermented.

The team also recommends comparing the final results to additional wines on the market. This could be wine similar to Zoll Cellar's, different wines, or even Zoll Cellar's wine itself. That way it can be better gauged how similar or different the wine samples are to the sponsor's wines. This could help in the final stages of the project when the samples need to be analyzed. We would also recommend getting a more precise scale for measuring out the yeast, Fermaid K, and sugar that needs to go into the wine sample. That way there is a smaller margin of error with measuring the elements going into the sample. Finally, we suggest further fermenting on a larger scale so the wine samples can be taste tested. This could be another way for the wine samples to be differentiated, and they could be separated based on taste.

Finally, since Frank Zoll is looking for a dry Chardonnay the team recommends using D-47 yeast and experimenting with different additions of Fermaid K to balance sweetness and dryness. We recommend that another MQP team uses D-47 yeast and varies Fermaid K additions to confirm and perfect this theory. We also recommend further taste tasting to establish a flavor profile for each sample of wine. The future team could also experiment with this theory to see if the same conclusions can be applied to Riesling wine.

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8.0 Appendix

8.1 Detailed GC Results

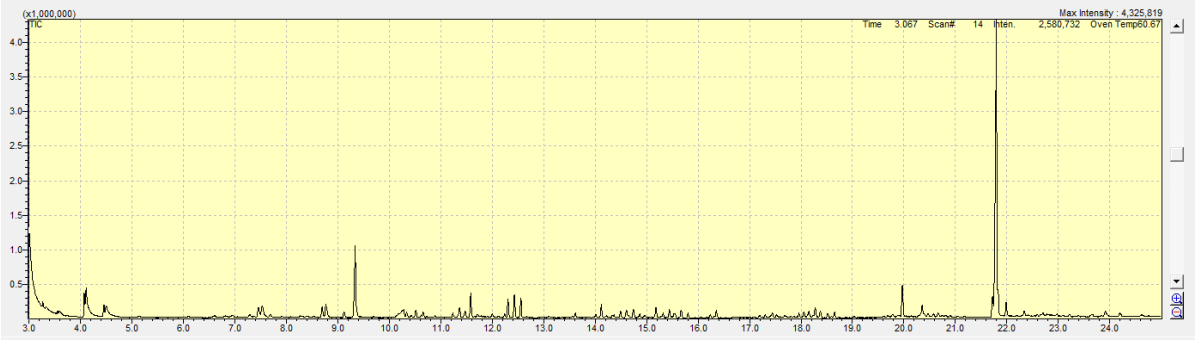
8.1.1 Vial 8

Retention time	Start time	End time	Area	Area %	Height	Height %	Name
4.063	4.04	4.08	264808	3.72	195688	5.35	Propanoic acid, 2-hydroxy-, ethyl ester, (S)-
4.099	4.08	4.16	432539	6.08	184429	5.04	Propanoic acid, 2-hydroxy-, ethyl ester, (S)-
9.321	9.275	9.395	1189923	16.72	588687	16.08	Phenylethyl Alcohol
16.352	16.325	16.39	120682	1.7	74236	2.03	Caryophyllene oxide
17.443	17.415	17.475	126281	1.77	76565	2.09	Heptadecane
19.967	19.935	20.015	447375	6.29	248240	6.78	Hexadecanoic acid, methyl ester
21.715	21.685	21.74	266266	3.74	130491	3.56	9,12-Octadecadienoic acid, methyl ester
21.778	21.74	21.87	4268581	59.98	2162605	59.07	9-Octadecenoic acid (Z)-, methyl ester

8.1.2 Vial 9

Retention time	Start time	End time	Area	Area %	Height	Height %	Name
4.078	4.055	4.095	423867	1.65	298470	2.73	Propanoic acid, 2-hydroxy-, ethyl ester, (S)-
4.112	4.095	4.205	765377	2.98	287664	2.63	Propanoic acid, 2-hydroxy-, ethyl ester, (S)-
9.123	9.095	9.155	98495	0.38	67436	0.62	Nonanal
9.337	9.285	9.41	1755209	6.84	870658	7.95	Phenylethyl Alcohol
11.91	11.885	11.94	114638	0.45	77372	0.71	Pentadecane
13.619	13.59	13.65	152471	0.59	103829	0.95	Tetradecane
14.121	14.09	14.155	210797	0.82	138205	1.26	Nonanoic acid, 9-oxo-, methyl ester
16.359	16.33	16.395	166956	0.65	104218	0.95	Caryophyllene oxide
17.446	17.415	17.48	195555	0.76	126890	1.16	Heptadecane
19.979	19.93	20.03	3037402	11.84	1791950	16.37	Hexadecanoic acid, methyl ester
21.729	21.68	21.75	1856765	7.24	873203	7.98	9,12-Octadecadienoic acid, methyl ester
21.81	21.75	21.9	15899539	61.99	5683320	51.9	9-Octadecenoic acid (Z)-, methyl ester
21.992	21.9	22.05	976209	3.81	524589	4.79	Methyl stearate

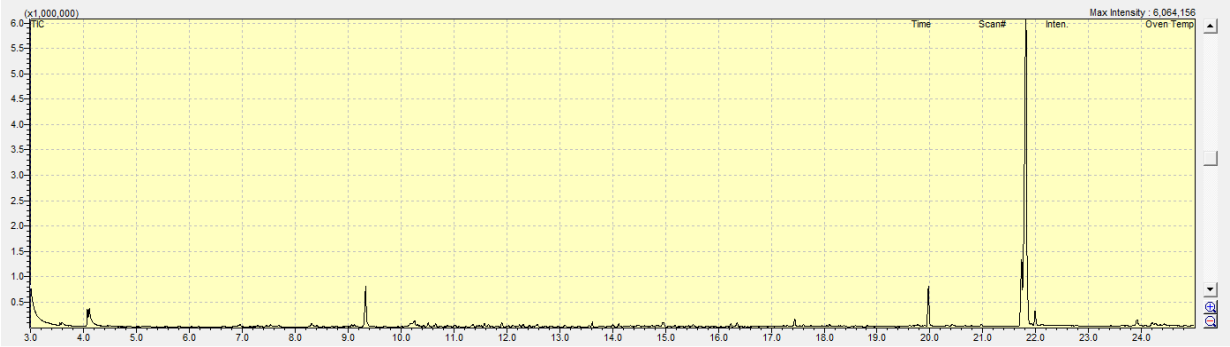
8.1.3 Vial 10



Retention time	Start time	End time	Area	Area %	Height	Height %	Name
3.273	3.25	3.305	88136	0.42	72257	0.68	Heptane, 4-methyl-
4.077	4.05	4.09	432481	2.05	332063	3.12	Propanoic acid, 2-hydroxy-, ethyl ester
4.112	4.09	4.205	1065300	5.06	391273	3.67	Propanoic acid, 2-hydroxy-, ethyl ester, (S)-
4.457	4.435	4.475	168395	0.8	127562	1.2	2,4-Dimethyl-1-heptene
7.463	7.42	7.5	328980	1.56	129629	1.22	Decane, 2,3,4-trimethyl-
7.534	7.5	7.595	420123	2	153044	1.44	Decane, 2,3,4-trimethyl-
8.697	8.66	8.735	311522	1.48	152830	1.43	1-Decene, 2,4-dimethyl-
8.77	8.735	8.83	429406	2.04	176913	1.66	1-Decene, 2,4-dimethyl-
9.34	9.29	9.41	2114670	10.05	1035230	9.72	Phenylethyl Alcohol
10.518	10.49	10.55	154982	0.74	101384	0.95	Propanoic acid, 2-(methoxymethoxy)-
11.367	11.34	11.405	182043	0.86	124020	1.16	Dodecane, 4,6-dimethyl-
11.583	11.545	11.625	573623	2.73	344478	3.23	Benzene, 1,3-bis(1,1-dimethylethyl)-
12.307	12.275	12.34	393805	1.87	259125	2.43	11-Methyldodecanol
12.433	12.34	12.475	587580	2.79	324113	3.04	2-Isopropyl-5-methyl-1-heptanol

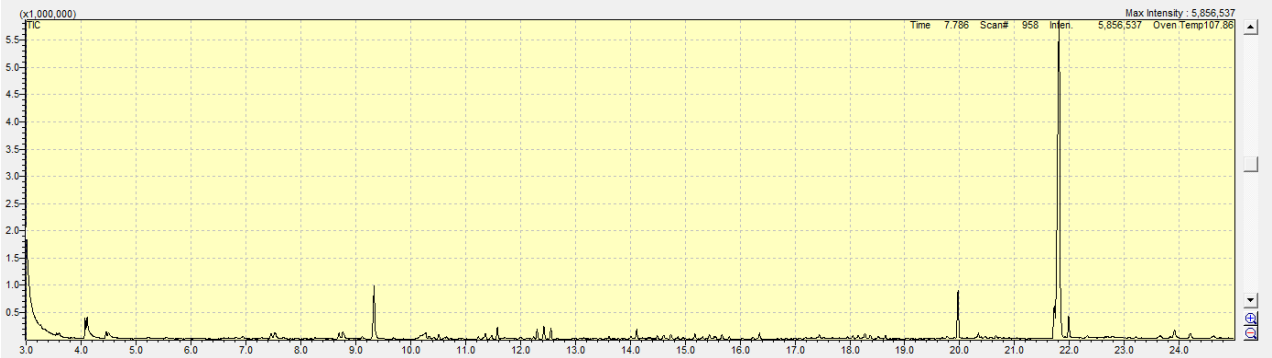
12.558	12.475	12.595	436883	2.08	279142	2.62	2-Isopropyl-5-methyl-1-heptanol
14.121	14.09	14.16	305112	1.45	191031	1.79	Nonanoic acid, 9-oxo-, methyl ester
14.495	14.465	14.525	129112	0.61	86126	0.81	Eicosane
14.605	14.525	14.72	252182	1.2	99155	0.93	Hexadecane, 2,6,11,15-tetramethyl-
14.743	14.72	14.79	212573	1.01	121316	1.14	Hexadecane, 2,6,11,15-tetramethyl-
15.183	15.15	15.22	221994	1.05	131411	1.23	Phenol, 2,4-bis(1,1-dimethylethyl)-
15.444	15.415	15.475	165223	0.79	111915	1.05	1-Dodecanol, 2-hexyl-
15.675	15.645	15.71	174744	0.83	103741	0.97	1-Dodecanol, 2-hexyl-
15.809	15.78	15.84	115459	0.55	72596	0.68	1-Dodecanol, 2-hexyl-
16.357	16.325	16.395	162359	0.77	99755	0.94	Caryophyllene oxide
18.277	18.245	18.31	206987	0.98	120931	1.14	1-Dodecanol, 2-hexyl-
18.375	18.345	18.415	158348	0.75	84584	0.79	1-Dodecanol, 2-octyl-
18.65	18.62	18.685	125508	0.6	78723	0.74	1-Dodecanol, 2-hexyl-
19.969	19.935	20.015	750160	3.56	452015	4.24	Hexadecanoic acid, methyl ester
20.357	20.325	20.38	183766	0.87	119503	1.12	Pentadecanoic acid
21.72	21.68	21.74	554294	2.63	295286	2.77	9,12-Octadecadienoic acid, methyl ester
21.793	21.74	21.875	9307705	44.24	4286789	40.27	9-Octadecenoic acid (Z)-, methyl ester
21.987	21.95	22.025	332134	1.58	193779	1.82	Methyl stearate

8.1.4 Vial 11



Retention time	Start time	End time	Area	Area %	Height	Height %	Name
4.077	4.05	4.09	430336	1.62	327185	3.19	Propanoic acid, 2-hydroxy-, ethyl ester
4.111	4.09	4.205	925151	3.49	337459	3.29	Propanoic acid, 2-hydroxy-, ethyl ester, (S)-
9.335	9.285	9.405	1624280	6.13	794744	7.75	Phenylethyl Alcohol
11.908	11.88	11.94	127238	0.48	80456	0.78	Tetradecane
13.618	13.59	13.65	142786	0.54	91087	0.89	Tetradecane
17.444	17.415	17.48	203338	0.77	131197	1.28	Heptadecane
19.97	19.93	20.02	1286673	4.86	775506	7.56	Hexadecanoic acid, methyl ester
21.73	21.675	21.75	2815310	10.63	1305904	12.73	9,12-Octadecadienoic acid, methyl ester
21.814	21.75	21.895	18134478	68.44	6027900	58.75	9-Octadecenoic acid (Z)-, methyl ester
21.988	21.895	22.035	562242	2.12	277609	2.71	Methyl stearate
23.912	23.875	23.955	242819	0.92	109612	1.07	cis-11-Eicosenoic acid, methyl ester

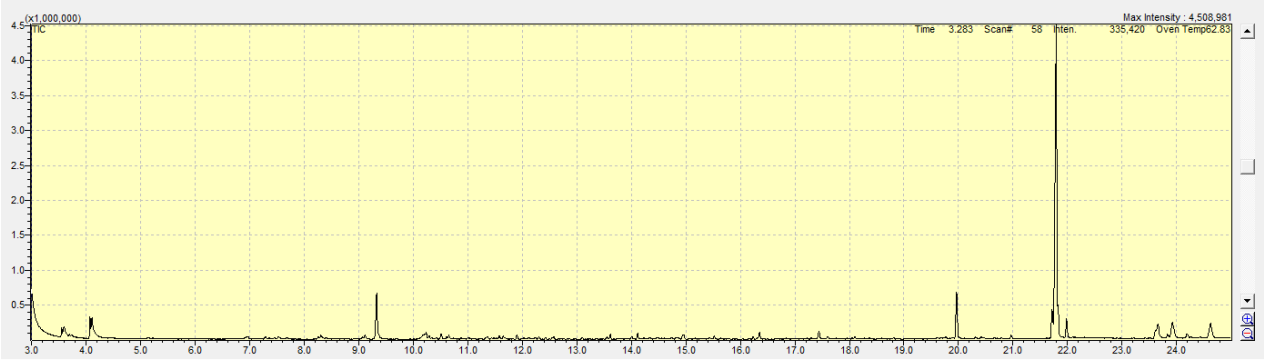
8.1.5 Vial 12



Retention time	Start time	End time	Area	Area %	Height	Height %	Name
4.075	4.05	4.09	486047	1.76	369275	3.29	Propanoic acid, 2-hydroxy-, ethyl ester
4.11	4.09	4.21	988199	3.58	376802	3.36	Propanoic acid, 2-hydroxy-, ethyl ester, (S)-
4.455	4.43	4.475	123387	0.45	98880	0.88	2,4-Dimethyl-1-heptene
7.45	7.42	7.505	161695	0.59	70956	0.63	Decane, 2,3,4-trimethyl-
8.695	8.665	8.73	170989	0.62	98918	0.88	1-Decene, 2,4-dimethyl-
9.338	9.285	9.41	2053147	7.44	972124	8.66	Phenylethyl Alcohol
10.516	10.49	10.55	124194	0.45	82411	0.73	Propanoic acid, 2-(methoxymethoxy)-
11.365	11.34	11.4	113576	0.41	83983	0.75	Dodecane, 4,6-dimethyl-
11.58	11.545	11.62	344836	1.25	218646	1.95	Benzene, 1,3-bis(1,1-dimethylethyl)-
12.304	12.27	12.34	265517	0.96	178642	1.59	2-Isopropyl-5-methyl-1-heptanol
12.43	12.34	12.47	401733	1.46	227552	2.03	2-Isopropyl-5-methyl-1-heptanol
12.556	12.47	12.59	313751	1.14	198895	1.77	2-Isopropyl-5-methyl-1-heptanol
14.119	14.085	14.155	270908	0.98	173957	1.55	Nonanoic acid, 9-oxo-, methyl ester

14.741	14.715	14.78	132630	0.48	81039	0.72	Hexadecane, 2,6,11,15-tetramethyl-
16.355	16.325	16.39	154453	0.56	97599	0.87	Caryophyllene oxide
18.275	18.245	18.305	142730	0.52	84230	0.75	1-Dodecanol, 2-hexyl-
19.969	19.93	20.02	1403293	5.09	876182	7.81	Hexadecanoic acid, methyl ester
21.722	21.675	21.74	1191703	4.32	585084	5.21	9,12-Octadecadienoic acid, methyl ester
21.81	21.74	21.89	17726138	64.26	5823864	51.92	9-Octadecenoic acid (Z)-, methyl ester
21.986	21.89	22.03	702630	2.55	393943	3.51	Methyl stearate
23.911	23.875	23.97	310742	1.13	128412	1.14	cis-11-Eicosenoic acid, methyl ester

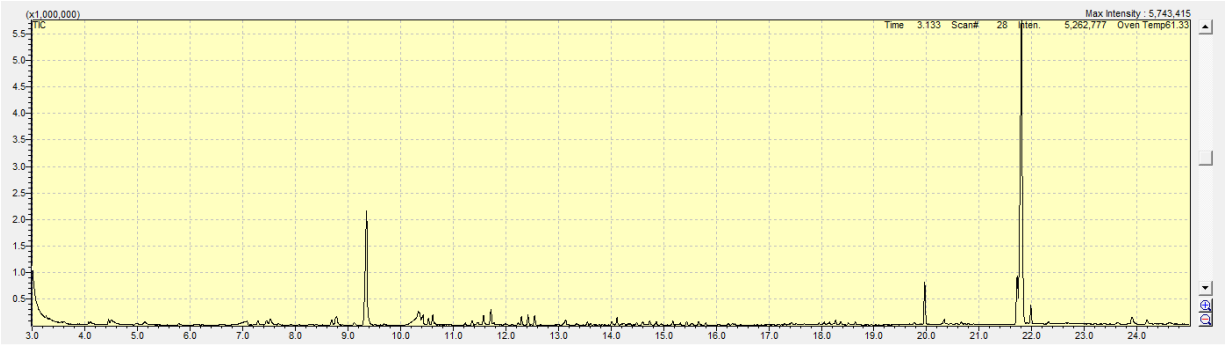
8.1.6 Vial 13



Retention time	Start time	End time	Area	Area %	Height	Height %	Name
3.555	3.54	3.57	100050	0.56	99933	1.24	2,3-Butanediol, [R-(R*,R*)]-
4.075	4.05	4.09	396450	2.2	307583	3.82	Propanoic acid, 2-hydroxy-, ethyl ester
4.11	4.09	4.2	728081	4.04	275922	3.42	Propanoic acid, 2-hydroxy-, ethyl ester, (S)-
9.331	9.285	9.4	1308912	7.27	642096	7.97	Phenylethyl Alcohol

14.116	14.09	14.145	114269	0.63	75530	0.94	Nonanoic acid, 9-oxo-, methyl ester
16.353	16.325	16.39	136279	0.76	82773	1.03	Caryophyllene oxide
17.441	17.41	17.475	160361	0.89	101466	1.26	Heptadecane
19.966	19.925	20.015	1139714	6.33	654438	8.12	Hexadecanoic acid, methyl ester
21.716	21.68	21.735	741978	4.12	402143	4.99	9,12-Octadecadienoic acid, methyl ester
21.793	21.735	21.875	10808552	60.03	4467287	55.44	9-Octadecenoic acid (Z)-, methyl ester
21.982	21.945	22.025	468691	2.6	271897	3.37	Methyl stearate
23.61	23.58	23.635	246726	1.37	96332	1.2	(R)-(-)-14-Methyl-8-hexadecyn-1-ol
23.657	23.635	23.705	400441	2.22	186973	2.32	Oxiraneoctanoic acid, 3-octyl-, methyl ester, cis-
23.923	23.875	23.995	684132	3.8	210573	2.61	Oxiraneoctanoic acid, 3-octyl-, methyl ester, cis-
24.623	24.57	24.685	573195	3.18	182714	2.27	2-Butyl-3-methyl-5-(2-methylprop-2-enyl)cyclohexanone

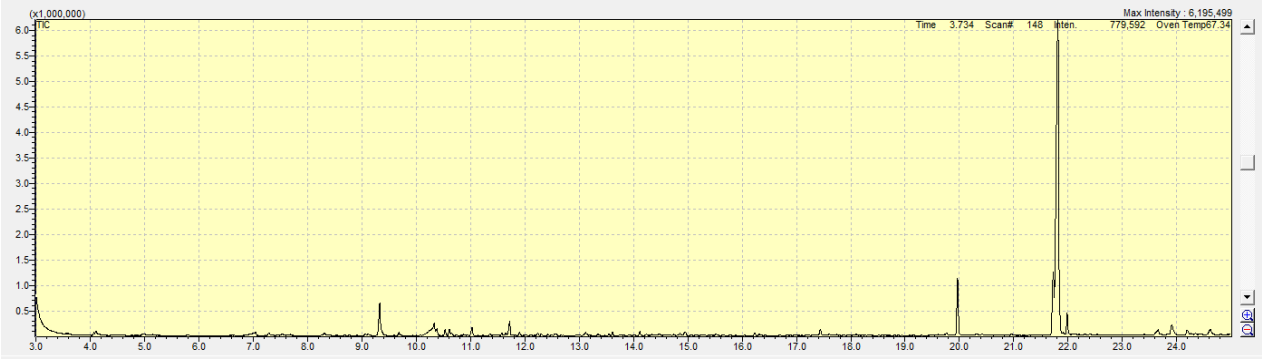
8.1.7 Vial 20



Retention time	Start time	End time	Area	Area %	Height	Height %	Name
4.453	4.425	4.475	110038	0.37	84175	0.71	2,4-Dimethyl-1-heptene

8.685	8.66	8.745	187310	0.63	74178	0.63	1-Decene, 2,4-dimethyl-
8.783	8.745	8.84	450111	1.52	157474	1.33	Benzaldehyde, 3-methyl-
9.358	9.28	9.435	5627132	18.96	2129447	17.97	Phenylethyl Alcohol
10.341	10.305	10.385	417137	1.41	147864	1.25	Butanedioic acid, diethyl ester
10.534	10.505	10.57	204548	0.69	125869	1.06	Ethanol, 1-(2-butoxyethoxy)-
10.613	10.58	10.64	253174	0.85	171653	1.45	Octanoic acid, ethyl ester
11.361	11.335	11.395	123189	0.42	79576	0.67	Dodecane, 4,6-dimethyl-
11.577	11.545	11.63	324731	1.09	176072	1.49	Benzene, 1,3-bis(1,1-dimethylethyl)-
11.719	11.67	11.755	454612	1.53	277555	2.34	Butanedioic acid, hydroxy-, diethyl ester, (.+/-.)-
12.301	12.27	12.335	229814	0.77	154676	1.31	11-Methyldodecanol
12.426	12.385	12.465	345602	1.16	192565	1.63	2-Isopropyl-5-methyl-1-heptanol
12.551	12.515	12.585	260076	0.88	171202	1.44	2-Isopropyl-5-methyl-1-heptanol
14.115	14.085	14.15	207614	0.7	136476	1.15	Nonanoic acid, 9-oxo-, methyl ester
19.964	19.925	20.01	1275388	4.3	795176	6.71	Hexadecanoic acid, methyl ester
21.721	21.67	21.74	1890750	6.37	909560	7.68	9,12-Octadecadienoic acid, methyl ester
21.805	21.74	21.885	16683715	56.24	5713051	48.21	9-Octadecenoic acid (Z)-, methyl ester
21.981	21.885	22.025	626351	2.11	352223	2.97	Methyl stearate

8.1.8 Vial 21



Retention time	Start time	End time	Area	Area %	Height	Height %	Name
9.328	9.28	9.42	1442162	4.97	623644	5.86	Phenylethyl Alcohol
10.332	10.305	10.36	167836	0.58	127745	1.2	Butanedioic acid, diethyl ester
10.532	10.5	10.57	203846	0.7	121467	1.14	Ethanol, 1-(2-butoxyethoxy)-
10.612	10.585	10.635	140418	0.48	98198	0.92	Octanoic acid, ethyl ester
11.027	10.99	11.065	280869	0.97	152682	1.43	Benzaldehyde, 2,4-dimethyl-
11.718	11.68	11.755	401228	1.38	253278	2.38	Butanedioic acid, hydroxy-, diethyl ester, (.+/-.)-
14.113	14.085	14.145	112449	0.39	72260	0.68	Nonanoic acid, 9-oxo-, methyl ester
17.44	17.41	17.47	165077	0.57	108851	1.02	Heptadecane
19.967	19.925	20.015	1844651	6.36	1097324	10.3	Hexadecanoic acid, methyl ester
21.725	21.67	21.745	2730529	9.42	1236024	11.6	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
21.813	21.745	21.89	20154950	69.53	6164955	57.89	9-Octadecenoic acid (Z)-, methyl ester
21.983	21.89	22.035	817457	2.82	420883	3.95	Methyl stearate
23.909	23.865	23.985	531350	1.83	173580	1.63	cis-11-Eicosenoic acid, methyl ester