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Flavor Compound and Compositional Analysis of Wine

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Flavor Compound and Compositional Analysis of Wine

A Major Qualifying Project
Submitted to the Faculty of
Worcester Polytechnic Institute
In partial fulfillment of the requirements for the Bachelor of Science Degree in Chemical Engineering

By

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Benjamin List

Date:
April 15, 2015

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Professor Stephen J. Kmiotek, Project Advisor
Abstract

Flavor compounds represent a small amount of the total composition of wine. Over 1000 compounds make up wine flavor. The goal of this project was to find a method to analyze the detection limits of these compounds on a GC-MS as well as a method to concentrate these compounds and remove water from the wine samples. A sensitivity analysis was performed on a GC-MS instrument with 8 different flavor compounds. The detection limit of the GC-MS instrumentation was found to exist between 10 mg/L and 0.1 mg/L concentration of the flavor compounds tested; however some compounds encountered solubility and testing issues. Further investigation is required to increase the accuracy of the data presented in this report and to investigate potential flavor concentration and water removal techniques.
Acknowledgements

We would like to thank, first and foremost, our advisor Stephen Kmiotek for his continuous support. Thank you for putting up with us as we come by your office with new ideas to bounce around and problems to ask for help with every day and for all your patience with us and all of the advice that you gave us on everything.

We would also like to thank Professor Timko for all of his help with our project. Thank you for all the great ideas you provided us with, help you gave us with understanding the GC-MS and separations, and all of the general support throughout the latter part of our project. We owe a big thank you to the graduate student who helped us with our initial understanding of the GC-MS procedure, analysis, and all the support he provided us with in the lab, so thank you Alex Maag.

Thank you to our amazing Chemical Engineering Department for all they do and the constant support. Thank you to Felicia and Tiffany for making sure we always received everything we needed in a timely manner. Finally, thank you to everyone that supported us along the way.
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1. Introduction

As a society, we understand to a very general degree that if we change parts of the wine process, the resulting wine will have different flavors. The largely surprising fact however, is that we do not truly understand the chemical makeup of wine or how these process changes will affect it. The changes to the process are made without truly understanding the impact it will have on the chemical or flavor profiles. We change the grape, the yeast strain, process durations, and process conditions and hope that the resulting wine is better. This trial and error methodology is a way that a lot of wineries, especially the smaller ones, produce new wines. Even the best practices that are generally accepted are rooted in a tradition based on trial and error rather than scientifically figuring out the best way to produce a certain wine with a certain flavor.

This begs the question of how does one engineer a better wine? The first step to engineering a better wine requires an understanding of what wine is made of. We know that wine can be anywhere between 9 and 15% alcohol by volume. This is useful information, but it doesn’t provide a full picture of what wine really is. What are these flavor compounds and how many of them are there? Research has shown that there are over one thousand identified chemical compounds (Styger, 2011) and eight hundred aromatic compounds (Barbe, 2008) that make up the flavor and aroma of wine. There is the definite possibility that there are more compounds that are still yet to be identified, but for the purposes of this project, we will focus on the identified compounds.

The presence of all of these compounds does not mean that they all necessarily can be tasted by a human tongue. The goal then becomes being able to detect these compounds down to the levels that are noticeable to a human tongue. General taste thresholds for different types of compounds can be compared to the detection limits of a machine such as a gas chromatograph. If we want to make a better wine, we will have to understand what makes a good wine. This requires a more quantitative method for flavor profile analysis than the current qualitative methods used by sommeliers. While a sommelier will say a wine is fruity, nutty, earthy, and so on, it is hard to look at a wine and know what to add or adjust in the process to get a fruitier wine or a nuttier wine other than just changing grapes entirely.

The goal of this project is to determine suitable methods that will allow WPI students, and eventually others, to quantitatively analyze wines and their flavor profiles. This project does not seek to fully understand the flavor network of wine, but rather understand the potential components that can be used to start down the road of understanding. The road to understanding the vast flavor network of wine is long and will most likely take many projects to complete. With the minimal amount of research available on the many compounds that create wine’s unique flavor, the lack of information on the detection limits of the available gas chromatograph, and the challenge of running a water rich substance through a GC-MS system, these projects have many challenges to tackle.
2. **Background**

When trying to understand the flavor profile of wine it is important to understand the wine making process, the composition of wine, and methods that can be used to aid in the analysis. The wine making process is a very old process and relies mostly on tradition and trial and error than it does scientific research; this is especially true at smaller wineries. The full composition of wine is still at least partially shrouded by mystery and the impact of this composition on flavor is an enigma. The last major section of understanding required is knowledge of the tools and methods that can be incorporated in a lab setting to aid in the flavor analysis.

2.1 **The Wine Making Process**

The conversion of fruit to wine is a very old process. Wine has been an integral part in human culture for centuries. The process is often considered an art form, though it is founded in science. The actual process of winemaking is not the main focus of this research; however it does greatly affect the results of the compositional analysis that will be performed. The winemaking process has a few overarching steps: Growing, Fermenting, Clarification, and Aging (Jackisch, 1985). Each of these phases will have a different impact on flavor. The flavor changes will be due to different chemicals being present in the wine due to occurrences in these stages of the process. If one wants to eventually be able to use knowledge of the impact of certain compounds on flavor, they must understand which phase will produce that compound unless they want to artificially add the compound later. Manipulating these phases to produce a wine with the compounds that produce the desired flavor can result in a wine that has a better flavor that is closer to what is desired than in the trial and error methods currently used.

The first phase, growing, is essentially determined by biology. The grapes have to grow and ripen before they can be harvested to make wine. The quality of the grape here will largely determine the wine’s quality and the chemical composition of this grape will greatly impact the chemicals present in the final product (Jackisch, 1985). The acids and sugars present here will play a key role in determining the flavor of the final product. The environment the grape is grown in can play a large role in determining the final chemical composition of the grape. Grapes grown in cooler climates or climates with large temperature swings like New England will be very different from grapes grown in warmer and more consistent climates like California. (Mira de Orduña, 2010)

The second phase is fermentation in which microorganisms are used to breakdown the sugars into alcohol and carbon dioxide. The microbes that typically used for fermentation are yeast. This process allows for multiple different wines that vary in flavor, alcohol content, and sweetness to be made from the exact same batch of grapes (Jackisch, 1985). The formation of different flavor compounds depends more on the yeast strain used than the actual raw materials (Nykänen, 1986). The yeast strain plays a large role in determining the flavor, texture, aroma,
and body of the wine. For example, the yeast strain D-47 is associated with riper fruit flavors and creamier textures while RC 212 is associated with acidic and fruity flavors (Lagassey, 2014).

The third phase is clarification and is where pressing of the grapes occurs. This process involves removing stems and leaves and then crushing the grapes to get the fruit to contact the microbes. The physical separation process occurs during the press part of this phase. The grapes are pressed to separate them from the skins. This phase also includes the reduction of particulates through filtration and precipitation. Removing particulates will affect the final product’s flavor and composition.

The final phase is aging, where the wine is allowed to sit in a vessel, often times a wooden barrel, to contact with oxygen and develop its flavor profile. The oxygen will cause oxidation of different substances in the wine which can greatly alter flavor and composition. The chemicals in the wine can also interact with each other. If the wine is aged in a wooden barrel, some of the compounds in the wood can dissolve and change the flavor of the wine as well. After this stage, the wine is bottled and ready for consumption (Jackisch, 1985).

The product that comes from the aging stage is the wine that is of interest for this research. The impact of the individual stages on flavor and composition is not what is actually important, however the final composition is the main concern. Knowing how wine is made is crucial in predicting the types of compounds that will be present. Predicting the composition of wine will greatly impact the choices of how to extract the flavor compounds and analyze them. The overall determination of a method for compositional analysis of wine and its relation to flavor is the overall goal of this research. In order to properly design a method for compositional analysis, it is imperative to understand the different types of compounds that can be present in wine.

2.2 Flavor Composition of Wine

There have been over one thousand identified chemical compounds (Styger, 2011) and eight hundred aromatic compounds (Barbe, 2008) that make up the flavor and aroma of wine. All of these compounds interact with each other to create an overall flavor profile. The concentrations of these compounds range from the milligrams per liter to the nanogram per liter level (Barbe, 2008). Some of the common functional groups in the flavor compounds are esters, ketones, aldehydes, alcohols, aromatics, and glycerols (Styger, 2011). The sheer number of possible flavor compounds that exist in these minute quantities make analytically understanding the flavor profile of wine an extremely complex endeavor.

The overarching functional groups, such as ketones, aldehydes, and esters, have been correlated to certain wine flavors that tend to occur when those compounds are present. Volatile esters have been shown to be the main contributor to fruity aromas in wine (Lambrechts, 2000). Aldehydes have been shown to correlate to a floral and woody flavor and aroma in wine (Ubeda, 2000) (Regodón, 2006). While these general correlations between groups of compounds are helpful shortcuts, they do not make correlations between individual compounds and flavor.
There has been work done that has correlated chemical compounds to odor characteristics in wine. Two tables extracted from the Styger et al. paper that relate specific molecules and branched amino acids to odors in wine can be seen below. While the research on these compounds is useful, the number of molecules studied is quite small and there is little to no analysis done on the effect of concentration on the flavor or the possible interactions between compounds to affect flavor and odor. It is fairly well documented that our sense of smell can impact flavor so understanding odoristic compounds can be useful for understanding flavor to a degree as well. The two tables below, Table 1 and Table 2, were extracted from the Styger 2011 paper to show some examples of odor compounds in wine.

**Table 1: Odoristic Qualities of Molecules in Wine -- Extracted from Styger (Styger, 2011)**

<table>
<thead>
<tr>
<th>Odorant</th>
<th>Impact Compound</th>
<th>Cultivar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Floral</td>
<td>Linalool</td>
<td>Muscat</td>
</tr>
<tr>
<td>Citrus</td>
<td>Geraniol</td>
<td>Muscat</td>
</tr>
<tr>
<td>Citrus</td>
<td>Nerol</td>
<td>Muscat</td>
</tr>
<tr>
<td>Germanium Oil</td>
<td>Tetrahydro-4-methyl-2-(2-methyl-1-propenyl)-2,5-cis-2H-pyran (cis-Rose oxide)</td>
<td>Gewurztraminer</td>
</tr>
<tr>
<td>Kerosene</td>
<td>1,1,6-Trimethyl-1,2-dihydronaphthalene</td>
<td>Reisling</td>
</tr>
<tr>
<td>Bell Pepper</td>
<td>3-Isobutyl-2-methoxypyrazines</td>
<td>Sauvignon blanc</td>
</tr>
<tr>
<td>Coconut, woody</td>
<td>3,6-Dimethyl-3a,4,5,7a-tetrahydro-3H-1-benzofuran-2-one</td>
<td>Gewurztraminer</td>
</tr>
<tr>
<td>Black Currant</td>
<td>4-Methyl-4-mercaptopentan-2-one</td>
<td>Sauvignon blanc</td>
</tr>
<tr>
<td>Grapefruit</td>
<td>3-Mercapto-1-hexanol (R isomer)</td>
<td>Sauvignon blanc</td>
</tr>
<tr>
<td>Passion Fruit</td>
<td>3-Mercapto-1-hexanol (S isomer)</td>
<td>Semillon</td>
</tr>
<tr>
<td>Black Pepper</td>
<td>Rotundone</td>
<td>Shiraz</td>
</tr>
</tbody>
</table>
Table 2: Odoristic Qualities of Branched Amino-Acids in Wine -- Extracted from Styger (Styger, 2011)

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Compound</th>
<th>Concentration (mg/l)</th>
<th>Odor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucine</td>
<td>Isovaleraldehyde</td>
<td>Traces</td>
<td>Fruity, nut-like</td>
</tr>
<tr>
<td>Valine</td>
<td>Isovaleraldehyde</td>
<td>Traces</td>
<td>Slightly apple-like</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>2-Methylbutyraldehyde</td>
<td>NR</td>
<td>Green (herbaceous), malty</td>
</tr>
<tr>
<td>Valine</td>
<td>Isobutyric acid</td>
<td>Traces</td>
<td>Sweet, apple-like</td>
</tr>
<tr>
<td>Leucine</td>
<td>Isobutyric acid</td>
<td>&lt;3</td>
<td>Rancid, cheese, rotten fruit</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>2-Methylbutanoic acid</td>
<td>NR</td>
<td>Fruity, waxy, sweaty fatty acid</td>
</tr>
<tr>
<td>Leucine</td>
<td>Isoamyl alcohol</td>
<td>45-490</td>
<td>Alcohol</td>
</tr>
<tr>
<td>Valine</td>
<td>Isobutanol</td>
<td>40-140</td>
<td>Fruity, alcohol, solvent-like</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>Amyl alcohol (active)</td>
<td>15-150</td>
<td>Marzipan (almond)</td>
</tr>
<tr>
<td>Leucine</td>
<td>Isoamyl acetate</td>
<td>0.03-0.81</td>
<td>Banana, pear</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>2-Phenyl acetate</td>
<td>0.01-4.5</td>
<td>Rose, honey, flowery</td>
</tr>
<tr>
<td>Leucine</td>
<td>Ethyl isovalerate</td>
<td>0-0.07</td>
<td>Apple, fruity</td>
</tr>
<tr>
<td>Valine</td>
<td>Isobutyl acetate</td>
<td>0.01-0.08</td>
<td>Banana</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>Ethyl 2-methylbutanoate</td>
<td>0-0.09</td>
<td>Strawberry, pineapple</td>
</tr>
</tbody>
</table>

The general relationships of functional groups to flavor types as well as the link of certain chemical compounds to different aromas are just the tip of the iceberg when tackling the enormous feat of characterizing the chemical profile of wine. To truly understand this age old consumable, it is necessary to find direct flavor correlations. Previous experiments have shown the massive number of compounds present in wine. The main flavor contributors in wine represent only a small fraction of the total composition of wine. With all of these compounds present in wine, it is extremely difficult to directly correlate one flavor to one compound, especially when it is possible that interactions exist. When the compounds exist in such small quantities, how does one ensure that a lab instrument is seeing all of the compounds? Concentrating these flavors is one way to attempt to see all of the compounds, even the ones that are present in just trace amounts in the wine. The next challenge is to separate out the flavor
compounds from the water and ethanol. This sounds easy enough, however when it is necessary not to impact the flavor compounds or change them in any way, many of the traditional options are eliminated.

2.3 Reverse Osmosis

Reverse osmosis is the only filtration process to separate on the molecular level (Bergman, 2006). It uses a selectively permeable membrane to separate small molecules, typically water, from a solution (Bergman, 2006). The process results in two streams which are shown below in Figure 1: a permeate which is water-rich and non-permeate which is solute-rich. Its most common application is recovering the water-rich stream as in a desalination plant or in water purification.

![Figure 1: General Reverse Osmosis diagram](image)

Reverse osmosis can also be used to recover the solute-rich stream as a means to concentrate a solution. Reverse osmosis has already been used in the wine industry to dealcoholize wine. It’s used to dealcoholize wine over conventional distillation as it does not heat the wine and which can change the flavor compounds (Gil, 2013). For these reasons, reverse osmosis can be an effective tool to concentrate wine. By concentrating wine, the relative concentrations of the flavor compounds increases which makes for easier detection. The removal of water can also be important if the sample is to be run through devices that are incompatible with water, such as mass spectrometers. This method is useful in the analysis of wine for two major reasons; it extracts the water from the wine which allows for the use in a GC-MS as previously mentioned, but it also concentrates the flavor compounds which can artificially increase the detection limits on the GC-MS for these compounds.
2.4 Gas Chromatography

A Gas Chromatograph, usually referred to as a GC, is an instrument that performs a type of chromatography which uses a stationary phase and a mobile phase. The mobile phase in GC is made up of the vaporized sample and an inert carrier gas. The stationary phase is specific to each GC unit, but is usually solid. GC works due to the different rates at which species move through the stationary phase. This movement is related to the diffusivity of the particular species in the stationary phase. If a species readily diffuses into the stationary phase, it requires a higher residence time to pass through the column as it spends more time in the stationary phase. Conversely, if a species does not readily diffuse into the stationary phase, it will have a lower residence time as it just passes over the stationary phase without much transfer of the species into the stationary phase. The GC instrument can use a variety of detectors to determine the quantity of substance exiting the column. One popular detector is an FID, Flame Ionization Detector, which can measure the amount of carbon leaving the column, by burning the sample in a hydrogen flame and measuring the electric potential generated by the ionized gases. This method has limited applications as it destroys the sample in the combustion, but benefits from high sensitivity. Based on the difference in residence times, different compounds will leave at different points in time, this allows the detector to show the relative amounts of each component based on when they leave the column. The peaks that are obtained from a GC can then be integrated; each integral is then divided by the total integration area of the peaks to get a relative concentration. This type of detector will only work for carbon based compounds so the concentrations may not be 100% accurate if there are other nonorganic contaminant compounds in the solution.

Gas chromatographs can also come with an attached mass spectrometer; this is referred to as GC-MS. The effluent stream from the GC column is fed into a mass spectrometer (MS). This stream is given a charge and sped up through a magnetic field. The sample is then passed fired an electric field and detected on a plate. Depending on the position it contacts the plate, a mass to charge (m/z) ratio can be determined. The intensity of the detection in the plate is used to determine the peak height. The m/z value is run against a data base along with the retention time to match the sample to a known compound. While the mass spectrometer is a valuable tool for determination of compounds present in the solution, it does add some limitations to the instrument. The main issue presented by a mass spectrometer for wine analysis, is the inability to process water without damaging the column. Water can damage certain mass spectrometer columns due to the hygroscopic packing involved in the MS. This means that processing water can lead to damage to the mass spectrometer.

An important aspect for the purpose of wine flavor analysis with a GC-MS system is the detection limit of the instrument. The GC-MS has to be able to detect the compounds at least down to the same concentration that the human tongue’s sensory limit can. The level at which a compound can be considered detected is typically when the peak is 3 times the height of the noise, a 3 to 1 signal to noise ratio. These guidelines come from the International Conference on
Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) (ICH, 2005). This is based of guidelines used in the pharmaceutical industry for GC-MS.

2.5 Zeolites

Another potential way to filter water out of wine and concentrate flavor compounds is through the use of zeolites. Zeolites are a group of minerals that are made of aluminosillicate compounds of the IA and IIA group metals. The crystalline structure of zeolites is made up of tetrahedrally coordinated atoms that form subunits through the sharing of oxygen atoms. (Pálinkó, 2013) The way that these subunits arrange into a larger framework results in the formation of pores. These pores are crucial to the uses of zeolites. Zeolites can be used as molecular sieves to filter molecules of a certain size out of a solution. The main zeolite of interest in this project is Zeolite A. Zeolite A can be used to filter water out of solutions. This specific zeolite can be used to concentrate the wine flavors through the removal of water. Concentrating the flavor compounds allows for an artificial increase in detection limits on the GC-MS by making it easier for the instrument to detect, in a similar manner to the use of Reverse Osmosis. It can also reduce the risk of column damage from water by lessening how much water passes through the column. These are another potential way to extract water from the sample and concentrate the solution, however due to time constraints were not tested in this paper, but could serve as a potential resource for future testing.
3. Methodology

To determine a suitable method for the characterization of wine, we will investigate the viability of using GC-MS at neat concentrations. We will also be investigating means to concentrate the sample and mitigate issues with water content in samples for instrumentation. This methodology covers the steps that will be taken to investigate these aforementioned goals.

3.1 GC-MS Sensitivity Analysis of Wine Flavor Compounds

To determine the viability of using GC-MS (see Figure 2 below) for wine characterization, the detection limits of the instrument need to be comparable if not better to the sensory threshold of the compounds for the human tongue. Determining the exact sensory threshold and detection limit of each individual compound in wine would require extreme amounts of time. Instead, representative sample compounds were chosen from known compounds found in wine for varying size, carbon content, and functional groups. The list of compounds that were ordered for testing in this study and a short description of each compound is provided in Table 3 below. Only eight of these compounds were tested throughout the duration of our project.

Figure 2: GC-MS used for these analyses
Table 3: List of compounds that were ordered to be tested for detection limit

<table>
<thead>
<tr>
<th>Compound</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vanillin</td>
<td>Main compound in vanilla flavoring. C\textsubscript{8} compound with aldehyde group</td>
</tr>
<tr>
<td>Quercetin</td>
<td>C\textsubscript{15} compound with phenol functional groups</td>
</tr>
<tr>
<td>Isobutanol</td>
<td>By-product of yeast fermentation. C\textsubscript{4} alcohol.</td>
</tr>
<tr>
<td>Tartaric Acid</td>
<td>Antioxidant, gives a sour taste. C\textsubscript{4} carboxylic acid</td>
</tr>
<tr>
<td>Fructose</td>
<td>Common sugar found in fruit. C\textsubscript{6} sugar</td>
</tr>
<tr>
<td>Acetophenone</td>
<td>Ketone found in many foods. C\textsubscript{8} compound</td>
</tr>
<tr>
<td>Isoamyl Acetate</td>
<td>Banana flavoring. C\textsubscript{7} ester</td>
</tr>
<tr>
<td>Cirtric Acid</td>
<td>Acid found in many fruits, especially citrus. C\textsubscript{6} acid</td>
</tr>
<tr>
<td>Isoamyl Alcohol</td>
<td>Precursor to Isoamyl Acetate. C\textsubscript{5} alcohol</td>
</tr>
<tr>
<td>Glucose</td>
<td>Common sugar known as “grape sugar”. C\textsubscript{6} sugar</td>
</tr>
</tbody>
</table>

3.1.1 Sample Prep

Sample preparation is a crucial step in the process of forming a calibration curve and finding the detection limit of a compound. Knowing exactly the amount of the compound in solution is critical to a successful analysis. The GC-MS has to be able to detect the compounds at or below the sensory threshold for the human tongue for each compound. The sensory thresholds can vary between a few nanograms per liter to milligrams per liter depending on the compound (Polaskova). When aiming for concentrations this low, precision is key as one very small error in sample preparation can result in a fairly large difference in concentration from what is expected. The solutions are prepared using absolute, 200 proof, ethyl alcohol as a solvent. Ethyl alcohol is used as a solvent specifically because it is also found in fairly large quantities in wine. This means that any way that the GC-MS reading is impacted by the presence of the alcohol can be accounted for in our data.

The dilutions for each compound follow the dilution scheme set forth in Table 4 below. In order to accomplish these dilutions, an automatic micropipette is used to measure all of the liquid volumes. Each sample is stirred to ensure homogeneity. A small amount of each sample is removed and put into a vial for testing. Note that only samples 1 through 4 were used for GC-MS testing and sample 0 was used to make sample preparation easier and less prone to error in measuring of initial compound weight.
Table 4: Dilution Process for GC-MS Analysis

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Solute</th>
<th>Diluent</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.1 gram of compound</td>
<td>100 ml of ethyl alcohol</td>
<td>1 gram/liter</td>
</tr>
<tr>
<td>1</td>
<td>0.1 ml of Sample 0</td>
<td>100 ml of ethyl alcohol</td>
<td>1 mg/liter</td>
</tr>
<tr>
<td>2</td>
<td>0.1 ml of Sample 1</td>
<td>100 ml of ethyl alcohol</td>
<td>1 µg/liter</td>
</tr>
<tr>
<td>3</td>
<td>0.1 ml of Sample 2</td>
<td>10 ml of ethyl alcohol</td>
<td>10 ng/liter</td>
</tr>
<tr>
<td>4</td>
<td>0.1 ml of Sample 2</td>
<td>100 ml of ethyl alcohol</td>
<td>1 ng/l</td>
</tr>
</tbody>
</table>

Once these samples are obtained and labeled in vials, they must be submitted for GC-MS. Running the GC-MS in a time-efficient manner requires an estimation of residence time in the stationary phase of the GC. This allows for the total run time to be minimized for increased amount of tests and a higher level of convenience for others using the GC column. In order to determine the required run time, initial tests were run at a long enough test time to see anything that could possibly come out in the sample. Then, the run was stopped once the peaks for the sample were seen. The new test times for the actual sample tests were set to the retention time found in the initial test plus a 2 minute period of time to act as a safety margin. The GC-MS conditions are seen in Figure 3.

These samples represented the initial dilution scheme for the sensitivity analysis. As the tests were run and the relative detection limits were approached or surpassed for each compound, a secondary dilution scheme with higher concentrations was required. This scheme was incorporated into the testing due to the lack of detection of the compounds by the GC-MS at lower concentrations. The concentrations used in this new scheme were 500 mg/L, 10 mg/L and 100 µg/liter. The samples were created in the same manner as the first dilution but made with varying amounts of solvent in order to obtain the correct target concentrations.
3.2 Water Mitigation and Concentrating Methods

For the GC-MS in Goddard Hall, the mass spectrometer’s packing is hygroscopic and the performance of the instrument will decrease if water is present in the samples. The aforementioned sample preparation procedure limits the samples to 2 compounds, ethanol and a carbon based flavor compound. However, when moving beyond this phase of testing, actual wine will have to be analyzed. Wine is composed mostly of water which needs to be removed to run in this specific GC-MS. It may also be necessary to remove water for testing on any GC-MS, even without hygroscopic packing, just to concentrate the compounds to allow detection above the minimum GC-MS limit. Increasing composition in a known way by a known amount allows for a controlled means of artificially increasing detection limit of the GC-MS instrument. The following section will discuss two potential ways of removing water from the wine samples. This is not to say that these two methods are the only or the best ways to remove water from the samples, but they are the ones that were thought of and available for testing.

3.2.1 Reverse Osmosis

For reverse osmosis, there were two different experimental methods that we pursued to test the membrane. The first being in the wound capsule format that the membrane comes in when purchased. The other method that was explored was to unravel the membrane and cut single layer disks from it. In both cases, the initial feed wine was sampled for GC testing for a comparison basis.

3.2.1.1 Wound Capsule

For the wound capsule reverse osmosis tests, the membrane was used in its “as-purchased” housing. The capsule (see Figure 4 b below) involved several square feet of wound membrane. The membrane that was used for this testing was a DOW Filmtec RO membrane. An 80 psi diaphragm pump (see Figure 4 a below) was attached to the inlet side of the capsule to provide the pressure needed to overcome the osmotic barrier. Initially only tap water was run through the membrane to get an idea of flow rates and initial feasibility studies. Then, white wine, specifically NY Chardonnay and NY Riesling, were run through the membrane. Any effluents from both streams, filtrate and retentate, were tested on a GC to compare for differences.
Figure 4: a) 80 PSI diaphragm pump used for wound capsule testing and b) wound capsule membrane format

3.2.1.2 43 mm disks

The wound capsule was unraveled and disks with an approximate diameter of 43mm were cut out of the membrane. These disks were placed inside a tangential flow filter housing (see Figure 5 below) in which the water or wine would flow over the top and what passed through the membrane would exit underneath. The wine was filtered in a batch style setup. A small sample of wine was pulled into a syringe and used to prime the membrane housing with the wine. A second syringe full of wine is then extracted and attached to the inlet nozzle of the housing. The effluent nozzle is manually blocked to allow for pressure to build inside the housing as the plunger of the syringe is depressed. As pressure builds, filtrate runs through the membrane and is collected in a beaker. As filtration occurs, the pressure required to push the sample across the membrane increases as osmotic pressure of the sample rises due to concentration increases. Once the pressure became insurmountable and separation no longer occurred, the effluent retentate stream was collected in a beaker. The two samples are analyzed on a GC. The housing is cleaned out with water and a new membrane disk is inserted to ensure no cross contamination across tests.

Figure 5: 43 mm disk membrane format housing used for testing of RO membrane cutout
3.2.3 GC Analysis

The reverse osmosis product streams and initial feed samples were run on a separate GC column (shown below in Figure 6) for a 3 minute run time using parameters seen in Table 5 below. This GC column did not have a mass spectrometer. This allows for samples containing water to be run. However, because of the lack of a mass spectrometer, the GC peaks could not be identified for specific compounds. If the membrane was completely rejecting of organic compounds, the GC spectrum of the filtrate would have no peaks since only water would pass through the membrane and water not show up on the GC. If only ethanol and water pass through there should be one peak. If there are several peaks on the filtrate spectrum, this means some of the other organic compounds are passing through the RO filter. By comparing the gas chromatograph results of the effluent, retentate, and original sample, the overall performance of the membrane was qualitatively determined. This method will determine on a higher level if compounds are making it through the membrane, but not the actual size selectivity of the membrane.

![Gas Chromatograph](image)

**Figure 6:** Gas Chromatograph used for this part of the study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Set Value</th>
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<tbody>
<tr>
<td>Oven Temperature</td>
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</tr>
<tr>
<td>Inlet Temperature</td>
<td>250°C</td>
</tr>
<tr>
<td>Detector Temperature</td>
<td>300°C</td>
</tr>
<tr>
<td>H₂ Flow</td>
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</tr>
<tr>
<td>Air Flow</td>
<td>300 ml/min</td>
</tr>
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</table>
4. Results and Discussion

4.1 GC-MS Sensitivity Analysis of Wine Flavor Compounds

In the GC-MS Sensitivity Analysis, 8 compounds were tested in varying concentration levels to determine the detection limit as well as form a makeshift calibration curve for each compound. The compounds that were tested in this sensitivity analysis are shown below in Table 6. A discussion of the testing results for each of these compounds on an individual basis follows the table. The primary testing compound with the most analysis performed on it was Vanillin due to the timing of compound reception. Any test data not mentioned explicitly in this section can be found in the Appendix, this includes peak identifications and GC-MS Spectra for all of the tests.

Table 6: Compounds tested in GC-MS Sensitivity Analysis
(All images in this table are courtesy of commons.wikimedia.org except for glucose which is courtesy of the website of Sigma Aldrich)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isobutanol</td>
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</tr>
<tr>
<td>Tartaric Acid</td>
<td>C₄H₆O₆</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>Glucose</td>
<td>C₆H₁₂O₆</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>Isoamyl Acetate</td>
<td>C₇H₁₄O₂</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>Isoamyl Alcohol</td>
<td>C₅H₁₂O</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>Vanillin</td>
<td>C₈H₈O₃</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>Acetophenone</td>
<td>C₈H₈O</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>Quercetin</td>
<td>C₁₅H₁₀O₇</td>
<td><img src="image" alt="Structure" /></td>
</tr>
</tbody>
</table>
4.1.1 Isobutanol

Isobutanol was run at concentrations of 500 milligrams per liter, 10 milligrams per liter, and 100 micrograms per liter. The isobutanol had a low response on the GC with the area of the 500 milligram per liter run registering at 12.7 million while the other compounds registered around 30-40 million at this concentration. The isobutanol retention time was found to be around 3.4 minutes. This low retention time means that there is some interference from the solvent peak of ethanol. Interference from the ethanol peak results from the overlap of the solvent peak and the sample peak, which could have led to broader peaks and elevated peak height compared to isobutanol that was tested in a different solvent. The resulting spectra of these tests are shown below in Figure 7.

![Figure 7: Isobutanol GC-MS spectrum for a) 500 milligrams per liter b) 10 milligrams per liter and c) 100 micrograms per liter.](image)

Due to the low responsiveness and decreasing concentration it is evident that the peak for isobutanol is undetectable at the 100 micrograms per liter level which would put the detection limit somewhere between 100 micrograms per liter and 10 milligrams per liter as the peak was still present at 10 milligrams per liter. Another important aspect to note is the peak at a retention time of 4 minutes, which represents a contaminant. This contaminant was constant across the board at a height of around 2 million and can actually be used as a reference. The source of the contamination was never identified; however the contaminant itself was identified by the MS to most likely be a silane compound or organic acid.
4.1.2 Tataric Acid

The analysis of tartaric acid presented some intriguing data. An initial test was run to determine the residence time of the acid. The resulting spectrum showed a residence time of around 14.4 minutes with a fairly odd shaped peak which is shown in Figure 8 below. The peak had an extremely low height for a considering the relatively large concentration of compound used in the initial test. The other interesting aspect of all of the spectra for tartaric acid was the presence of other peaks that were relatively large and not correlated through MS with tartaric acid. The MS identified these peaks as correlating to compounds such as propanoic acid, a furandione compound, and other unexpected compounds. The full list of these peak identifications is present in the appendix in the form of screen captures from the MS for each peak. The final important note is the lack of a peak at 14.4 minutes in the 500 milligrams per liter concentration solution of tartaric acid which is shown in Figure 9 below. This would mean that the detection limit of tartaric acid is above the highest concentration considered. The source of these interesting issues was unclear, however some ideas of what could cause these issues are: there is some sort of interaction with tartaric acid and the column, a contaminant compound present in the solution or column, poor detection of tartaric acid by the column, contamination of raw materials, and a reaction between tartaric acid and the solvent.

Figure 8: High Concentration test spectra to determine residence time of tartaric acid to be 14.4 minutes

Figure 9: 500 mg/L test spectra of tartaric acid showing no tartaric acid peak at 14.4 minutes residence time
4.1.3 Glucose

While preparing the samples of glucose for GC-MS testing, it became apparent that the glucose was not readily soluble in the ethanol solvent. Some of the glucose remained crystalline in the vial. Without the water usually present in wine, the glucose would not fully go into solution. This posed a fairly large problem with the idea of getting a detection limit in ethanol since it was not possible to really get a calibration curve for glucose in ethanol. It also posed the problem for concentrating wine flavors with water removal. If the water were to all be removed in order to run on the GC-MS, the glucose would precipitate out to a certain degree and will affect the concentration of flavor compounds in the remaining solution. One possible way to remedy this that could be pursued in the future is to incorporate a secondary co-solvent for the solution in order to allow compounds such as glucose to stay in solution. There could be potential other issues with this recommendation that would have to be analyzed through experimentation.

4.1.4 Isoamyl Acetate

Isoamyl acetate was run on the GC-MS at 500 mg per liter, 10 milligrams per liter, and 100 micrograms per liter. The initial run of isoamyl acetate showed a residence time of 5.6 minutes. Isoamyl acetate has the highest response of the compounds, with a total area of 45.4 million on the 500 milligrams per liter run. The isoamyl acetate peak at 500 milligrams/liter seems to have an irregular shape as seen in Figure 10. This could be a result of isoamyl acetate having a high responsiveness in this specific GC column. The compound could be saturating the column and broadening the peak, similar to what happens in the solvent peak.

Figure 10: GC Spectrum of 500 milligrams per liter concentration isoamyl acetate
4.1.5 Isoamyl Alcohol

Isoamyl alcohol, with an experimentally derived retention time of 4.1 minutes, was run on the GC-MS at 500 milligrams per liter, 10 milligrams per liter, and 100 micrograms per liter. During the runs, there was a contaminant peak with a slightly shorter residence time than the isoamyl alcohol which can be seen in Figure 11. This contaminant peak could have interfered with the isoamyl alcohol peak, slightly increasing the integration on the 10 milligram per liter run. On the 100 microgram per liter run, there is a large broad peak at lasting until 6 minutes as seen in Figure 12. The MS identified the peak as n-butanol. This peak could be attributed to lab error as n-butanol was being used as a substitute solvent for other samples and could have been used to dilute the isoamyl alcohol instead of ethanol.

![Figure 11: Spectra of Isoamyl Alcohol at 10 milligrams/liter](image1)

![Figure 12: 100 microgram/liter run with n-butanol contamination](image2)
4.1.6 Vanillin

Vanillin was run through the GC-MS in concentrations of 1 ng/L, 10 ng/L, 1 µg/L, 100 µg/L, 1 mg/L, 10 mg/L, and 500 mg/L, as well as an initial test to find the residence time of the compound. Vanillin was run in the largest spread of concentrations due to the fact that it was one of the first compounds received in the lab. The test run for residence time confirmation showed an approximate time of 14 minutes to obtain the peak for Vanillin. This test is shown in Figure 13 below. This test had the Vanillin peak represented as an especially broad peak due to oversaturation and actual testing revealed the peak to actually occur at around 13.7-13.9 minutes.

![Figure 13: Vanillin test run on GC-MS to determine residence time](image)

Testing revealed that the noise consumed the peak starting down around 100 micrograms per liter. The spectrum for the 100 microgram per liter test is shown in Figure 14 below. The other interesting details to note on the 100 microgram per liter test are the increased noise compared to the other tests as well as the unexpected peaks around and before 4 minutes. If time permitted, it would be a smart idea to go back and rerun this test in order to see if it was an instrument error or contamination of that specific sample causing those peaks to occur as well.

![Figure 14: Vanillin GC-MS spectrum for 100 micrograms Vanillin per liter of ethanol](image)
Another issue that presented itself during the testing that is most apparent in the Vanillin tests is the accuracy of the MS at detecting what compound is present at lower concentrations. When the 500 milligrams per liter test was performed, the MS indicated the peak as Vanillin with a similarity value of 96. Dropping the concentration down to 100 micrograms per liter decreased similarity to 62. This led to Vanillin being the 5th recommendation from the MS as to what the compound could be as opposed to the first on the high concentration run. The similarity value for the Vanillin peak in the 100 micrograms per liter solution is shown in Figure 15 below.

![Figure 15: Vanillin peak MS similarity analysis for 100 micrograms per liter concentration](image)

### 4.1.7 Acetophenone

Acetophenone was run at concentrations of 500 milligrams per liter, 10 milligrams per liter, and 100 micrograms per liter. Even at the 100 micrograms per liter level, the GC-MS was still able to detect the compound. The residence time for the Acetophenone peak was around 8.8-9 minutes. The 500 milligrams per liter and the 100 micrograms per liter spectra are shown below to demonstrate how detection drops with concentration of Acetophenone. It also serves to illustrate an interesting point of unexpected peaks. These spectra are shown in Figure 16 and Figure 17 below.
The scale of the y axis changes from x1,000,000 to x100,000 which accounts for the height change in the peaks that are not Acetophenone. The peak height and area of the Acetophenone peak drops with concentration but is still noticeably above the noise. The extra peak that is shown to be the tallest peak in the 100 micrograms per liter spectrum was identified by the MS as a form of methylcyclohexanol. This was not a compound that was expected to be in the sample and could have resulted from contamination of sample during prep, though having it be consistently there across all samples at similar levels would say this is probably not the case. It is also possible that it resulted from contamination of raw materials or some sort of weird reaction within the vial or column. Another possibility is instrumental error, meaning that the MS just could not properly identify the peak.
4.1.8 Quercetin

Quercetin is a very large compound with the formula C\textsubscript{15}H\textsubscript{10}O\textsubscript{7}. When run on the GC-MS instrument, no peak was observed even at extremely long run times. It is believed that this is due to the extremely high melting point of the compound which is 316.5 degrees Celsius (national library medicine). The column cannot safely run hot enough, with a max temp of approximately 330 C, to get the compound to vaporize adequately to receive a peak. This made testing of Quercetin near impossible on the GC-MS that was available. This has the potential to pose a fairly significant problem in wine flavor analysis if other large flavor compounds suffer from the same issues that were encountered with Quercetin.
4.1.9 Relation between Area and Concentration

Using the data from the runs of each of the 8 compounds, it is possible to try to find a correlation between peak area and concentration. Finding a calibration curve for each compound would allow future tests to determine the concentration of an individual known compound within the wine. The current method used for this would be to take a known amount of a substance not present such as acetone and spike the sample with that, however these compounds could cause unwanted chemical interactions or interference with data collection. There is not one calibration curve that would allow someone to look at a GC spectrum and say this area corresponds to this concentration, the curve varies from compound to compound. The curves generated in this report were from only a few data points and are just a starting point for more detailed and accurate curves to be generated. Each test of the compounds that actually worked in the GC-MS column was analyzed for peak height and area and related to the known concentration of the sample. Some of these tests ran into errors and thus were not able to be used. The data was then used to plot area against concentration for the compounds which is shown below in Figure 18.

![Figure 18: Area plotted against concentration of sample](image)

The resulting graph shown above demonstrates a linear relationship between concentration and peak area. This is the result that was expected; however it also shows that every compound follows a different linear relationship, though some are very similar. These lines are based on 3-4 data points each and surely must be refined by adding in more data points.
The amount of data points was limited by the amount of time that was available to test in. Since there are two to three relatively small concentration points and 1 large concentration point on each, the perfect linear relationship is probably not entirely accurate at the moment. Future tests should generate the curve using a wider spread of concentrations for the data points for increased accuracy and examine the results at that point.

### 4.1.10 Relation between height and Concentration

An attempt was made to relate height and concentration the same way that area and concentration were related. The attempt was made in the same manner as the area attempt in which the data was collected from multiple samples, entered into an excel table, and plotted against concentration. This attempt was not as successful as the area and concentration relationship. The resulting points did not match up nicely to a linear trend as shown below in Figure 19. It may still be possible, if enough data points are collected, to create a calibration curve for each compound, though it will most likely not be linear. The data seemed to follow a trend with a sharper curvature at lower concentrations and level out later, however with so few data points, especially at higher concentrations, it is hard to tell for certain what shape it will take.

![Figure 19: Height plotted against concentration of sample](image-url)
4.2 Water Mitigation and Concentrating Methods

4.2.1 Reverse Osmosis:

The DOW Filmtec™ reverse osmosis membrane was tested for uses of concentrating wine flavor compounds as a means of artificially increasing the detection limits of the GC-MS and mitigating the effects of water on the hygroscopic MS packing. Two different forms of the membrane were tested throughout the course of our experiments. The first was the wound capsule, which is the format that the membrane initially comes in when purchased. The second was a disk of approximate diameter of 43 millimeters. The 43 mm disks were not 100% accurate as they were cut out of the sheet using an edge, not an exact 43 mm dye.

As mentioned in the Methodology, the feed and effluent streams from these tests were sampled and run on a Gas Chromatograph. The results of these GC runs are discussed in this section as well. This section aims to outline the raw GC spectra, what it tells us, and how we might be able to improve the performance in future tests.

4.2.1.1 Wound Capsule

The wound capsule membrane was initially run with water on a peristaltic pump. It was found that the pressure applied by the pump, approximately 25-30 psi based on the pump rating, was insufficient to overcome the resistance within the membrane filter. In order to overcome this resistance, a 12 volt diaphragm pump rated at 80 psi was attached to the inlet side connection of the membrane capsule. This new pump was able to apply enough pressure to obtain an effluent flow for the water tests. The water in these tests flowed from both the retentate and filtrate streams of the capsule, which proved that water could fit through the membrane.

The wound capsule was then put to the test with an ethanol-water-glycerol mixture. This mixture was composed of 85% water, 14% ethanol, and 1% glycerol. This mixture was to see if a compound as large as glycerol could make it through the mixture. These tests also served to examine the possibility of ethanol passing through the membrane as well since it is relatively close in size to water. It was found that there was only flow through the retentate path of the capsule. This means that the mixture was flowing tangentially over the membrane, but not passing through the membrane itself to reach the filtrate stream.

In order to test if the addition of the large glycerol molecule was resulting in the problem, a water-ethanol mixture was made and run through the membrane capsule. This solution was composed of 85% water and 15% ethanol. The resulting effluent flow was once again only exiting through the retentate stream and not passing through the membrane into the filtrate stream. This led us to believe that the filter would require a higher pressure to obtain a filtrate flow due to the impurities in the mixtures resulting in an increased osmotic pressure when compared to tap water. To obtain a filtrate stream would require a higher pressure, however for
safety reasons we did not want to elevate the pressure beyond 80 psi in our experiments. Furthermore, there were concerns that a pressure far greater than 80 psi would be needed to obtain a near anhydrous sample as the osmotic pressure would increase dramatically with the decreasing concentration of water.

### 4.2.1.2 43 mm disks:

The 43 mm disks were cut from the membrane that was wound up in filter capsule. The first major issue with this method arose from the inaccuracy of cutting the disks out with a razor edge. The disks could not be assured to be the exact right size without a dye, and this could lead to potential leaks if the membrane disk was too large for the housing. However, this method has its advantages as well. The decreased size of the membrane reduces the required pressure at the inlet and separation could actually be achieved. Another advantage to this method is the reduction of dead space in the apparatus which decreases the amount of sample required per run. The reduction in sample size is fairly dramatic with the capsule requiring samples on the order of a liter and the disks on the order of 40 to 50 milliliters.

### 4.2.1.3 GC Analysis:

The reverse osmosis filtration method incorporating 43mm disks was applied to samples of Zoll Cellars Hard Apple Cider and Fox Run Riesling wine. These samples were run through the membrane housing to obtain a retentate and a filtrate stream sample. The filtrate, retentate, and a sample of the initial feed of either cider or wine were run through the gas chromatograph in the lower level of the WPI Unit Operations Lab for 3 minutes. The resulting spectra from the Zoll Cellars Cider are seen below in Figure 20, Figure 21, and Figure 22.

![Figure 20: GC Spectrum of the Zoll Cellars Cider Original sample](image)
The peak which appears to correspond to Ethanol is located at approximately 1.35 minutes into the test. The justification for this observation is the relative height of the peak and consistency of the peak’s presence in the cider samples and the wine samples. Ethanol is known to be in quite high concentrations in both the cider and the wine. With a concentration by volume of approximately 15% in wine and 6% in cider, Ethanol is the most abundant organic compound present in our samples which should match up to the largest height peak or peak with the largest integral which are both true of this peak at 1.35.

The other peaks present in the filtrate sample revealed that more than water and ethanol was coming through the membrane. This showed that even though the membrane was separating the solution to some degree, the size selectivity was not good enough to completely exclude flavor molecules. Despite the knowledge that these peaks show the presence of other compounds, the identity of these compounds remains unknown. If these unknown flavor compounds were being removed, then the resulting flavor profile of the wine would not have been accurate.
The cider analysis provided some support for the idea of using a membrane to concentrate the solution by removing water and ethanol, but also showed that this specific membrane may not have been tight enough. To further test these assumptions, the same tests were performed on the Riesling wine. The resulting GC spectra are shown in Figure 23, Figure 24, and Figure 25 below.

**Figure 23:** GC Spectrum of the Riesling Wine Original sample

**Figure 24:** GC Spectrum of the Riesling Wine Retentate sample
The wine tests were less conclusive and had some interesting issues. It appears that the filtrate has peaks that were not present in the original sample or had peak splitting of some sort. Some research was performed and peak splitting is something can apparently happen, however it is unknown why it would occur in the wine sample for filtrate or retentate and not the original sample. This test should most likely be run again with a few different wines and on a tighter membrane to determine if reverse osmosis can actually work for concentrating wine flavors.
5. Conclusions and Recommendations

A working system to dehydrate wine is still needed for testing of wine on GC-MS. Initial tests on the capsule housing showed that filtrate could not be produced at pressures below 80 psig. For these reasons, it is not recommended to pursue future testing using this method. The 43mm disc housing was able to produce sufficient volumes of sample at low pressures. While filtrate was produced, tests through a GC-FID showed the DOW Filmtec® reverse osmosis (RO) membrane allowed for organic compounds to pass through. This suggests that the pore size of this membrane is too large, as detailed in Results and Discussion. Despite the results of this test, RO still remains a viable means to separate water from wine since some separation was seen in the GC analysis. A membrane with smaller pore size could provide the required separation needed for the removal of water without the loss of flavor compounds.

If a wine sample could be dehydrated, solubility of flavor compounds could become a technical hurdle. In the GC-MS tests of glucose, results showed that ethanol was not a sufficient solvent to dissolve the glucose. A proper co-solvent could mitigate this issue of solubility. As such, a proper co-solvent is a point of investigation that we recommend for future studies should the solubility become an issue, which based on glucose tests it most likely will.

Results from the GC-MS sensitivity analysis indicated that there was linearity in the between the concentration and response as seen in Figure 18. Linearity between concentration and response is already documented in literature and was not a surprising find (EPA, 2007). The literature also indicated that there should be linearity between peak height and concentration. This was not found to be true in our tests as seen in Figure 19. An explanation for this behavior is at higher concentrations the column becomes saturated, causing the peak to broaden and the height to drop. The 500 mg/l data points appear to have a lower value than the expected, which supports the notion of peak broadening. Further testing of relatively dilute samples, 0.5mg/l-50mg/l, could verify linearity of peak height to concentration. If this linearity can be established, the detection limit for each compound can be found by extrapolating the peak height to three times the noise limit1.

During testing the GC-MS sensitivity testing, there was a need for a wine compound list database to be complied. When the software processes the MS results, it runs the m/z against a database of compounds. If the select flavor compound has a weak signal, the software may determine the likely identity of the compound to be one with a similar m/z as seen in Figure 15. If the GC-MS results were run against a database of known wine compounds, instead of a database of all known organic compounds, the incorrect identification of the compound could be decreased.

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1 3:1 detection limit is in align with the standards of ICH
5.2 Summary of recommendations

Throughout testing, numerous ideas of potential future tests or experiments arose. Our recommendations for future items of research are based upon issues that arose during our experiments and testing that we wanted to do, but did not have the time to do. The recommendations we make are:

1. Test a tighter Reverse Osmosis Membrane in a similar manner to our tests in order to determine if RO membranes can be used to concentrate flavor compounds and remove water from wine
   a. Use disk based membranes over capsule membranes to reduce dead space in such experiments
   b. Run these experiments in a much more controlled methodology with a higher sample number to determine viability of RO as a separation and concentration mechanism for wine
2. Test alternative concentration methods such as zeolites
3. Investigate potential solutions to solubility issue with water removal leaving ethanol as the solvent, particularly investigate the idea of a co-solvent that won’t greatly affect GC-MS results but will allow the flavor compounds to remain in solution.
4. Continue to make calibration curves of area against concentration and investigate further into detection limits of known wine compounds using more data points than the curves present in this paper in order to increase accuracy and predictive power
5. Finally, make an independent GC-MS database of chemical compounds known to be in wine. This database would be searched first and if no results matched, the GC-MS could expand into the current database.
   a. This would potentially address the issue of a known compound occurring as the 5th most likely match in the GC-MS database currently
   b. A separate database could be helpful in order to prioritize compounds that are definitively known to occur in wine. Many of these compounds are not common in other experiments and thus may not be considered as likely results. This was an issue run into specifically with flavor compounds such as vanillin
6. Bibliography


7. Appendix

7.1 GC-MS Sensitivity Results

7.1.1 Isobutanol

Figure 26: Isobutanol Initial test with isobutanol peak identified

Figure 27: Isobutanol 500 mg/l test

Figure 28: Isobutanol 10 mg/l test
7.1.2 Tartaric Acid

Figure 29: Isobutanol 100 microgram/l test

Figure 30: Tartaric acid initial test

Figure 31: Tartaric acid initial test- 7.8 min peak
Figure 31: Tartaric acid initial test - 8.9 min peak

Figure 32: Tartaric acid initial test - 3.9 min peak

Figure 33: Tartaric acid initial test - 4.6 min peak
Figure 34: Tartaric acid initial test - tartaric acid peak

Figure 35: Tartaric Acid 500 mg/l

Figure 36: Tartaric Acid 10 mg/l
Figure 37: Tartaric Acid 100 microgram/liter

7.1.3 Glucose

Figure 38: Glucose initial test

Figure 39: Glucose initial test- glucose peak
7.1.4 Isoamyl Acetate

**Figure 40**: Isoamyl Acetate 500 mg/l

**Figure 41**: Isoamyl Acetate 500 mg/l

**Figure 42**: Isoamyl acetate 100 micrograms/l
7.1.5 Isoamyl Alcohol

**Figure 43:** Isoamyl alcohol 500mg/l

**Figure 44:** Isoamyl alcohol 10mg/l

**Figure 45:** Isoamyl alcohol 100 micrograms/l - faulted run
7.1.6 Vanillin

**Figure 46:** Vanillin initial test

**Figure 47:** Vanillin initial test - peak determination

**Figure 48:** Vanillin 500mg/l
Figure 49: Vanillin 10mg/l

Figure 50: Vanillin 100 micrograms/l

Figure 51: Vanillin 100 micrograms/l - peak ID
7.1.7 Acetophenone

**Figure 52:** Acetophenone 500 mg/l

**Figure 53:** Acetophenone 500 mg/l - peak ID

**Figure 54:** Acetophenone 10 mg/l
Figure 55: Acetophenone 100 micrograms/l

Figure 56: Acetophenone 100 micrograms/l- peak ID